Key Performance Indicators

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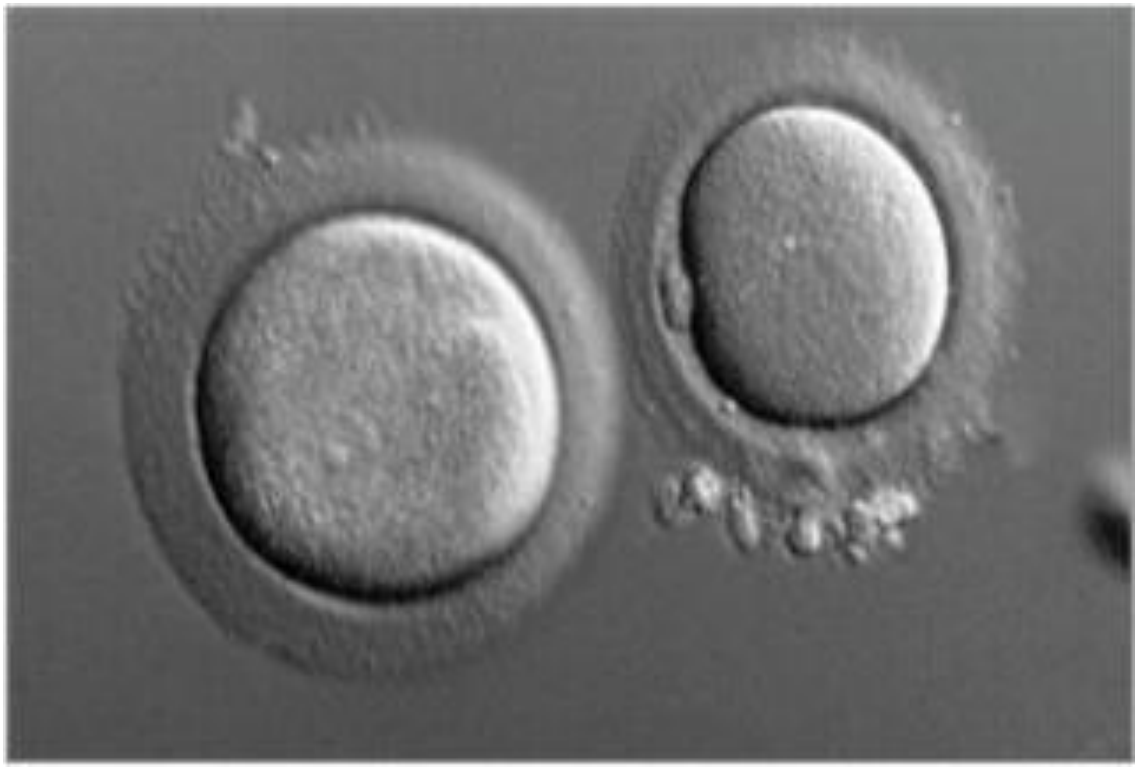
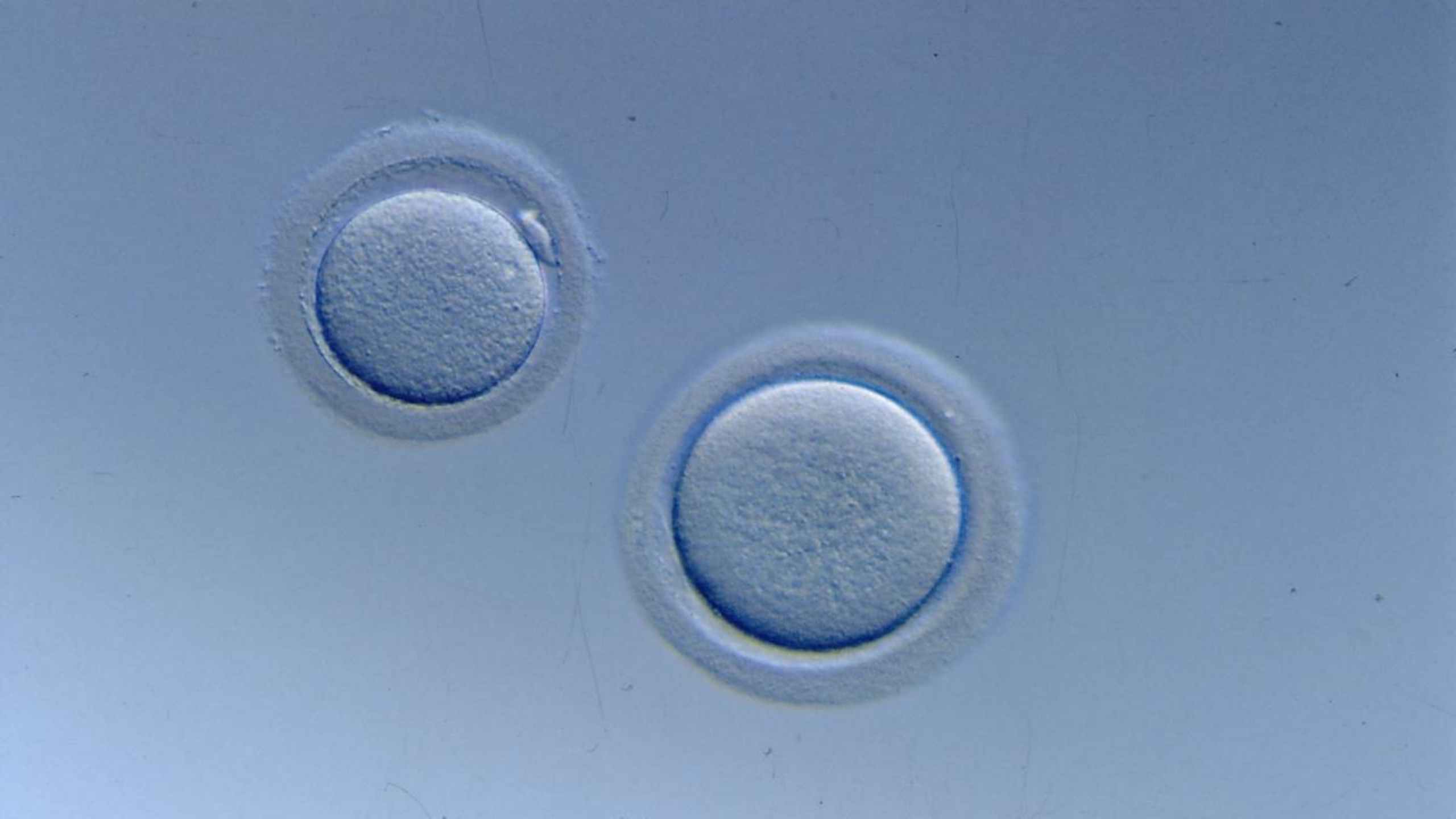
Oocytes

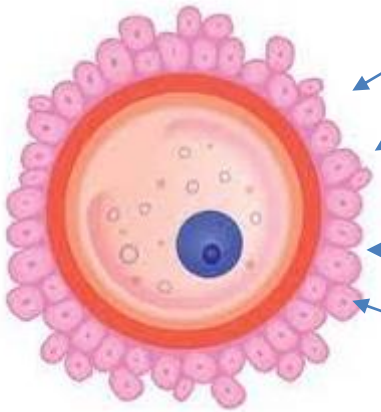
- Not all oocytes collected from a patient following ovarian stimulation for ART will have the same developmental competence.
- only 5% of oocytes collected eventually result in a live birth.
- Intrinsic oocyte competence is derived not only from the degree of nuclear maturity of the oocytes, but also from their cytoplasmic maturity.





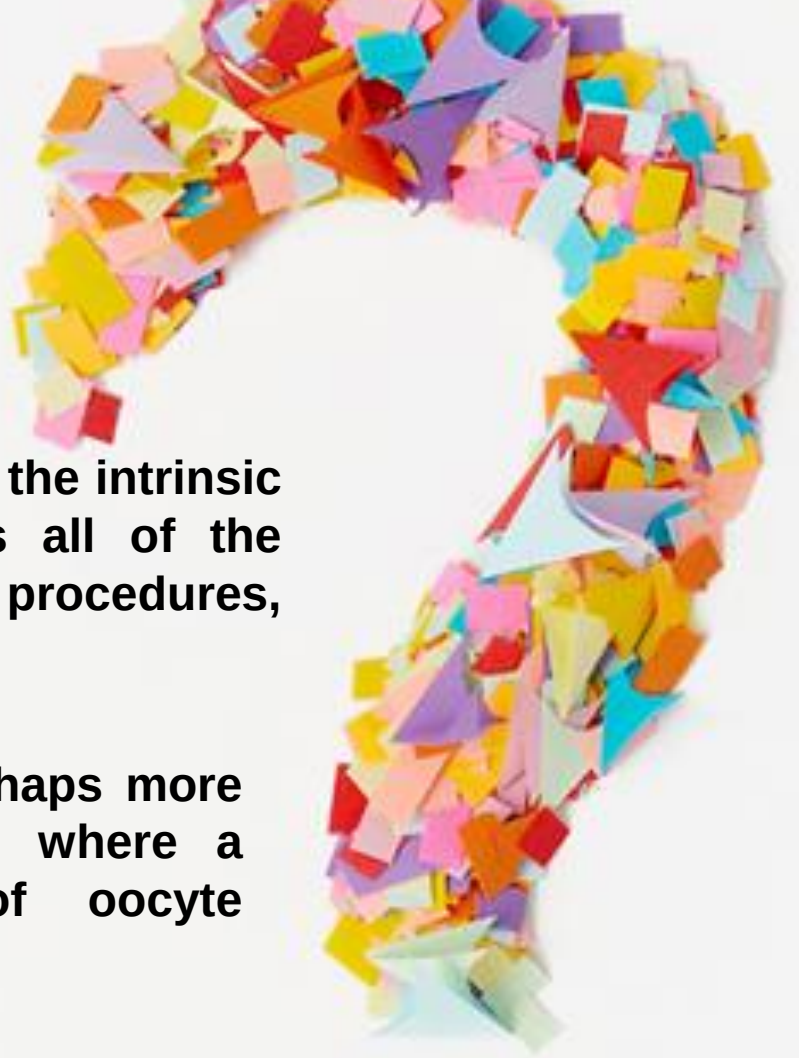
Intrinsic

1. age
2. BMI
3. lifestyle factors
4. type of infertility



1. ovarian stimulation
2. laboratory procedures (such as oocyte retrieval, denudation,cryopreservation, preparation for ICSI)
3. culture conditions (temperature, pH, pO2),
4. environmental conditions (light, air quality, humidity) and culture medium.

Extrinsic

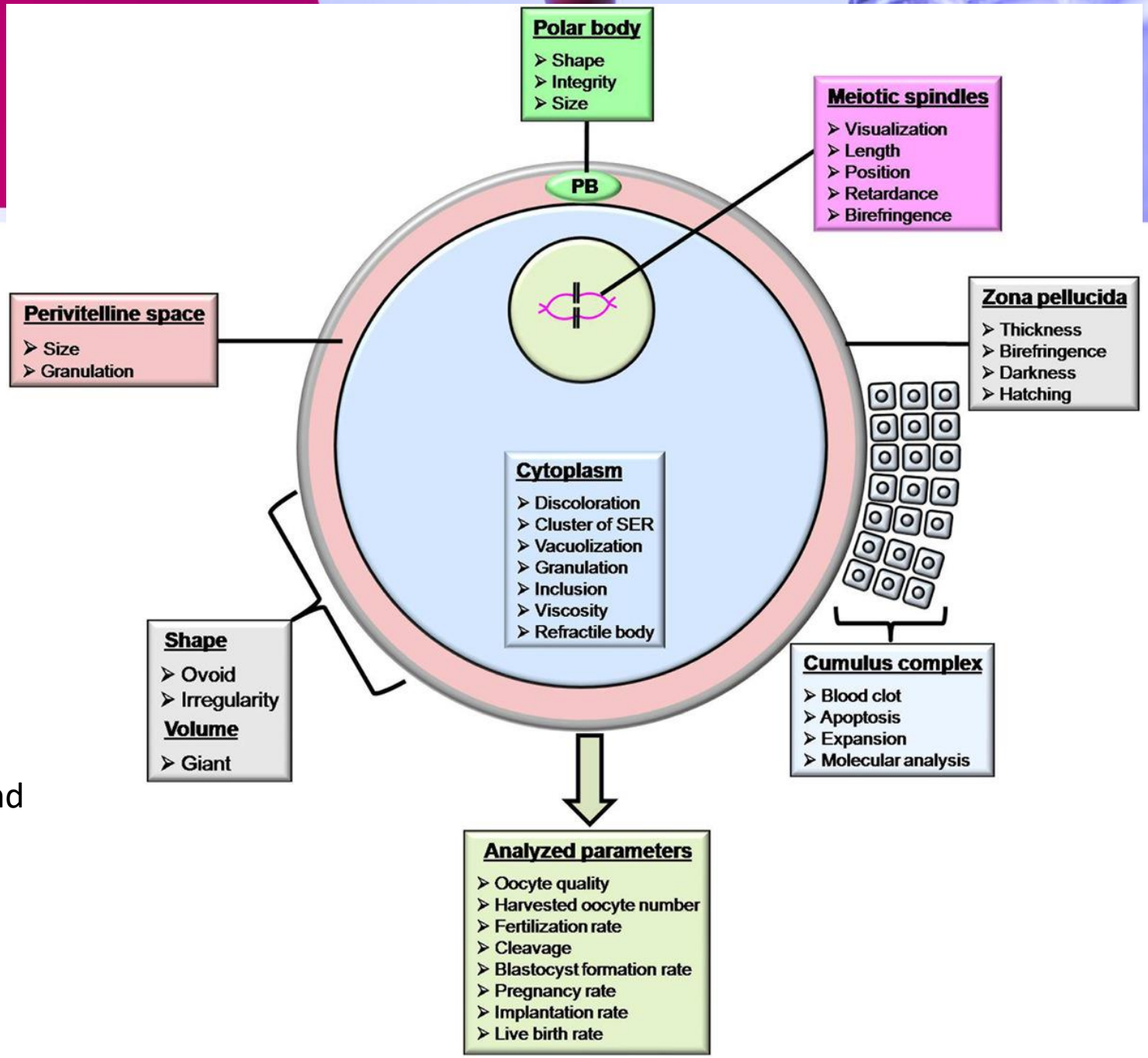


Whether any laboratory indicator can provide a measure of the intrinsic oocyte competence at the time of oocyte retrieval, as all of the subsequent events could be influenced by laboratory procedures, and/or by the genetic contribution of the spermatozoon?

In other words, is quality measurable for oocytes, or perhaps more pertinently, is there any measure that could pinpoint where a dysfunction occurred during the long process of oocyte development?

Oocyte competency

- biochemical markers
- gene expression
- oxygen uptake
- assessment of oocyte morphology
- largely research-based, and have not found widespread application in clinical service



KPIs after insemination by ICSI

normal fertilization rate

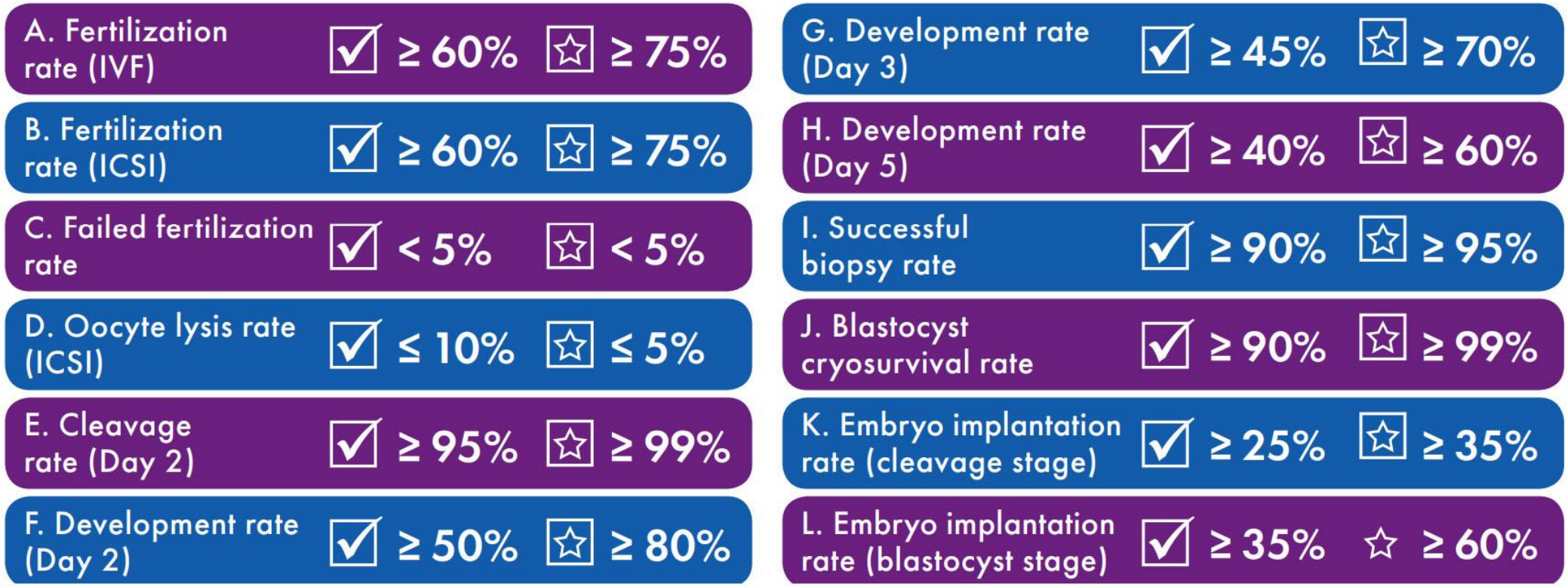
oocyte degeneration rate

KPIs after insemination by ICSI

failed fertilization rate

poor fertilization rate

Figure 3: *Key performance indicators (Adapted from 2)*



Definitions:

A. 2PN/COC retrieved
B. 2PN/COC retrieved
C. Cycles without any 2PNs/Stimulated cycles
D. MII lysis/MII injected
E. Cleaved embryo/2PN
F. 4-cell embryo/2PN
G. 8-cell embryo/2PN
H. Blastocyst/2PN
I. DNA amplified/biopsy
J. Intact blastocyst/warmed blastocyst
K. Sacs at ultrasound/embryos transferred
L. Sacs at ultrasound/blastocysts transferred

Key:

☑ Competency (%)

☆ Benchmark (%)

ICSI normal fertilization rate

$$= \frac{\text{no. oocytes with 2PN and 2PB}}{\text{no. MII oocytes injected}} \times 100$$

B. Fertilization rate (ICSI)	☑ ≥ 60%	☆ ≥ 75%

informative indicator of:

- gamete quality
- operator competence.
- ICSI 2PN rate does depend on the various criteria used for performing ICSI, which can be considered a weakness of the indicator.

Fertilization after insemination by ICSI

- except for the Spanish Registry and the Istanbul Consensus which include the observation of two PBs in the definition.
- The UK's Association of Clinical Embryologists proposed benchmark for the 2PN rate is >65% including only patients below 40 years of age with at least three oocytes collected.

ICSI damage rate or oocyte degeneration rate

D. Oocyte lysis rate (ICSI) ☑ ≤ 10% ☆ ≤ 5%

$$= \frac{\text{no. damaged or degenerated}}{\text{all oocytes injected}} \times 100$$

- Oocyte damage can be observed at three time points:

1. from the start at stripping
2. during ICSI

→ operator's competency •

3. at the fertilization assessment on Day

↓

operator competence, oocyte quality, and laboratory performance

Poor fertilization rate

Poor fertilization rate

- Is defined as the proportion of cycles in which <25% of the injected oocytes are fertilized. Poor fertilization rate can give an indication of operator competence and reflect gamete quality.

Poor fertilization rate

5 to 20% ☑ 0 to 15% ✶

Failed fertilization rate

- $= \frac{\text{no. cycles with no evidence of fertilization}}{\text{no. of stimulated IVF cycles}} \times 100$

C. Failed fertilization rate	☑ < 5%	☆ < 5%

indicator

- The indicator can be informative of gamete quality/ function / operator skill.
- A deficiency in the mechanism of oocyte activation is regarded as the principal cause of ICSI fertilization failure or abnormally low fertilization. Complete (‘or virtually complete’) fertilization failure with ICSI occurs in 1–5% of cycles.

Failed fertilization rate

- exclude cases where reduced fertilization rates are anticipated,

1. vitro matured metaphase I oocytes (although inconclusive data),
2. Artificially activated oocytes,
3. the use of testicular sperm
4. globozoospermia and asthenozoospermia

Conclusion

from the surveys and collected evidence

ICSI damage rate and
ICSI normal fertilization rate

>

ICSI low/failed fertilization rate
as a KPI is less clear

KPI	Calculation	Competency value (%)	Benchmark value (%)
ICSI damage rate	$\frac{\text{no. damaged or degenerated}}{\text{all oocytes injected}} \times 100$	≤10	≤5
ICSI normal fertilization rate	$\frac{\text{no. oocytes with 2PN and 2PB}}{\text{no. MII oocytes injected}} \times 100$	≥65	≥80
IVF normal fertilization rate	$\frac{\text{no. oocytes with 2PN and 2PB}}{\text{no. COCs inseminated}} \times 100$	≥60	≥75
Failed fertilization rate (IVF)	$\frac{\text{no. cycles with no evidence of fertilization}}{\text{no. of stimulated IVF cycles}} \times 100$	<5	
Cleavage rate	$\frac{\text{no. cleaved embryos Day 2}}{\text{no. 2PN/2PB oocytes on Day 1}} \times 100$	≥95	≥99
Day 2 Embryo development rate	$\frac{\text{no. 4-cell embryos on Day 2}}{\text{no. normally fertilized oocytes}^{a}} \times 100$	≥50	≥80
Day 3 Embryo development rate	$\frac{\text{no. eight cell embryos on Day 3}}{\text{no. normally fertilized oocytes}^{a}} \times 100$	≥45	≥70
Blastocyst development rate	$\frac{\text{no. blastocysts Day 5}}{\text{no. normally fertilized oocytes}^{a}} \times 100$	≥40	≥60
Successful biopsy rate	$\frac{\text{no. biopsies with DNA detected}}{\text{no. biopsies performed}} \times 100$	≥90	≥95
Blastocyst cryosurvival rate	$\frac{\text{no. blastocysts appearing intact}}{\text{no. blastocysts warmed}} \times 100$	≥90	≥99
Implantation rate (cleavage-stage)[b]	$\frac{\text{no. sacs seen on ultrasound}^{c}}{\text{no. embryos transferred}} \times 100$	≥25	≥35
Implantation rate (blastocyst-stage)[b]	$\frac{\text{no. sacs seen on ultrasound}^{c}}{\text{no. blastocysts transferred}} \times 100$	≥35	≥60

Fertilization after (routine) IVF insemination

- This section deals with:
- normal fertilization rate
- polyspermy rate,
- Poor fertilization rate
- zygote morphology after routine IVF insemination

Fertilization after IVF insemination

- Pronuclear formation
- occurs 1.5–2.0 h earlier in ICSI compared with IVF
- relative to the time elapsed since insemination (recommended as 17 ± 1 h)

IVF normal fertilization rate

- $= \frac{\text{no. oocytes with 2PN and 2PB}}{\text{no. COCs inseminated}} \times 100$

A. Fertilization rate (IVF)	☑ ≥ 60%	☆ ≥ 75%

This parameter provides an indication of the ability of the culture system to support sperm capacitation and sperm-oocyte interaction in IVF cycles

three or more pronuclei

1. Is indicative of an abnormal fertilization
2. arising from either nondisjunction (failure to extrude the second PB) or polyspermy.
3. Polyspermy may be the result of either oocyte immaturity (causing failure of the cortical reaction), oocyte over maturity and/or an extremely high concentration of motile spermatozoa in the insemination volume.
4. From the literature, the incidence of ≥3PN is 4–7% in IVF . This agrees well with the median values from the Alpha survey of <9% for competence, and <4.5% as a benchmark

Single pronucleus after IVF

- occurs in 1–5% of cases
- can be indicative of :

1. asynchronous appearance of pronuclei (an extremely rare event, as evidenced by the use of time-lapse microscopy),
2. parthenogenetic activation.

- The incidence of diploidy in 1PN oocytes following conventional IVF has been reported to be in the range of 45–50%. In contrast, 1PN oocytes arising after ICSI have a reported diploidy rate of only 7–14%, with genetic abnormalities in the subsequent embryos.

Incidence of poor fertilization

- The incidence of poor fertilization (<25% of inseminated COCs with 2PN) or total failure of fertilization (no oocytes with signs of fertilization)
- could be indicative of a problem with sperm function, too few motile spermatozoa during insemination, or failure of oocyte activation (Ebner et al., 2015).

poor and failed fertilization of 14 and 6%☑ 8 and 4%✶

Cleavage-stage embryos

- Early cleavage rate is defined as the proportion of cleaved zygotes at the early cleavage check on Day 1 (26 ± 1 h post-ICSI or 28 ± 1 h post-IVF)

- $$= \frac{\text{cleaved zygotes at the early cleavage check on Day 1}}{\text{no. 2PN/2PB oocytes on Day 1}} \times 100$$

E. Cleavage rate (Day 2)	☑ ≥ 95%	☆ ≥ 99%

Cleavage rate should be calculated frequently in a laboratory (at least once per month).

Cleavage-stage embryos

- This indicator reflects the ability of the culture system to support early cleavage of fertilized oocytes and the viability and quality of the embryos
- There are conflicting results on the importance of early cleavage. Studies have shown that early cleavage, together with other factors, can be used as an embryo selection method .
- Early cleavage rate has also been shown to correlate with blastocyst implantation and pregnancy rates and it is a better independent marker of implantation potential than zygote morphology
- premature occurrence of early cleavage can be negatively, instead of positively, associated with embryo implantation potential.
- it was not a reliable predictor for embryo implantation rate when good quality embryos are transferred, or when using a GnRH antagonist protocol

Embryo development rate

- Day 2 Embryo development rate:
- $\frac{\text{no. 4–cell embryos on Day 2}}{\text{no. normally fertilized oocytes}} \times 100$
- measured at 44 ± 1 h post-insemination

F. Development rate (Day 2) ☑ ≥ 50% ☆ ≥ 80%

G. Development rate (Day 3) ☑ ≥ 45% ☆ ≥ 70%

- Day 3 Embryo development rate
- $\frac{\text{no. 8–cell embryos on Day 3}}{\text{no. normally fertilized oocytes}} \times 100$
- Measured at 68 ± 1 h post-insemination
- Morula stage embryos on Day 4, 92 ± 2 h post insemination

Embryo development rate

- This indicator reflects the ability of the culture system to support cleavage stages and the quality and viability of embryos, especially for Day 2 or 3 transfer, while less important for blastocyst transfer.
- Possible confounders:

1. are the timing of laboratory observations
2. the type of culture media used.

- it reflects the overall laboratory performance

Blastocyst development

definition

- The blastocyst development rate, defined as the proportion of 2PN zygotes (not just of cleaved zygotes) which are at the blastocyst-stage at Day 5 (116 ± 2 post-insemination).

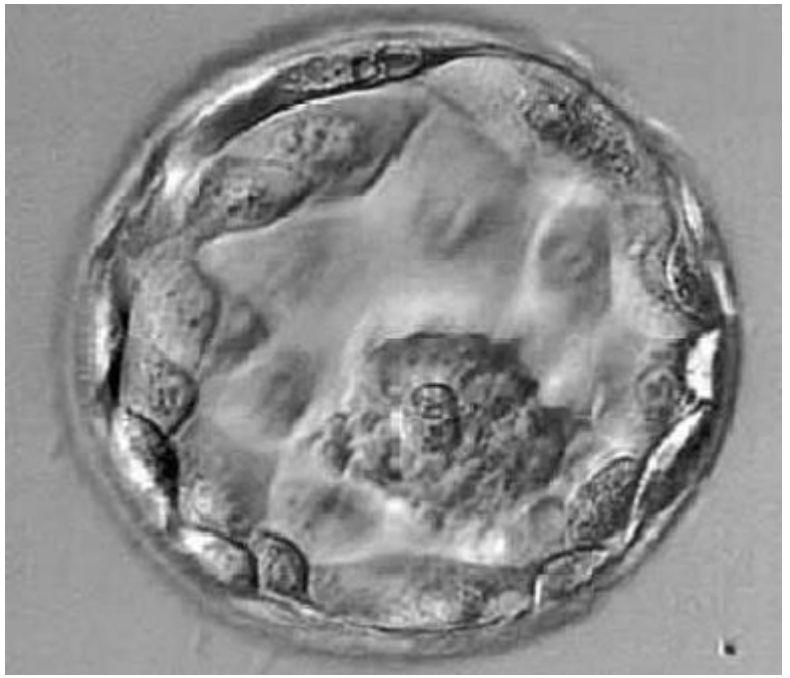
importance

- it reflects the efficiency of the whole culture system.
- Confounders can be the timing of laboratory observation, the culture medium and the culture conditions (in particular the pO2 concentration).

H. Development rate (Day 5) ☑ ≥ 40% ☆ ≥ 60%

Blastocyst development

- Blastocyst quality should be based on three factors, namely blastocoele expansion, appearance of trophectoderm (TE) and appearance of inner cell mass (ICM).
- Although all three parameters have been shown to be significantly correlated to pregnancy and LBR (Van den Abbeel et al., 2013), only TE was found to be a statistically significant independent predictor of live birth outcome after adjustment for known confounders



PGT

PGD/PGS (globally indicated as preimplantation genetic testing—PGT—that includes PGD for single gene disorders or for chromosome structural abnormalities, and PGS for aneuploidy) cycles should be excluded from this calculation.

Successful biopsy rate

$$= \frac{\text{no. biopsies with DNA detected}}{\text{no. biopsies performed}} \times 100$$

I. Successful biopsy rate	☑ ≥ 90%	☆ ≥ 95%

- A successful biopsy rate is defined as the proportion of biopsied and tubed/fixed samples where DNA is detected.
- the cells can often not be inspected visually and will only be detected by the presence of DNA after amplification.

Rate of no biopsy

- The rate of no biopsy is defined as the proportion of intended PGD/PGS (PGT) cases where there were no embryos available to biopsy. This parameter was rated important,
- it reflects patient related factors and the ability of the culture system to support cleavage/ blastocyst formation,
- rather than the performance ability of the laboratory to perform a PGD/PGS (PGT) treatment/analysis.

competence value and benchmark were 20 and 10%,

last data collection of the ESHRE PGD Consortium, out of 45163 reported cycles, 2.8% were canceled before biopsy

Indicators for cryopreservation: addition to the previous consensus

- Blastocyst re-expansion rate is defined as the proportion of warmed blastocysts that show re expansion within a defined time period (e.g. 2 h).
- Recent evidence shows an impact on the performance results depending on the quality/expansion of the blastocysts which are cryopreserved (Coboet al., 2012). Also, in blastocyst fresh transfer, from multivariate analysis it was shown that the odds of live birth increased by 36% for each grade of expansion (P = 0.0061) and decreased by 29% for blastocysts with grade B TE compared with Grade A TE (P = 0.0099).
- Furthermore, after thawing, the odds of live birth increased by 39% (P = 0.0042) for each 10% increase in degree of re-expansion.

Indicators for cryopreservation: addition to the previous consensus

- blastocoel expansion and TE grade were selected as the most significant pre-freeze morphological predictors of live birth and degree of re-expansion was selected as the best post-thaw parameter for prediction of live birth (Ahlstrom et al., 2013).
- Confounding factors are time of observation, female age and fertilization method. These observations do not include embryos that had been biopsied on Day 3 as they have a different hatching dynamic

J. Blastocyst cryosurvival rate ☑ ≥ 90% ☆ ≥ 99%


Thank You!

Oocyte competence

- There are a number of possible markers for oocyte competence
- these are largely research-based, and have not found widespread application in clinical service.
- include assessment of biochemical markers in follicular fluid,
- gene expression studies of follicular cells
- oxygen uptake assessments.
- Other markers, such as assessment of oocyte morphology, spindle imaging, and polar body (PB) biopsy, can be incorporated into clinical service, but this is not a universal approach.

- When the results of the Alpha and ESHRE surveys were combined, respondents identified
- oocyte recovery rate
- oocyte maturity rate
- as the most important indicators for oocytes

ICSI damage rate or oocyte degeneration rate

Alternatively, the term ICSI oocyte survival rate can be used.

- In the Alpha survey, the minimum expected value and target value ranged from 3 to 30% and 0 to 10%, respectively.
- Oocyte damage can be observed at three time points during the ICSI process:
- from the start at stripping,
- during ICSI
- at the fertilization assessment on Day 1.is detected until the fertilization check.
- Damage at denudation/stripping can be monitored separately as it mainly reflects operator's competency, but it has a very low frequency.
- It is useful to monitor this indicator for operator competence, oocyte quality, and laboratory performance. The damage rate can also be indicative of technical problems (e.g. cumulus cell removal stress, vibration).

Cleavage-stage embryos

- Proposed indicators for cleavage-stage embryos are:
- early cleavage rate
- cleavage rate
- embryo development rates
- embryo fragmentation rate
- and rate of good quality embryos
- Early cleavage rate is defined as the proportion of cleaved zygotes at the early cleavage check on Day 1 (26 ± 1 h post-ICSI or 28 ± 1 h post-IVF)
- $= \frac{\text{cleaved zygotes at the early cleavage check on Day 1}}{\text{no. 2PN/2PB oocytes on Day 1}} \times 100$

Day 5 embryo transfer rate was defined as the proportion of cycles with ≥1 2PN zygotes on Day 1 that had ≥1 blastocyst for transfer on Day 5.
PGD/PGS (globally indicated as preimplantation genetic testing—PGT—that includes PGD for single gene disorders or for chromosome structural abnormalities, and PGS for aneuploidy) cycles should be excluded from this calculation.