In the name of God the merciful

Avicenna Research Institute
Avicenna Infertility Clinic

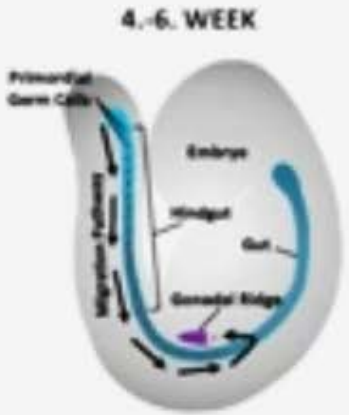
Contribution of Oocyte Quality to Success of ART Treatments

Sadeghi MR

Embryologist

Oogenesis

EMBRYONIC	PRENATAL	POSTNATAL/PREPUBERTAL			ADULT				
Primordial germ cells	Follicular stage	➡	Primordial	➡ Primary ➡	Secondary	➡	Antral	➡ Graafian	➡ Ovulatory



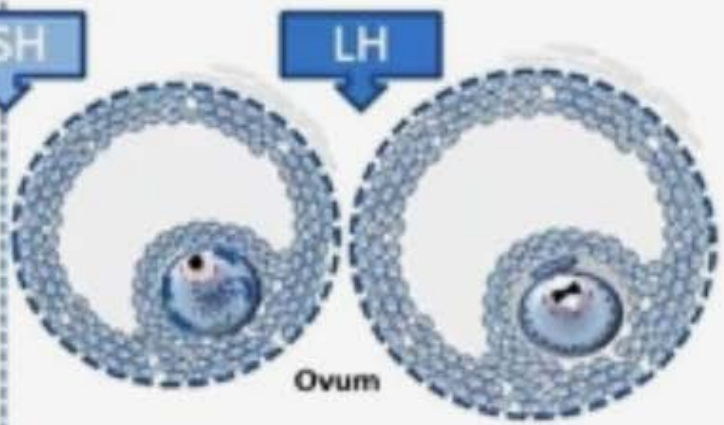
Migration to gonadal ridge

Oogonium

Cyst breakdown

Primary oocyte

Follicular growth



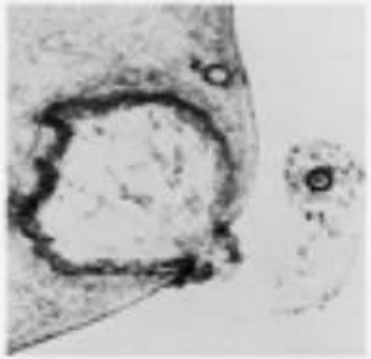
Ovum

Germinal vesicle

Pre-maturation

Metaphase II

Maturation



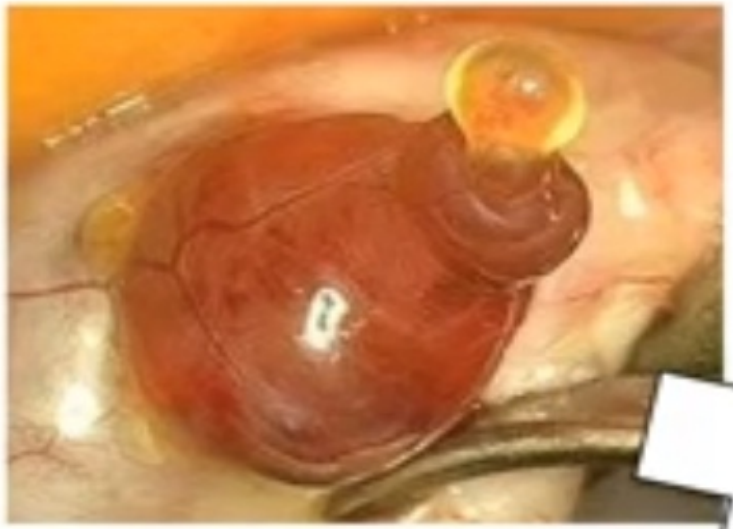
OVULATION

MITOSIS ➡ MEIOSIS | ARREST ➡ RESUMPTION ➡ ARREST

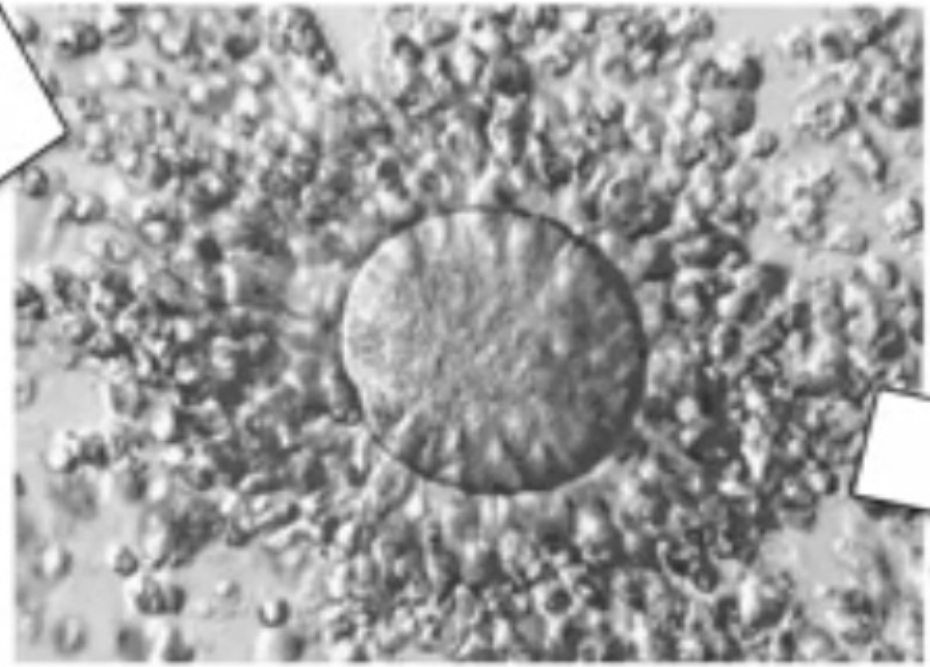
Wear et al. Journal of Ovarian research 2016
Knobil E, Neill JD. Physiology of Reproduction. 4th ed: Academic Press; 2015 (modified)

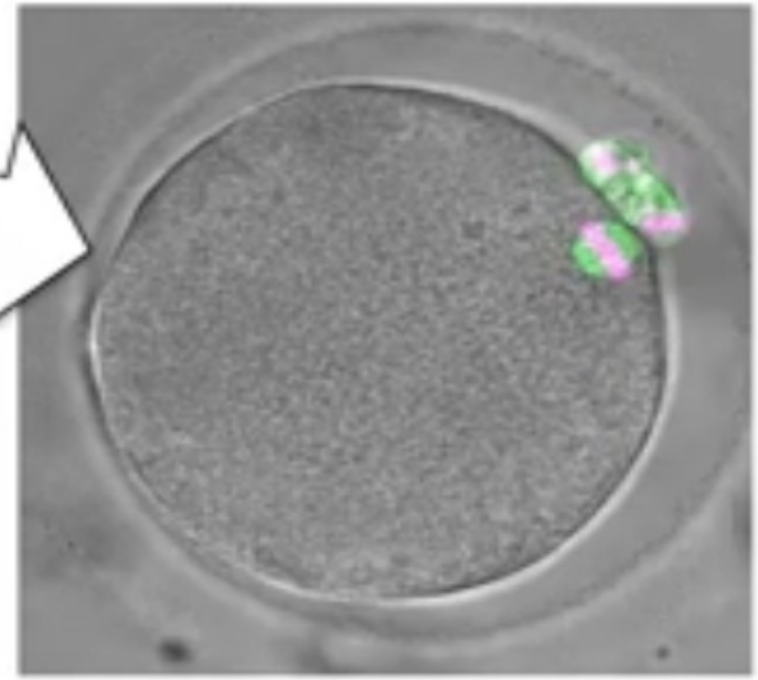
Oocyte maturation

IN VIVO



OVULATION

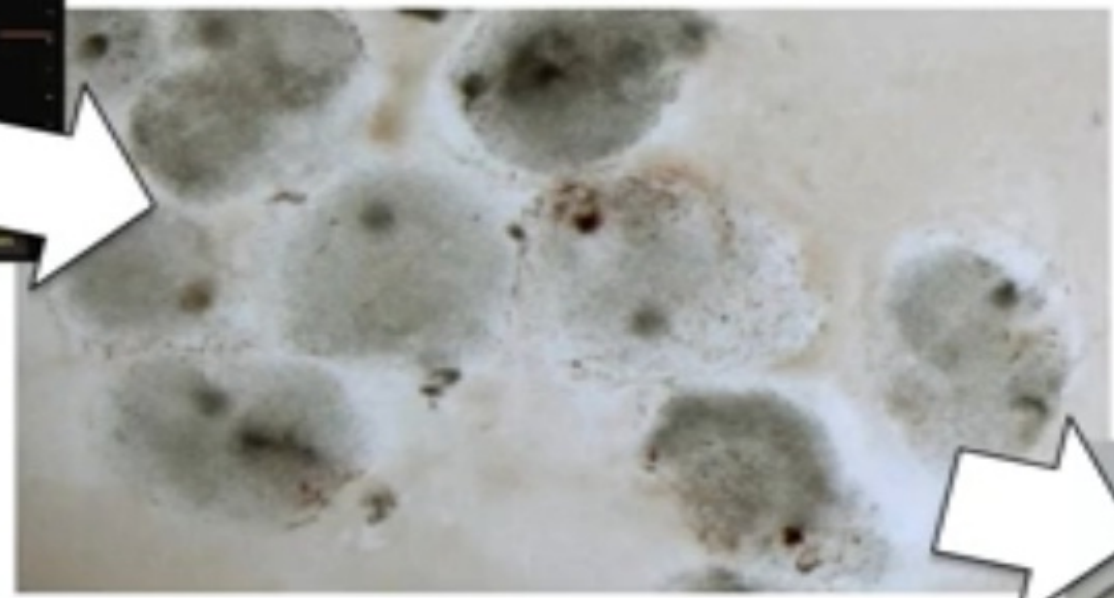


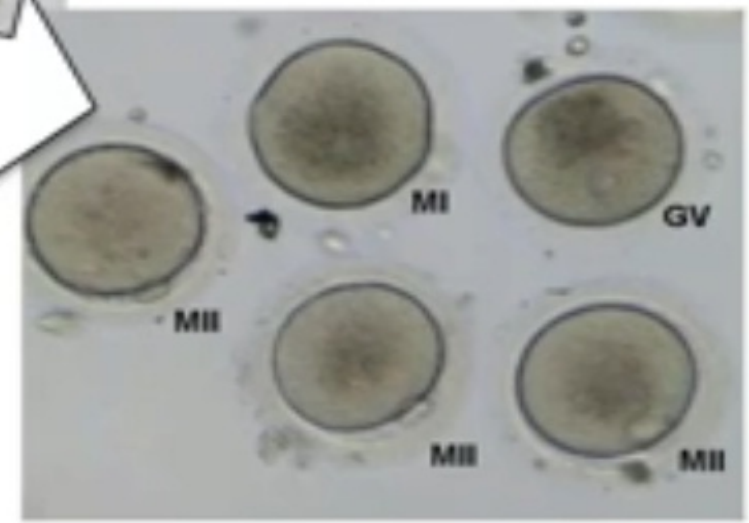


Oocyte maturation

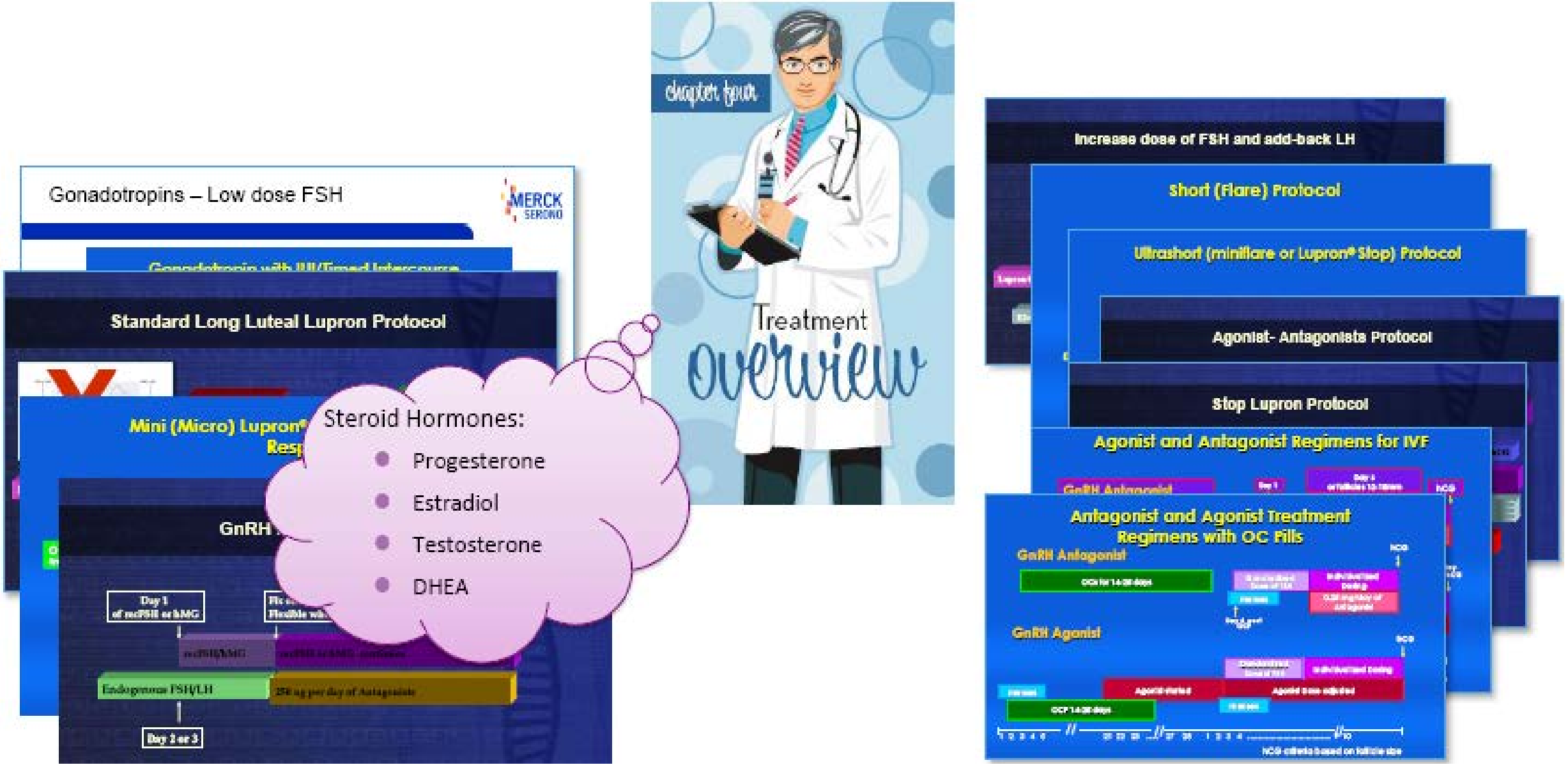
IN VITRO

PREOVULATORY FOLLICLES

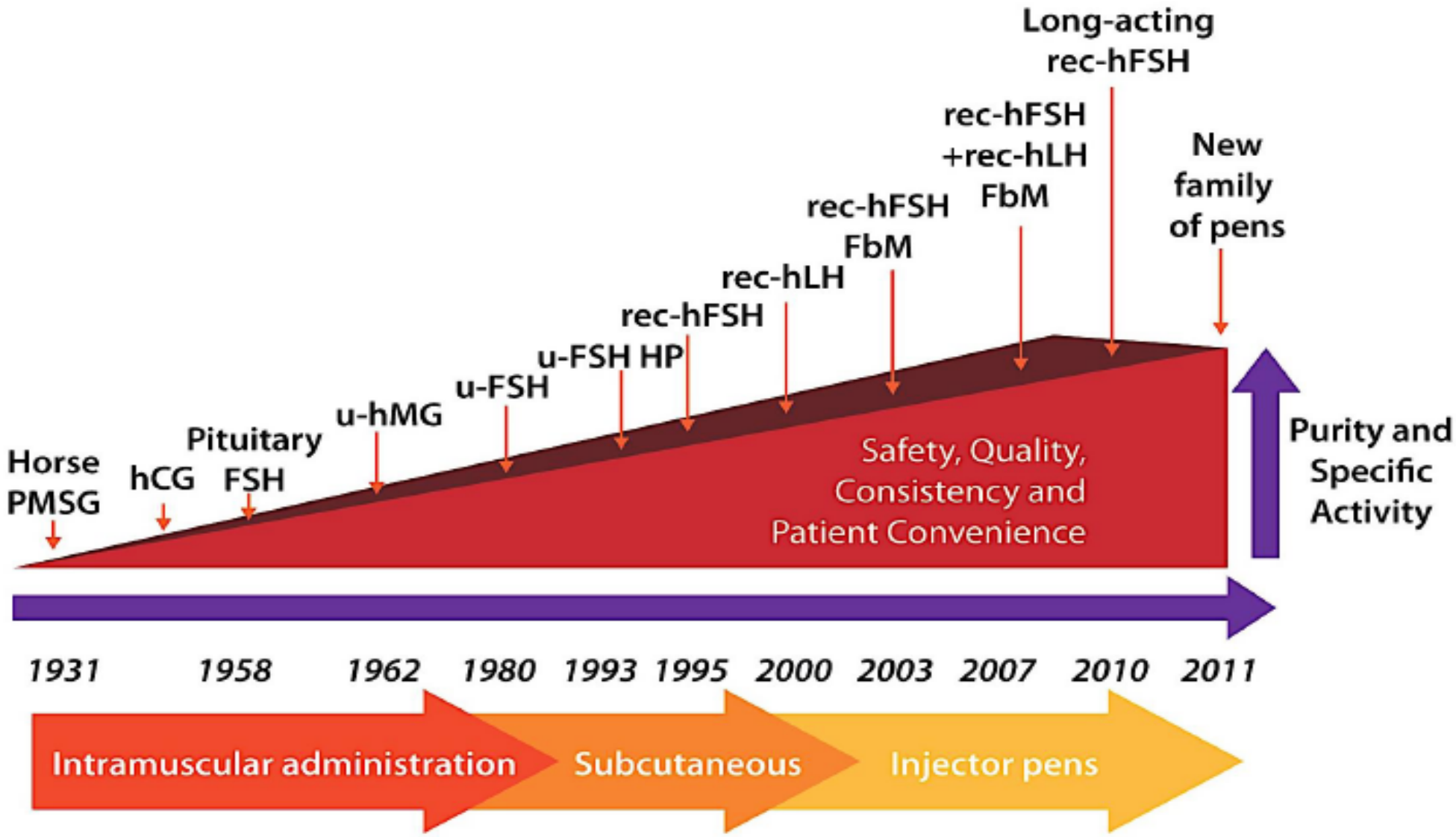




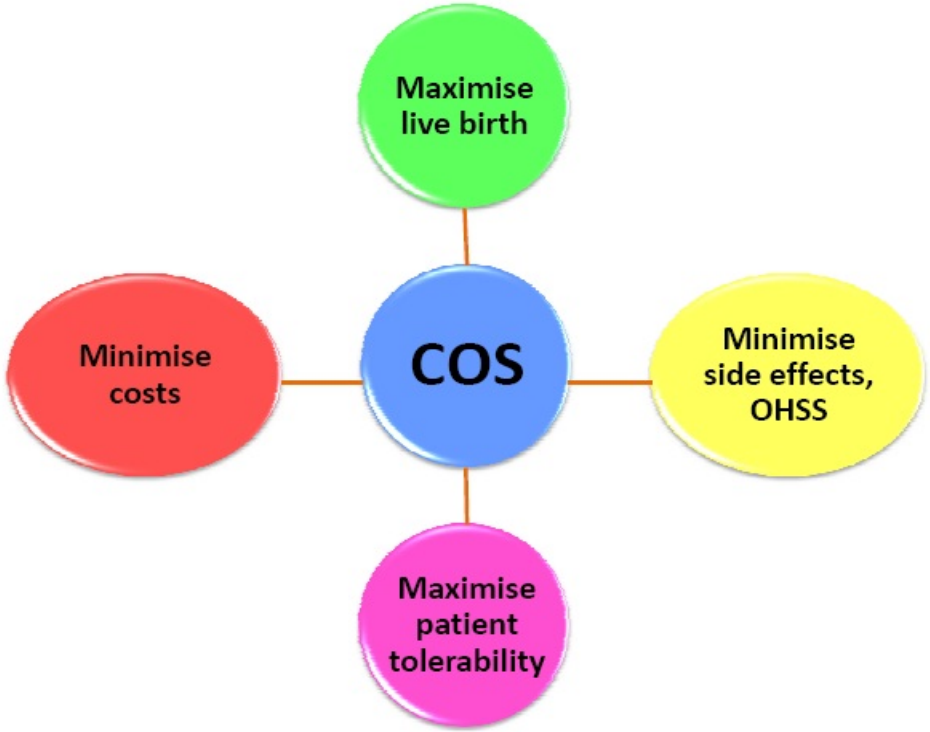
Multiple protocols of stimulation and drugs from different sources



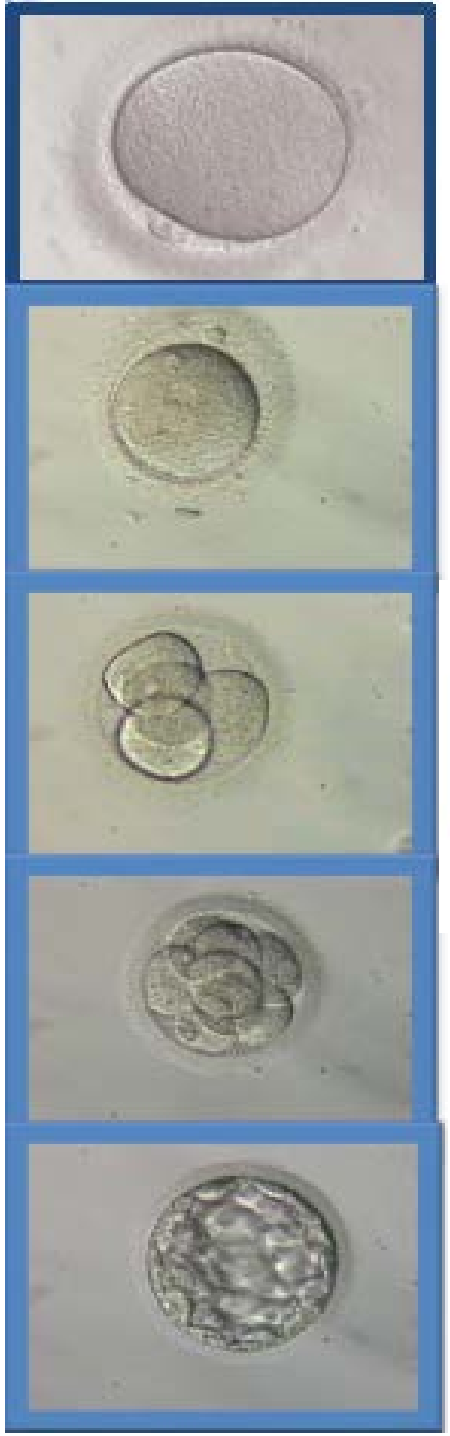
Gonadotropin Preparations and Oocyte Yield



Aim of COS




Normal oocyte



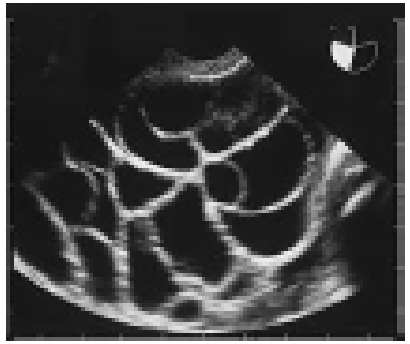
Oocyte quality means the ability to result in healthy live birth

iCOS
Central Paradigm

Minimize risks/complications

Poor Response

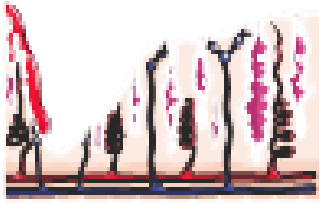
Hyper Response



Maximize Live Birth

Embryo Quality

Endometrium Quality



Ovarian Response & Oocyte Quality

AMH

HYPERINSULUNISM

AFC

FSHR,LHR GENOTYPE

AGE

ANDROGEN LEVELS

BMI

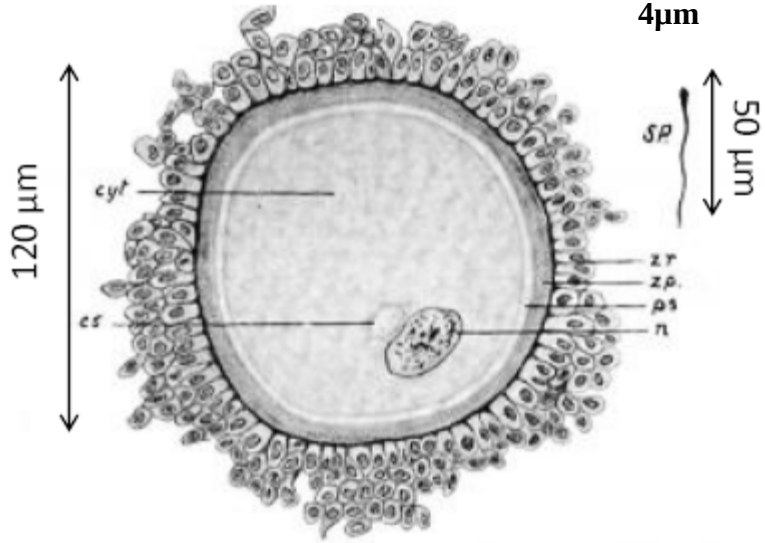
SMOKING

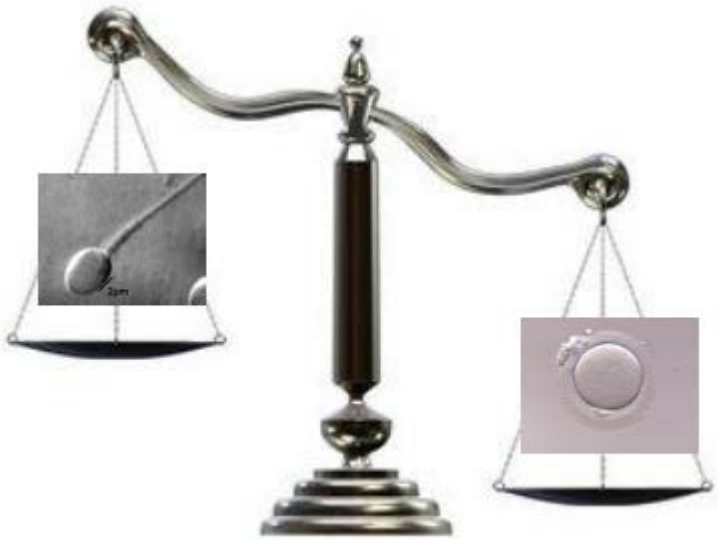
ETHNICITY

INFERTILITY DIAGNOSIS

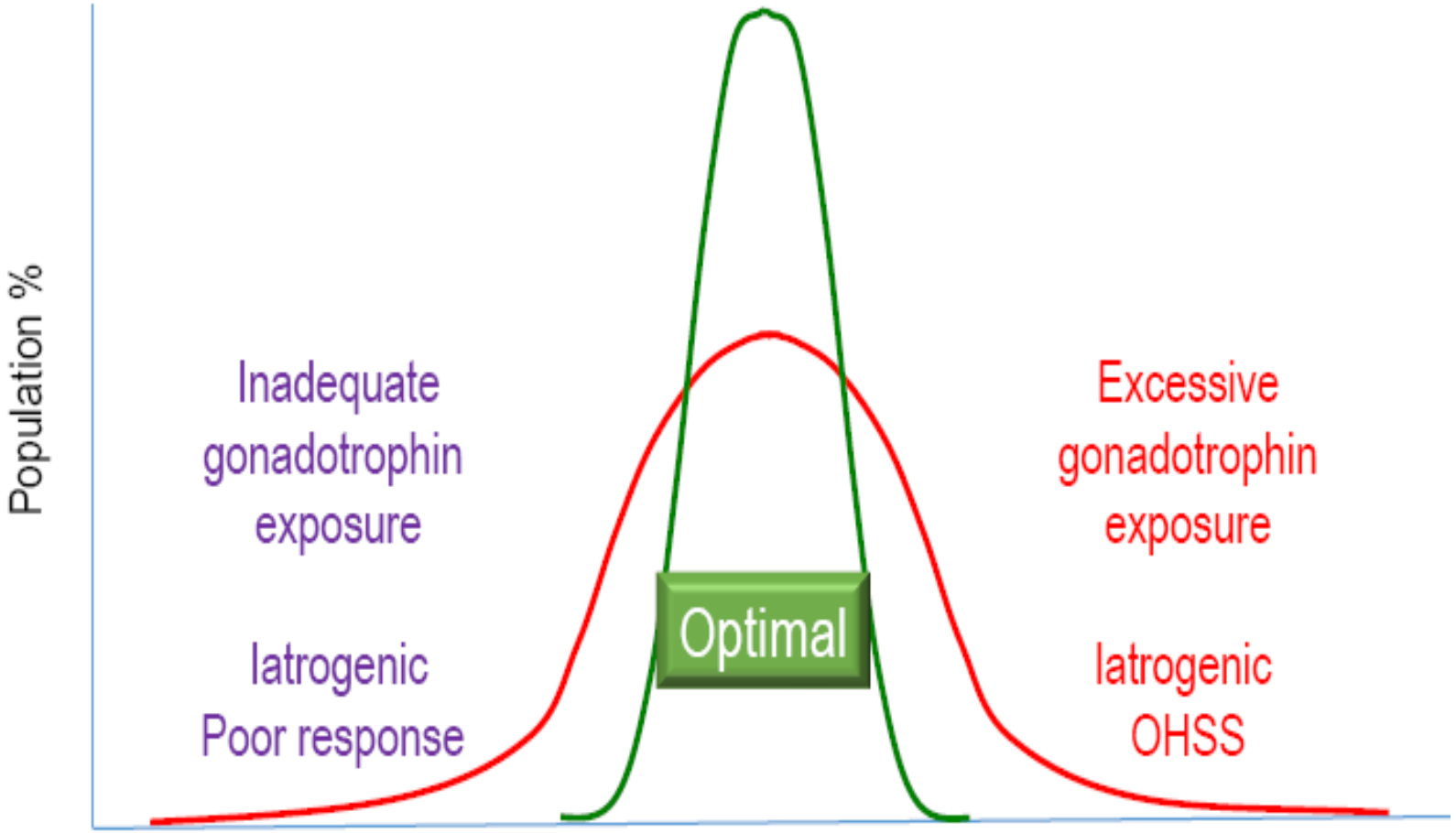
Sperm head

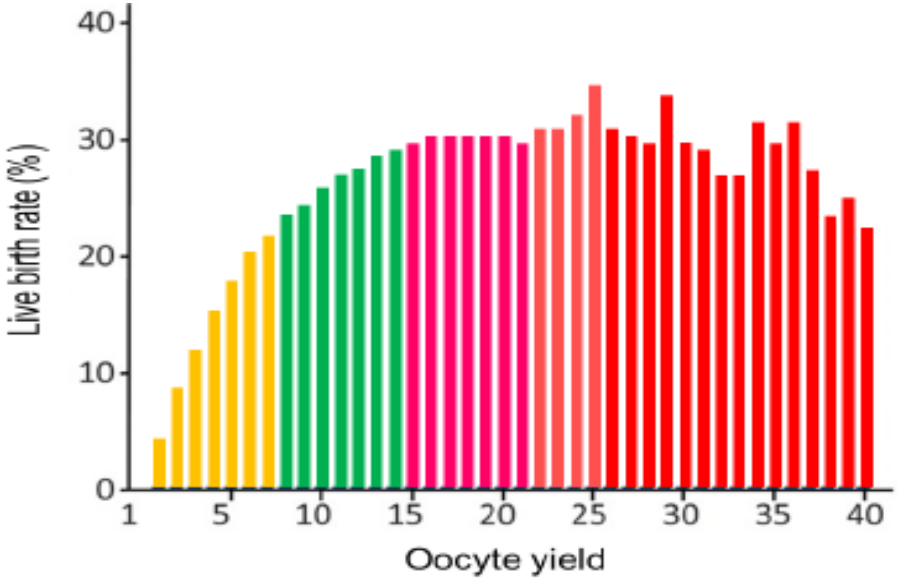
4µm





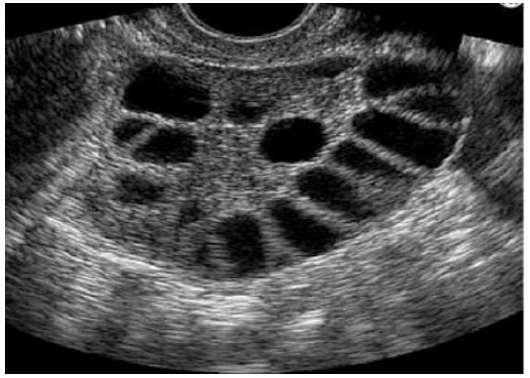
Individualized Controlled Ovarian Stimulation (iCOS)-Goals





Intrinsic factors influencing oocyte quality

- Polycystic Ovaries.



- Stimulation Protocols.

- Endometriosis.

- Advanced Maternal Age.

How many oocytes are needed to optimize PR?

- Van der Gaast et al-2006 - **13 oocytes**; below and above PRs are compromised
- Verberg et al-2009 - 5 for mild stimulation and **10 oocytes** for conve iation (meta-analysis ; mild-313 cycles; conventional-279 cycles)
- McAvey et al-2011 - Yielding **>6 M-II** oocytes d improve live birth rates (n=737)
- Bosch et al-2011 - LBR increase up to **15 oo** imize the chances of pregnancy (n=7954)
- Sunkara et al-2011 - LBR increase **ocytes**; plateaus between 15-20 and decline steadily beyond 20 (n=400,135)
- Ji et al-2013 Optimum - **6 ocytes** for LBR below and above PRs are compromised; however, cumulative LBR increase with increasing oocyte number (
- Fatemi - A high ovarian response **18 oocytes** does not jeopardize LBR in fresh ET's and even is associated with incre R (Engage; n=1,506)
- S ward et al-2014 - Retrieval of **>15 oocytes** significantly increases OHSS risk without improving LB rate in fresh autologous IVF cycles.

8-15 Retrieved Oocytes

What about iCOS for normo-responders?

- **Agonist vs Antagonist: No difference**

  Al Inani et al, 2011; Xiao [illegible]4

- **rFSH vs hMG in long protocol: No difference**

  Andersen et [illegible] (MERIT)

- **rFSH vs hMG in antagonist prootocol: No [illegible]ce**

  [illegible] et al, 2008; Devroey et al, 2012 (Megaset)

- **rLH supplementation in long pr[illegible] No difference**

  Kolibianakis et al, 2006

- **rLH supplementatio[illegible]agonist protocol: No difference**

  Griesinger et al, 2005; Bosch et al, 2010

- **Mild vs c[illegible]onal stimulation: No difference**

  Hohmann et al, 2003

- **[illegible] acting vs daily FSH: No difference**

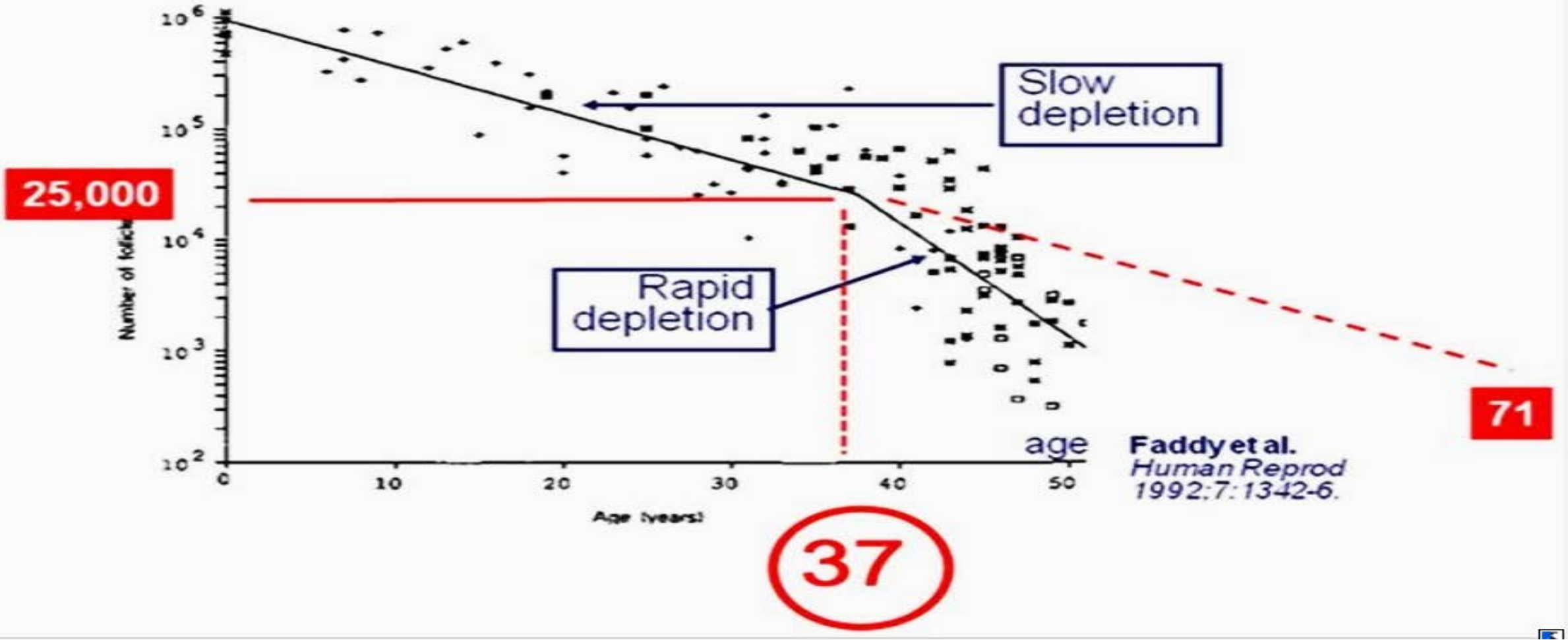
  Devroey et al, 2009

- **150 vs 200 IU/day of rFSH: No difference**

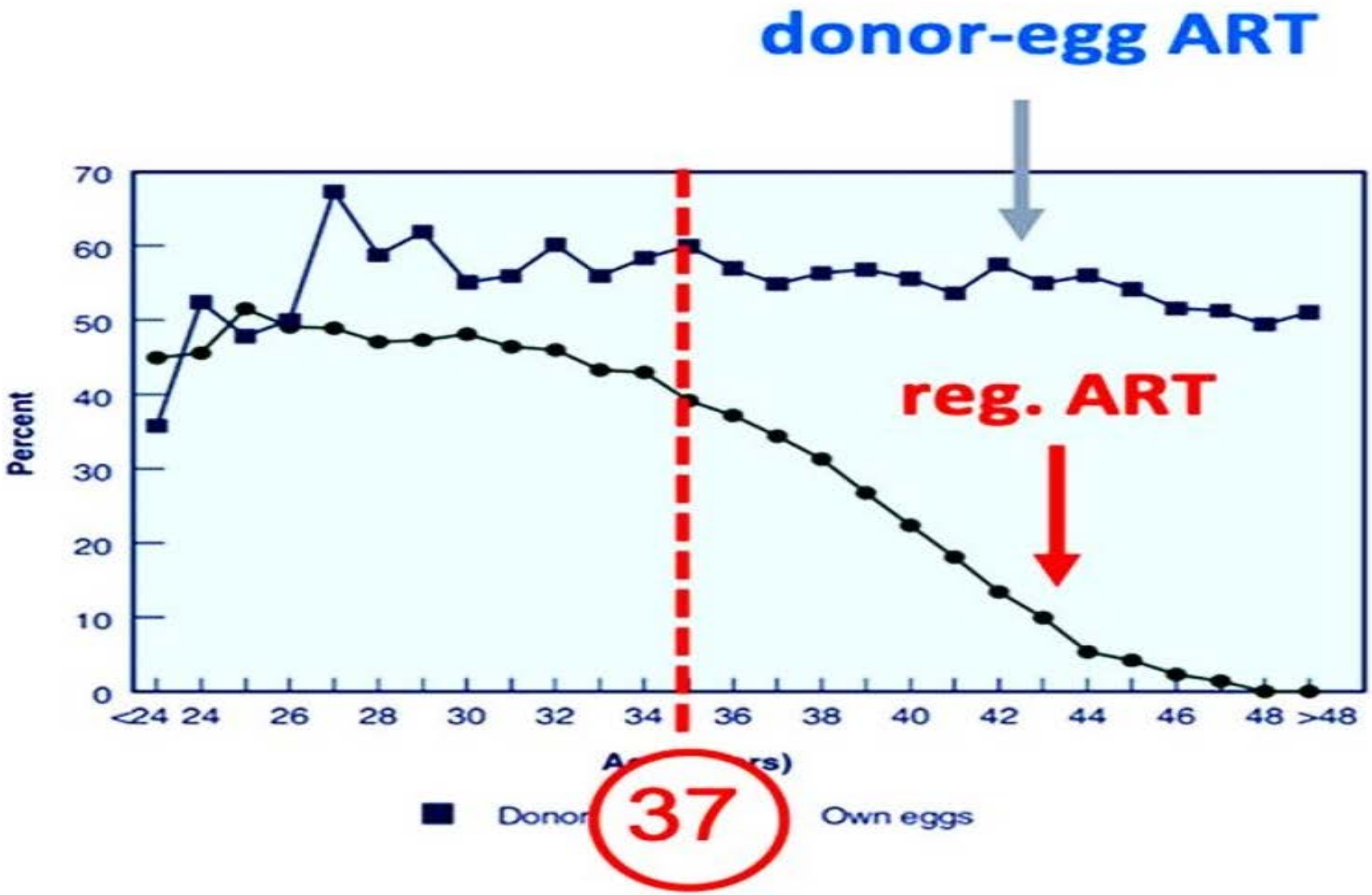
  Out el al, 2004

No Difference in Outcome…..

oocyte quantity



oocyte quality



Evaluation of oocyte quality

- Invasive and noninvasive methods are commonly used to select developmentally competent oocytes that can improve the take-home baby rates in assisted reproductive technology (ART) centers.
- One of the noninvasive methods conventionally utilized to determine competent oocytes is the morphological analysis of cumulus complex, first polar body, zona pellucida, perivitelline space, meiotic spindle, and ooplasm. Successful fertilization, early embryo development, uterine implantation, and healthy pregnancy depend on the quality of oocytes, and morphological evaluation is one of the options used to predict quality levels
- Ooplasm granulation patterns have predictive values for fertilization, pregnancy and live birth in IVF cycles, supporting integration of them into embryo selection, and suggesting that ooplasm granulation patterns reflect intrinsic features of oocytes that relate to oocyte quality, cytoplasmic maturity and developmental competence, but are largely independent of clinical co-variables.

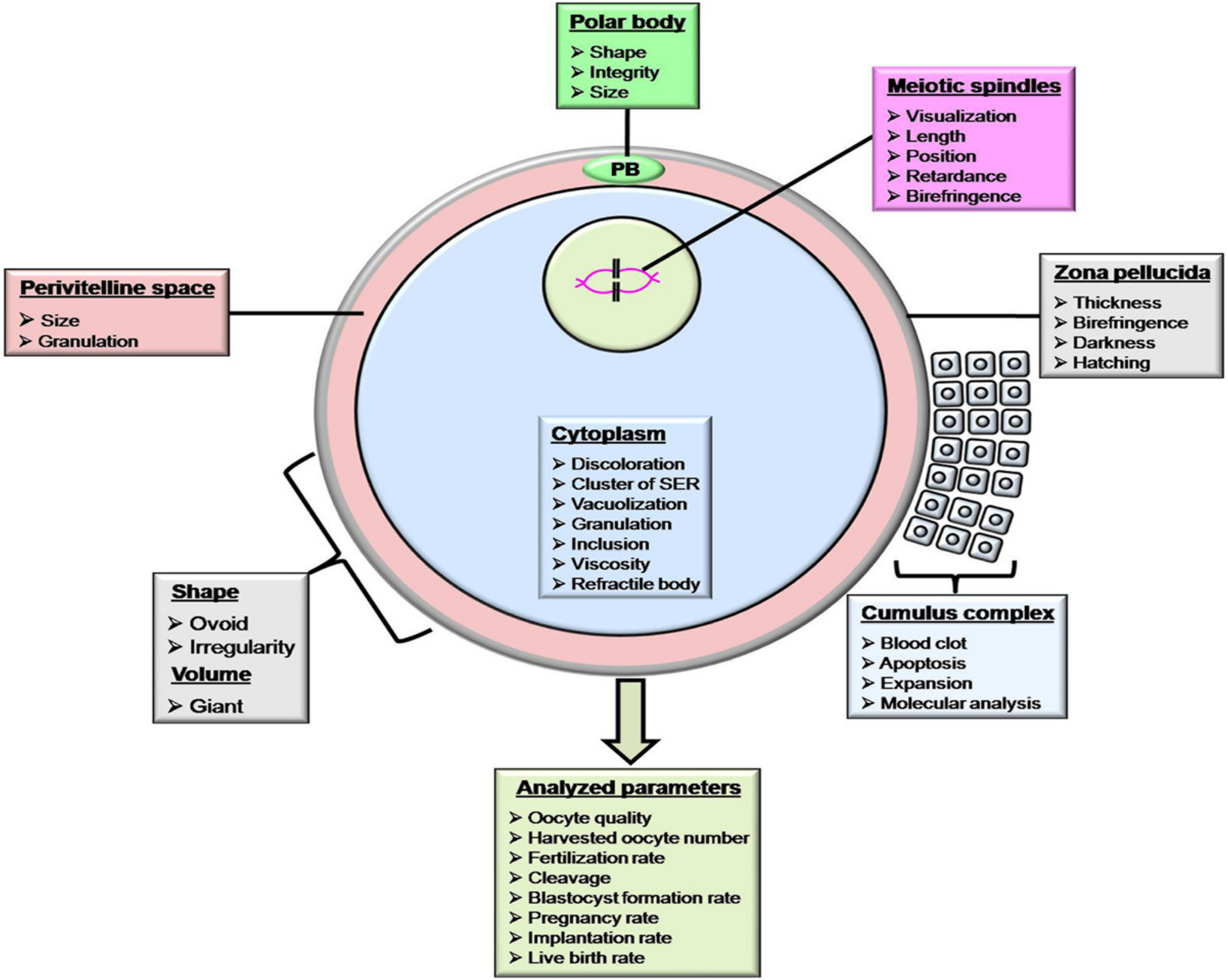
Evaluation of oocyte quality

- Several techniques have been engineered to evaluate oocyte quality during assisted reproductive technology (ART) procedures. The gold standard used to determine oocyte and embryo quality during ART is morphological assessment by trained embryologists.
- The evidence suggests that oocyte morphological assessment by embryologists can be useful in predicting in vitro outcomes but shows inconsistent success in predicting pregnancy or live birth.
- The modern approaches used to assess oocyte quality and prognosticate reproductive success include oocyte morphological evaluation, genomics or proteomics, and artificial intelligence.
- A considerable proportion of genomic- and proteomic-related articles identified promising cumulus cell biomarkers that may predict pregnancy and live birth.
- Machine learning(AI) is a promising new frontier that may minimize evaluator subjectivity and improve predictive ability, although further validation and standardization of this technology is needed before clinical use.

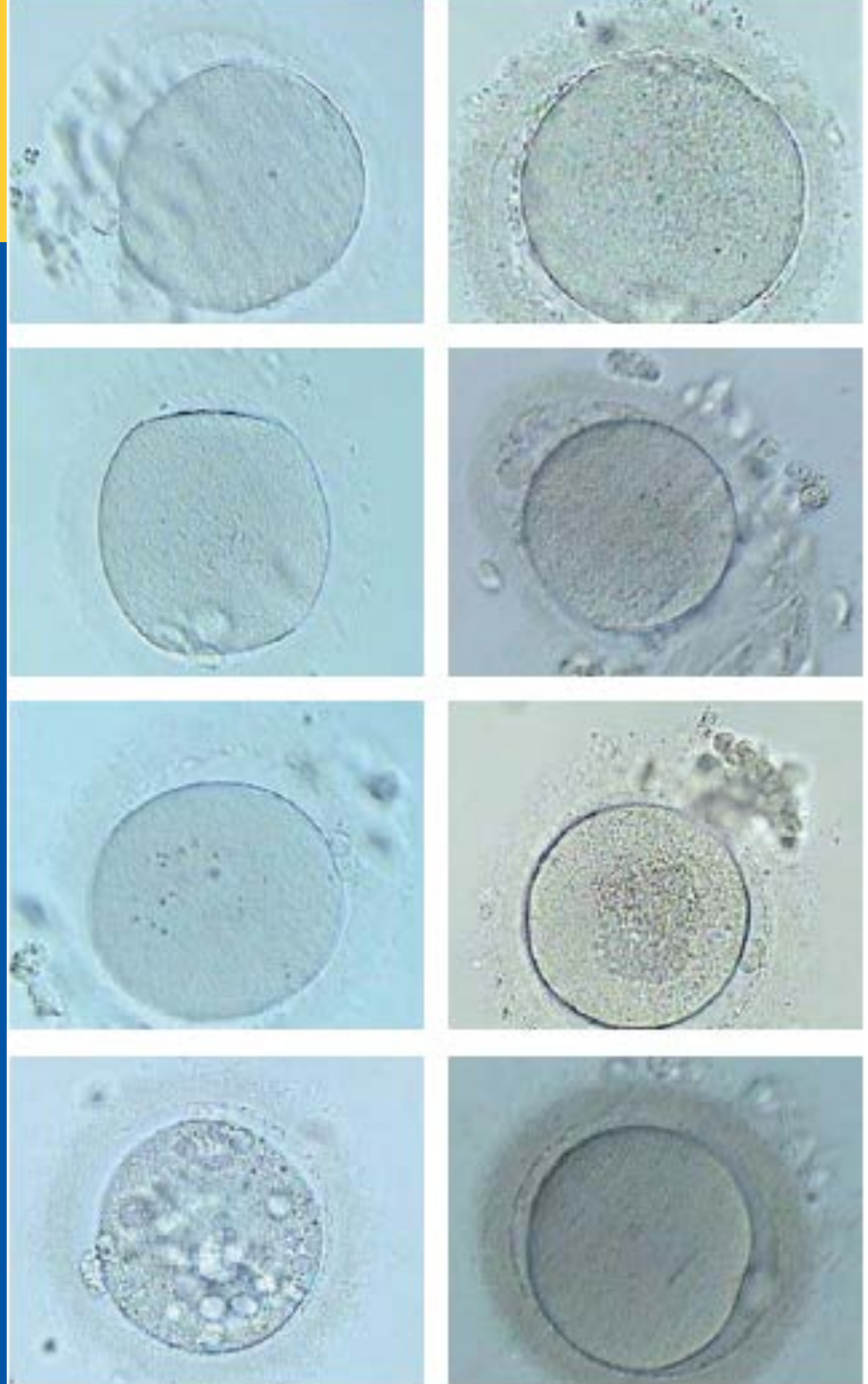
Important Parameters for Oocyte Grading

- The amorphic shape of oocyte
- Granularity in the perivitelline space
- Inclusions
- Vacuolization
- The dark color of the cytoplasm
- Changes in the color and construction of the zona pellucida
- Changes in the shape and size of the polar body (Gardener)

Selection of competent oocytes by morphological criteria for assisted reproductive technologies



Molecular Reproduction and Development, Volume: 87, Issue: 10, Pages: 1021-1036, First published: 09 September 2020, DOI: (10.1002/mrd.23420)

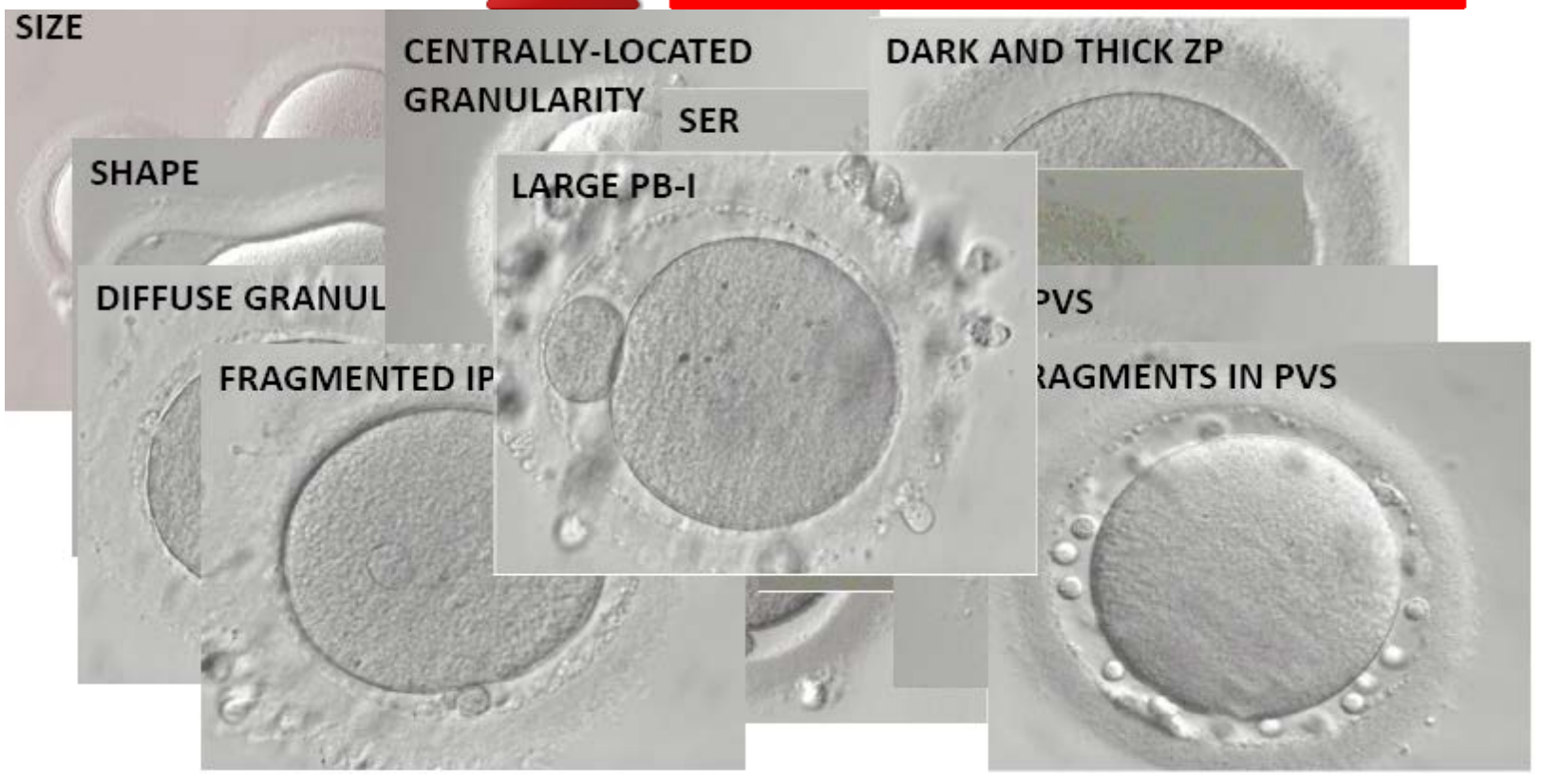


Oocyte morphology

An oocyte with a normal appearance should have a spherical shape, a homogenous cytoplasmic texture with no inclusions, a uniform zona pellucida constituted by three layers of glycoproteins and finally a small perivitelline space containing one single non-fragmented polar pody.
Swain and Pool, 2008

"the ideal oocytes"

ONLY 30% OF THE RETRIEVED OOCYTES HAVE IDEAL MORPHOLOGY



Extracytoplasmic parameters of human oocytes related to zona pellucida, perivitelline space, shape, and giantess

Extracytoplasmic factor	Property	Outcome
Zona pellucida	Thin	↑ Fertilization (Bertrand et al., 1995)
	Thick	↑ Oocyte development (Rama Raju et al., 2007; Shen et al., 2005) ↑ embryo quality (Host et al., 2002)
	High birefringence	↑ Meiotic spindle visualization (Madaschi et al., 2009) ↑ fertilization rate (Gonzalez-Ortega et al., 2016) ↑ embryo quality (Gonzalez-Ortega et al., 2016) ↑ blastocysts formation (Ebner et al., 2010) ↑ implantation (Madaschi et al., 2009; Montag et al., 2008) ↑ pregnancy (Madaschi et al., 2009; Montag et al., 2008) ↑ live birth (Madaschi et al., 2009; Montag et al., 2008)
	Low birefringence	↑ Miscarriage rate (Madaschi et al., 2009)
Perivitelline space	Large	↓ Fertilization rate (Xia, 1997) ↓ embryo quality (Xia, 1997); ↑ embryo quality (Ten et al., 2007)
	Presence of granules	↑ Oocyte maturity (Hassan-Ali et al., 1998) ↓ implantation rate (Farhi et al., 2002) ↓ pregnancy rate (Farhi et al., 2002)
Shape	Ovoid shape	↓ Cleavage (Ebner et al., 2008) ↑ delayed compaction (Ebner et al., 2008) ↑ delayed blastocyst formation (Ebner et al., 2008)
	Peripheral defect	↑ Degeneration (Ebner et al., 2001)
Giantess	Presence of giant oocyte	↑ Estradiol level (Balakier et al., 2002; Lehner et al., 2015) ↑ obtained oocyte number (Balakier et al., 2002; Lehner et al., 2015) ↑ abnormal embryonic cleavage (Machtinger et al., 2011)

The potential outcomes of morphological and structural changes in the meiotic spindles of human oocytes

Meiotic spindle	Outcome
Visualization	↓ZP birefringence (Madaschi et al., 2009) ↓fertilization rate (Braga et al., 2008; Garcia-Oro et al., 2017; Gonzalez-Ortega et al., 2016; Petersen et al., 2009; Rama Raju et al., 2007) ↓normal pronuclei appearance (Petersen et al., 2009) ↓cleavage rate (Petersen et al., 2009) ↓Day 3 embryo quality (Petersen et al., 2009) ↓ embryo quality (Gonzalez-Ortega et al., 2016) ↓blastocyst development (Braga et al., 2008; Garcia-Oro et al., 2017; Petersen et al., 2009; Rama Raju et al., 2007) ↓implantation rate (Garcia-Oro et al., 2017; Madaschi et al., 2008) ↓conception cycle (Gonzalez-Ortega et al., 2016) ↓pregnancy rate (Konc et al., 2004; Madaschi et al., 2008)
Longer spindle length	↓Fertilization success (Korkmaz et al., 2015) ↓blastocyst formation (Rama Raju et al., 2007)
Misaligned meiotic spindle position	↓Fertilization defect (Rienzi et al., 2003)
Localization to beneath or adjacent to PB	↓Fertilization rate (Fang et al., 2007)
Higher retardance	↓Better pronuclear score (Shen et al., 2006) ↓blastocyst formation (Rama Raju et al., 2007) ↓pregnancy rate (Shen et al., 2006)
Higher density	↓Pregnancy rate (Kilani et al., 2009)
Normally appearing spindles	↓Fertilization rate (Kilani et al., 2009; Tilia et al., 2020) ↓Day 3 embryo development (Tilia et al., 2020) ↓ blastocyst quality (Tilia et al., 2020) ↓euploid blastocyst number (Tilia et al., 2020) ↓implantation rate (Kilani et al., 2009) ↓pregnancy rate (Kilani et al., 2009) ↓live birth rate (Kilani et al., 2009)
Fine aligned	↓Embryo cleavage (Asa et al., 2017) ↓embryo quality (Asa et al., 2017) ↓embryo fragmentation (Asa et al., 2017) ↓implantation rate (Asa et al., 2017)
Birefringent	↓Pre-embryo number (Shen et al., 2006) ↓fertilization rate (Madaschi et al., 2008) ↓good quality pronuclei (Shen et al., 2006) ↓early embryo cleavage (Madaschi et al., 2008)

The potential outcomes of morphological changes in the first polar body of human oocytes

First polar body	Outcome
Intact	□Fertilization rate (Xia, 1997) □embryo quality (Xia, 1997) □Day 3 good quality embryo (Zhou et al., 2016) □blastocyst quality (Zhou et al., 2016) □available embryo number (Zhou et al., 2016)
Well-shaped morphology	□Implantation rate (Ebner et al., 1999) □pregnancy rate (Ebner et al., 1999) □multiple pregnancy rate (Ebner et al., 1999)
Intact and well-shaped morphology	□Fertilization rate (Ebner et al., 2000) □embryo quality (Ebner et al., 2000)
Intact and smooth surface	□Fertilization rate (Ebner et al., 2002) □embryo quality (Ebner et al., 2002) □implantation rate (Ebner et al., 2002) □pregnancy rate (Ebner et al., 2002)
Large	□Viability (Fancsovits et al., 2006) □fertilization rate (Rienzi et al., 2006; Navarro et al., 2009) □cleavage (Navarro et al., 2009) □embryo quality (Navarro et al., 2009)
Degenerated	□Fertilization rate (Rienzi et al., 2006)
Fragmented	□Developmental progress (Fancsovits et al., 2006)

Cytoplasmic changes	Outcome
Presence of vacuole	□Serum estradiol level (Otsuki et al., 2004) □ocyte cryosurvival (Balaban et al., 2008) □good quality blastocyst (Balaban et al., 2008) □zona pellucida hatching (Balaban et al., 2008) □biochemical pregnancy (Otsuki et al., 2004) □clinical pregnancy (Otsuki et al., 2004)
Presence of granulation	□Oocyte cryosurvival (Balaban et al., 2008) □fertilization rate (Wilding et al., 2007) □compromised pronuclear morphology (Rienzi et al., 2008) □good quality blastocyst (Balaban et al., 2008) □zona pellucida hatching (Balaban et al., 2008) □pregnancy rate (Serhal et al., 1997)
Presence of inclusion	□Fertilization rate (Xia, 1997) □embryo quality (Xia, 1997) □implantation rate (Serhal et al., 1997) □ pregnancy rate (Serhal et al., 1997)
Presence of SER aggregate	↓ Oocyte maturation (Setti et al. 2016) □fertilization rate (Sa et al., 2011) □embryo quality (Ebner et al. 2008b; Sa et al. 2011) □embryo transfer cancellation (Restelli, et al., 2015) □implantation rate (Setti et al. 2016) □pregnancy rate (Otsuki et al. 2004) □miscarriage rate (Braga et al. 2013) □transcriptome level (Stigliani et al., 2018)
High viscosity	□Fertilization rate (Ebner et al., 2003) □embryo quality (Ebner et al., 2003) □blastocyst formation (Ebner et al., 2003)
Presence of refractile body	□Fertilization rate (De Sutter et al., 1996; Otsuki et al., 2007) □blastocyst formation (De Sutter et al., 1996; Otsuki et al., 2007)
Presence of darkness	□Embryo quality (Ten et al., 2007)
Presence of multiple cytoplasmic dysmorphisms	□Embryo quality (Chamayou et al., 2006) □implantation rate (Serhal et al., 1997) □pregnancy rate (Serhal et al., 1997)
Texture, inclusion, vacuole and central granulation	□Embryo quality (Chamayou et al., 2006)
Granulation, inclusion, SER cluster and refractile body	□Implantation rate (Serhal et al., 1997) □pregnancy rate (Serhal et al., 1997)

The potential outcomes of morphological and molecular changes in the cumulus complex of human oocytes

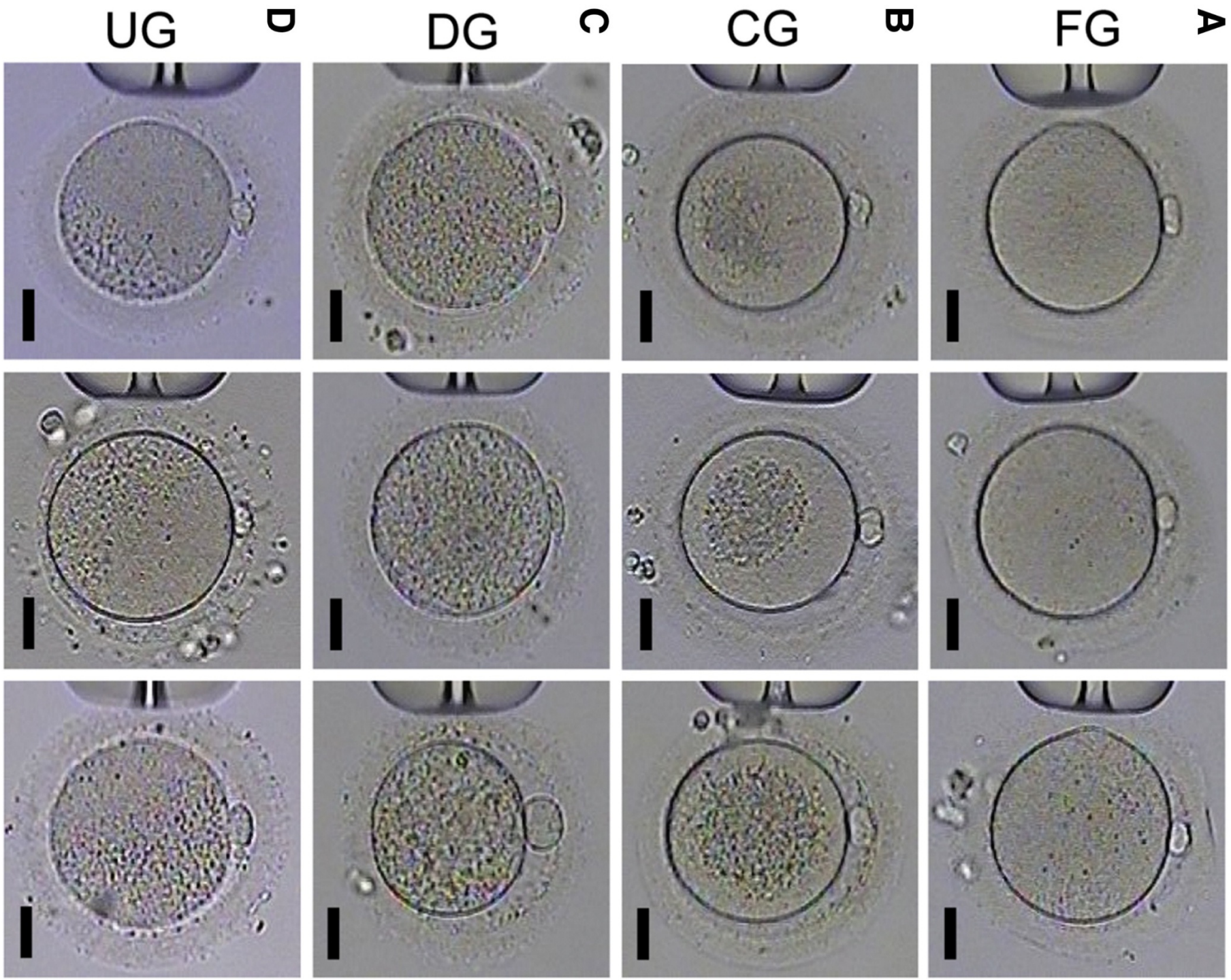
Cumulus complex	Outcome
Presence of blood clot	↓ Harvested oocyte number (Ebner et al., 2008) ↓ oocyte quality (Ebner et al., 2008) ↓ fertilization rate (Daya et al., 1990) ↓ cleavage rate (Daya et al., 1990) ↓ blastocyst formation rate (Ebner et al., 2008)
Higher apoptosis rate	↑ Immaturity (Host et al., 2002) ↓ fertilization (Host et al., 2002)
Expanded, fluffy cumulus cells and expanded corona cells	↑ Fertilization rate (Ng et al., 1999) ↑ pregnancy rate (Ng et al., 1999)
Expanded cumulus mass	↑ Implantation rate (Dal Canto et al., 2012) ↑ pregnancy rate (Dal Canto et al., 2012)
Higher expression of *COX2, HAS2,* and *GREM1* genes	↑ Embryo quality (McKenzie et al., 2004)
Higher expression *HAS2* and *GREM1* genes	↑ Day 3 embryo quality (Cillo et al., 2007) ↑ fertilization rate (Cillo et al., 2007)
Higher expression of *BCL2L11* and *PCK1* genes, and lower *NFIB* expression	↑ Embryo potential (Assou et al., 2008) ↑ successful pregnancy rate (Assou et al., 2008)
Higher *PTGS2* and lower *BDNF* genes expression	↑ Normal fertilization (Anderson et al., 2009)
Higher expression of *DPP8, HIST1H4C, UBQLN1, CALM1, NRP1,* and *PSMD6* genes	↑ Pregnancy rate (Assidi et al., 2011) ↑ implantation rate (Assidi et al., 2011)
Downregulation of *SPSB2* and *TP53I3* genes expression	↑ Chromosomally abnormal oocytes (Fragouli et al., 2012)
Relative ranking levels of *HAS2, FSHR, VCAN,* and *PR* genes	↑ Oocyte quality (Ekart, McNatty, Hutton, & Pitman, 2013) ↑ blastocyst formation rate (Ekart et al., 2013) ↑ live birth rate (Ekart et al., 2013)
Higher expression of *lncRNA* and *PSMD6* genes	↑ Pregnancy rate (Li et al., 2015)
Lower expression of *AMHR2* and *LIF* genes	↑ Embryo quality (Devjak et al., 2016)
Higher expression of *BAX* and *Caspase3*	↑ Embryos with uneven blastomere, cell fusion, and trichotomous mitoses (Faramarzi et al., 2019)
Higher expression of *CAMK1D*	↑ Blastocyst formation (Scarica et al., 2019)

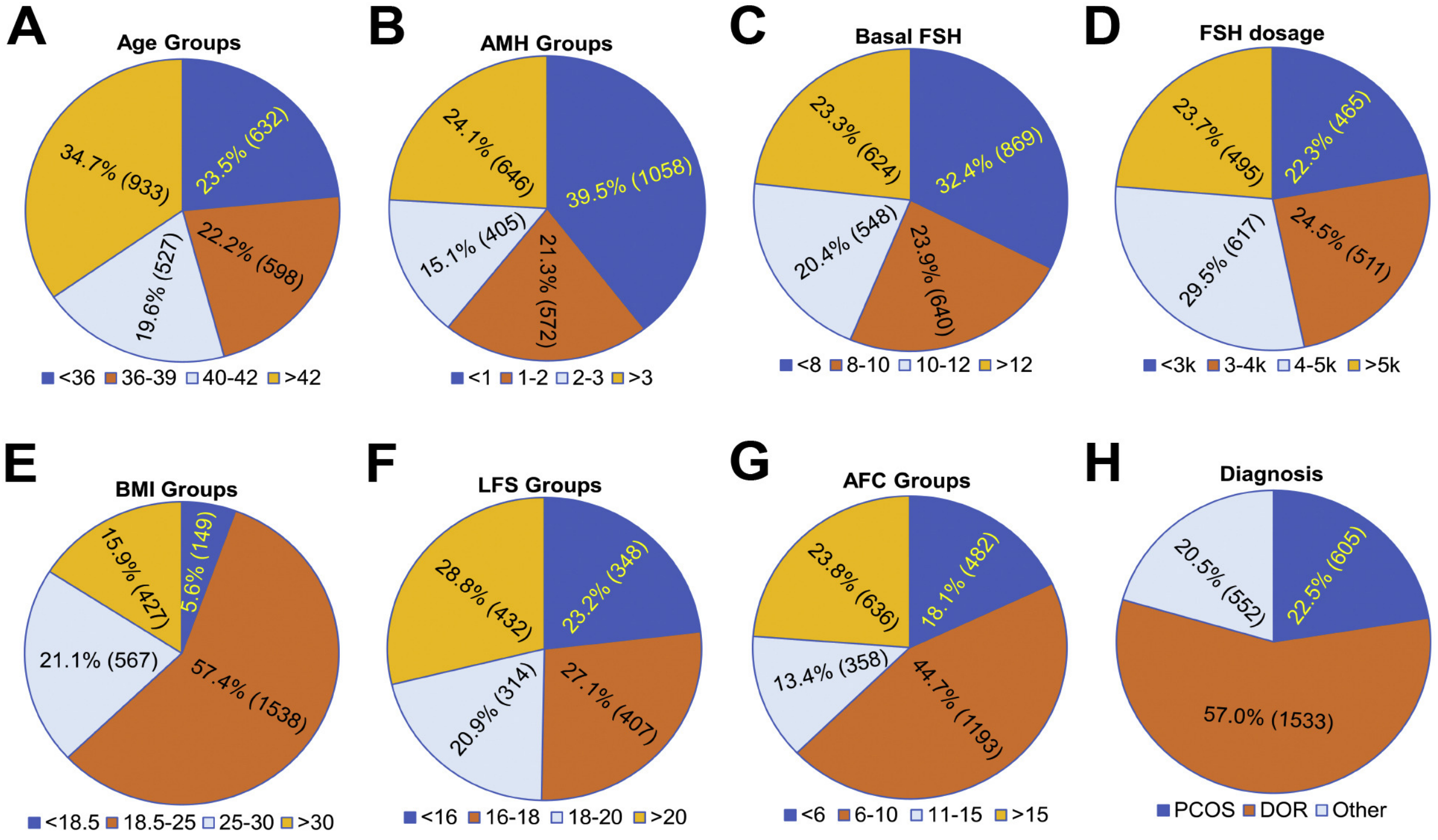
Multiple morphological, structural, and metabolic parameters in the human oocytes

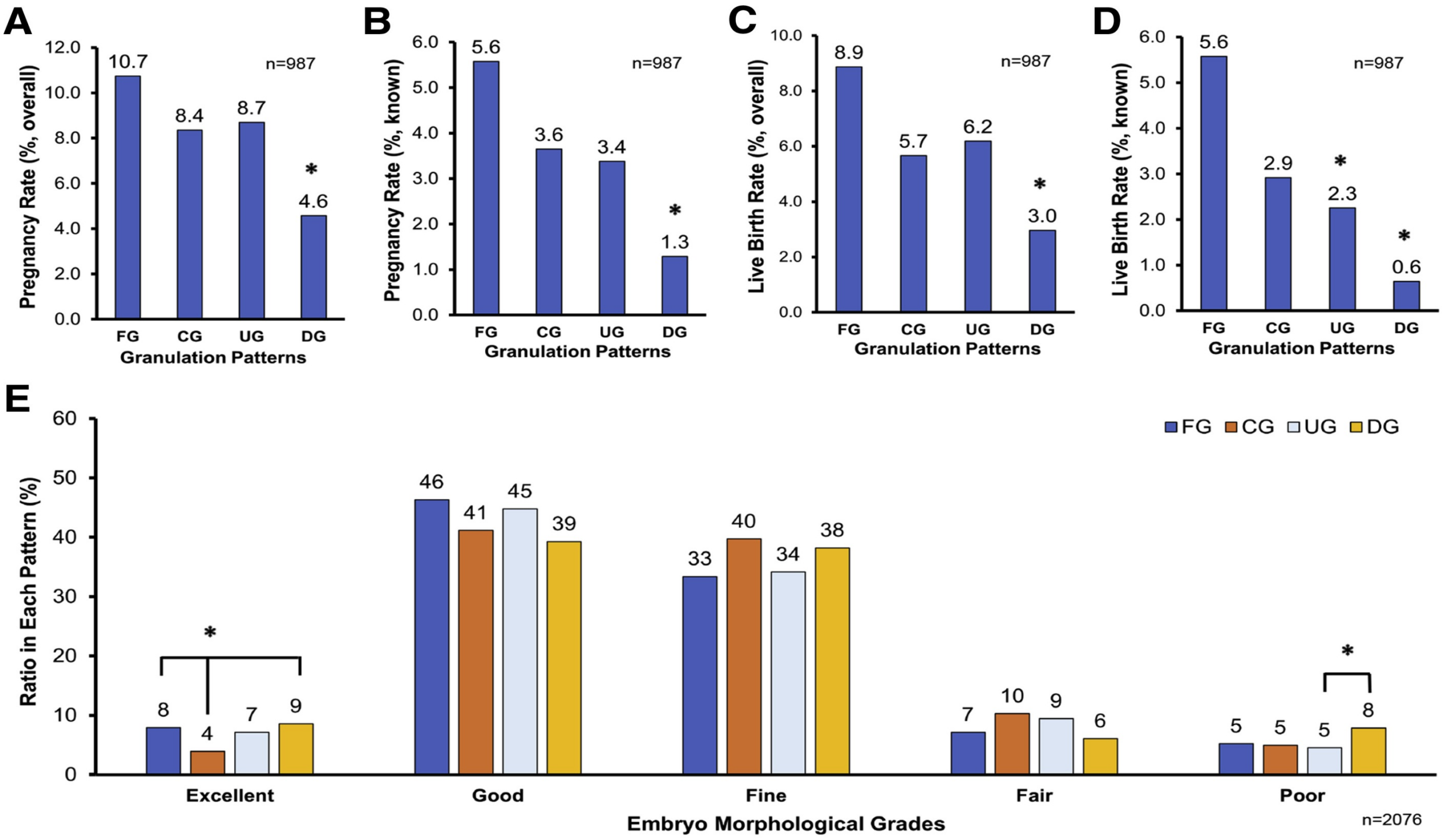
Multiple morphologies	Outcome
Large PS, fragmented PB, and cytoplasmic inclusion	↓Fertilization rate (Xia, 1997) ↓embryo quality (Xia, 1997)
Dark cytoplasm, many vacuoles or fragments in cytoplasm	↓Embryo quality (Loutradis et al., 1999) ↓Pregnancy rate (Loutradis et al., 1999)
Vacuoles, abnormal PB, large PS, diffused cytoplasmic granularity	↓Oocyte number (Rienzi et al., 2008) ↓clinical pregnancy (Rienzi et al., 2008) ↓basal FSH (Rienzi et al., 2008)
Single PB, normally appearing cytoplasm, proper ZP thickness, and PS	↓Transferable embryo number (Swain et al., 2008)
Dysmorphologies in meiotic spindle, ZP, PS, PB as well as the presence of vacuoles or granulations in the ooplasm	↓Blastocyst formation (Yakin et al., 2007)
Abnormal PB, existing vacuoles, and large PS	↓Fertilization rate (Rienzi et al., 2008)
Granularity, vacuoles, and inclusions in the ooplasm, and the resistance and fragility of the oocyte plasma membrane	↓Fertilization rate (Wilding et al., 2007) ↓embryo quality (Wilding et al., 2007) ↓clinical pregnancy rate (Wilding et al., 2007)
Large PS, diffused/central cytoplasmic granulation	↓Pronuclear quality (Rienzi et al., 2008) ↓Day 2 embryo quality (Rienzi et al., 2008)
Lower apoptotic index in cumulus cells and higher oxygen consumption of oocytes	↓Fertilization (Ruvolo et al., 2013) ↓embryo cleavage (Ruvolo et al., 2013)

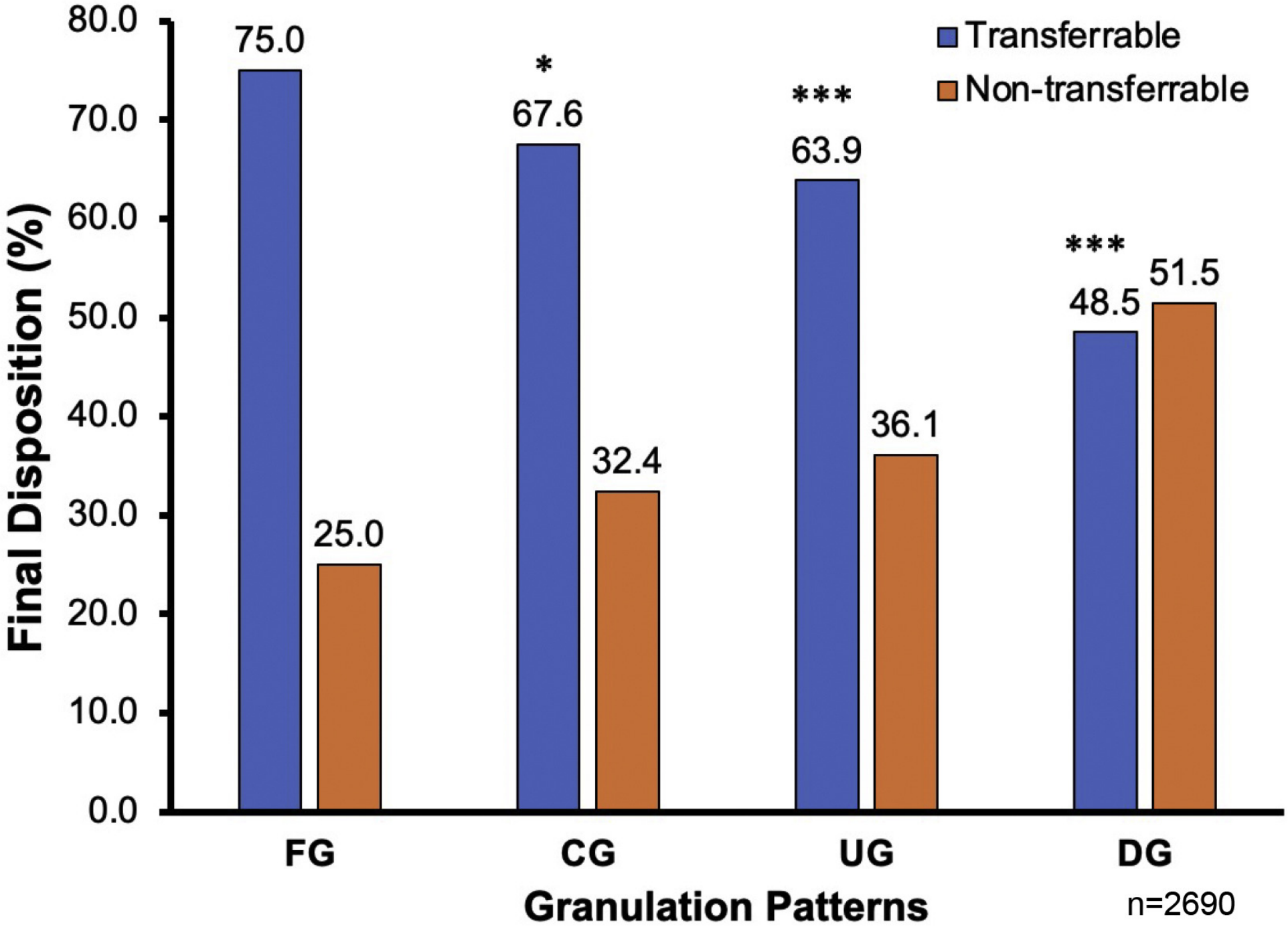
Four pattern-based of granulation in MII oocytes:

(**A**) Fine granulation (FG), homogenous cytoplasm with fine granules, featuring a ‘‘sandy’’ pattern.
(**B**)Central granulation (CG), a cluster of granules centrally localized in the cytoplasm, featuring a ‘‘ring’’ pattern.
(**C**) Dispersed granulation (DG), with the cytoplasm full of granules of a significant size, featuring a ‘‘rocky’’ pattern.
(***D***) Uneven granulation (UG), with granules of a significant size occupying only region(s) of the cytoplasm, whereas the rest is filled with fine granules, featuring a ‘‘rocky-sandy’’ pattern.



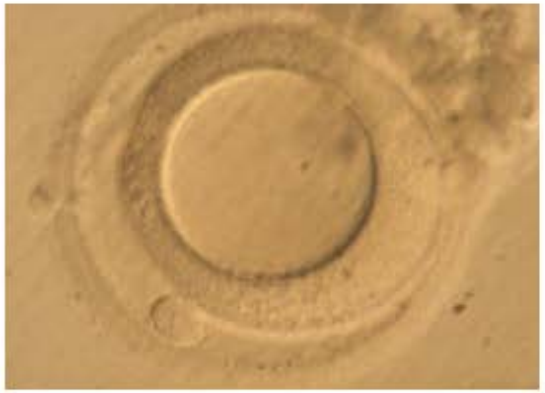

A
Age Groups
23.5% (632)
22.2% (598)
19.6% (527)
34.7% (933)
<36 36-39 40-42 >42
B
AMH Groups
39.5% (1058)
21.3% (572)
15.1% (405)
24.1% (646)
<1 1-2 2-3 >3
C
Basal FSH
32.4% (869)
23.9% (640)
20.4% (548)
23.3% (624)
<8 8-10 10-12 >12
D
FSH dosage
22.3% (465)
24.5% (511)
29.5% (617)
23.7% (495)
<3k 3-4k 4-5k >5k
E
BMI Groups
5.6% (149)
57.4% (1538)
21.1% (567)
15.9% (427)
<18.5 18.5-25 25-30 >30
F
LFS Groups
23.2% (348)
27.1% (407)
20.9% (314)
28.8% (432)
<16 16-18 18-20 >20
G
AFC Groups
18.1% (482)
44.7% (1193)
13.4% (358)
23.8% (636)
<6 6-10 11-15 >15
H
Diagnosis
22.5% (605)
57.0% (1533)
20.5% (552)
PCOS DOR Other

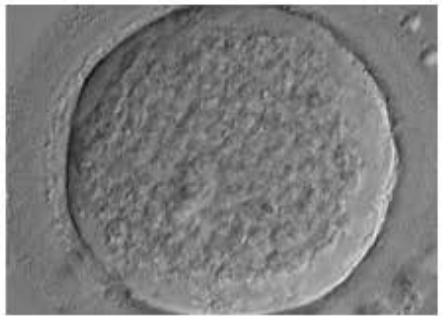

A
Pregnancy Rate (%, overall)
12.0
10.0
8.0
6.0
4.0
2.0
0.0
10.7
8.4
8.7
*
4.6
n=987
FG
CG
UG
DG
Granulation Patterns
B
Pregnancy Rate (%, known)
6.0
5.0
4.0
3.0
2.0
1.0
0.0
5.6
3.6
3.4
*
1.3
n=987
FG
CG
UG
DG
Granulation Patterns
C
Live Birth Rate (%, overall)
10.0
8.0
6.0
4.0
2.0
0.0
8.9
5.7
6.2
*
3.0
n=987
FG
CG
UG
DG
Granulation Patterns
D
Live Birth Rate (%, known)
6.0
5.0
4.0
3.0
2.0
1.0
0.0
5.6
2.9
*
2.3
*
0.6
n=987
FG
CG
UG
DG
Granulation Patterns
E
FG
CG
UG
DG
Ratio in Each Pattern (%)
60
50
40
30
20
10
0
*
8
4
7
9
46
41
45
39
33
40
34
38
7
10
9
6
*
5
5
5
8
Excellent
Good
Fine
Fair
Poor
Embryo Morphological Grades
n=2076

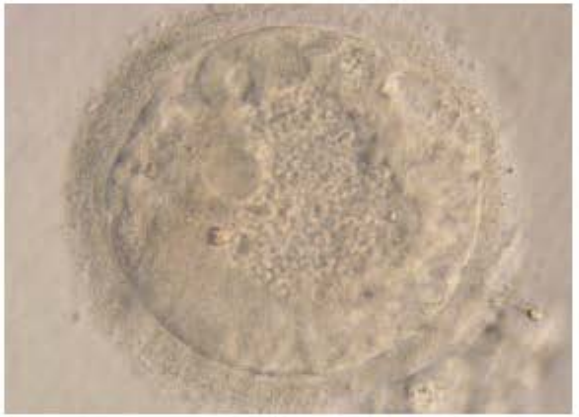

Transferrable
Non-transferrable
Final Disposition (%)
80.0
70.0
60.0
50.0
40.0
30.0
20.0
10.0
0.0
75.0
25.0
*
67.6
32.4
***
63.9
36.1
***
48.5
51.5
FG
CG
UG
DG
Granulation Patterns
n=2690

Oocytes Selection Criteria

- IMPORTANT: we learn from every cycle => laboratory feedback about oocyte quality from previous cycles before starting a new stimulation => **MULTIDISCIPLINARY WORK**

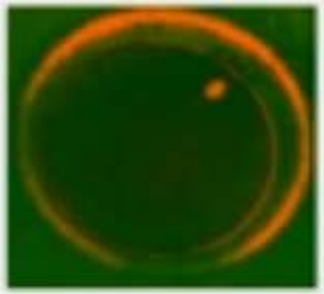


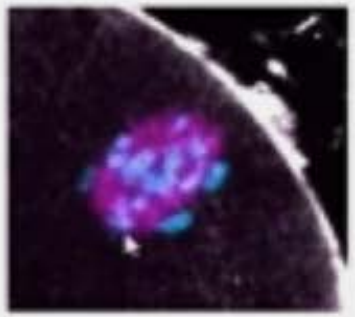
Limitations

Mitochondria

Egg maturity



Chromosome status



Sperm quality

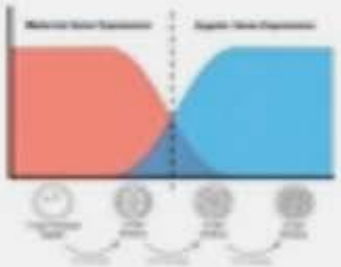
ICSI

Mitotic errors

Endometrium

Epigenetics

Genome activation



presence of the spindle <u>does not guarantee</u> successful pregnancy

Good things are worth waiting for...

Thank You for Your Attention