Stripping for Success: Does Oocyte Denudation Influence IVF Outcomes?

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Kindbody

Disclosures

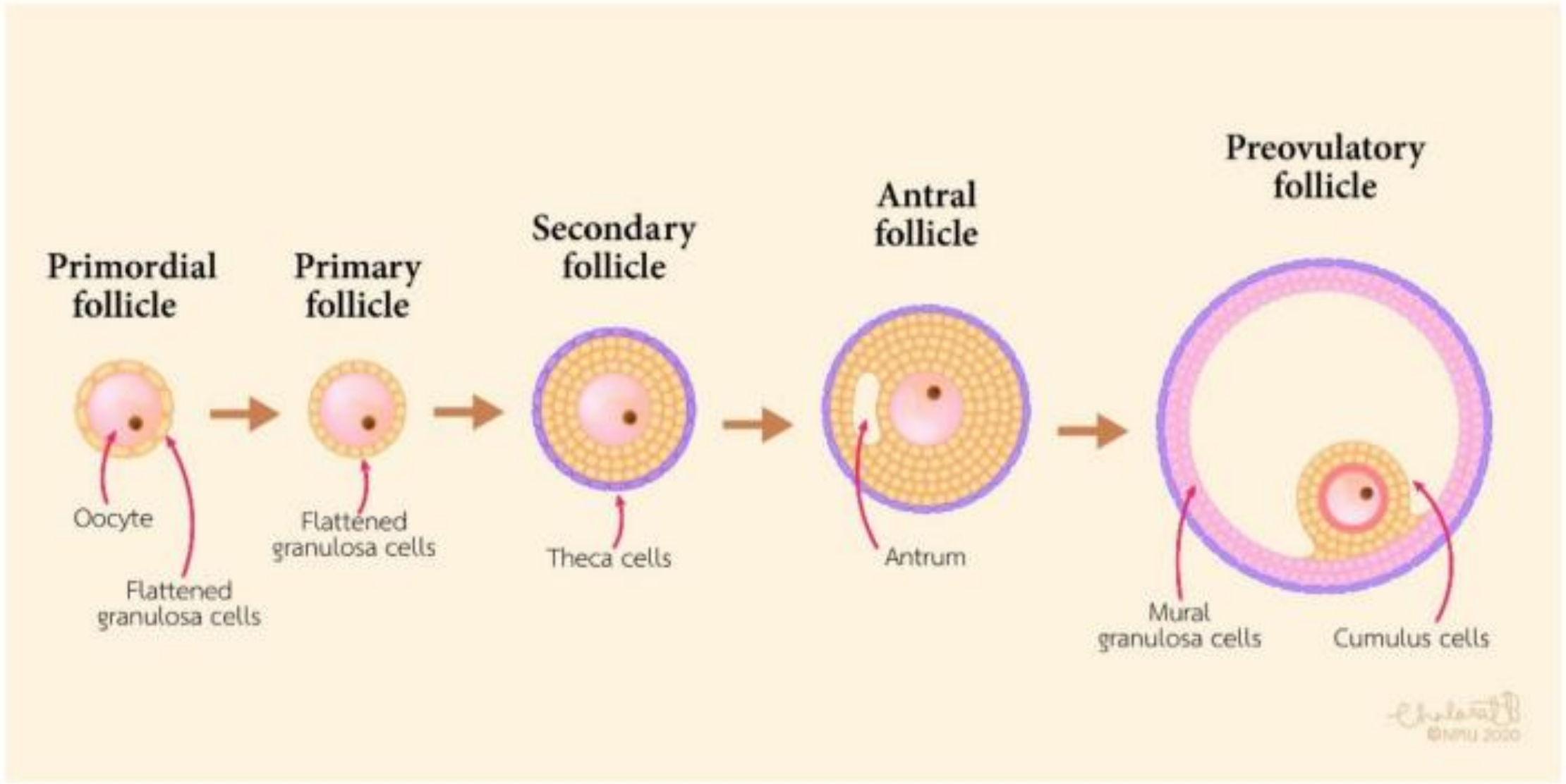
- I cannot break dance

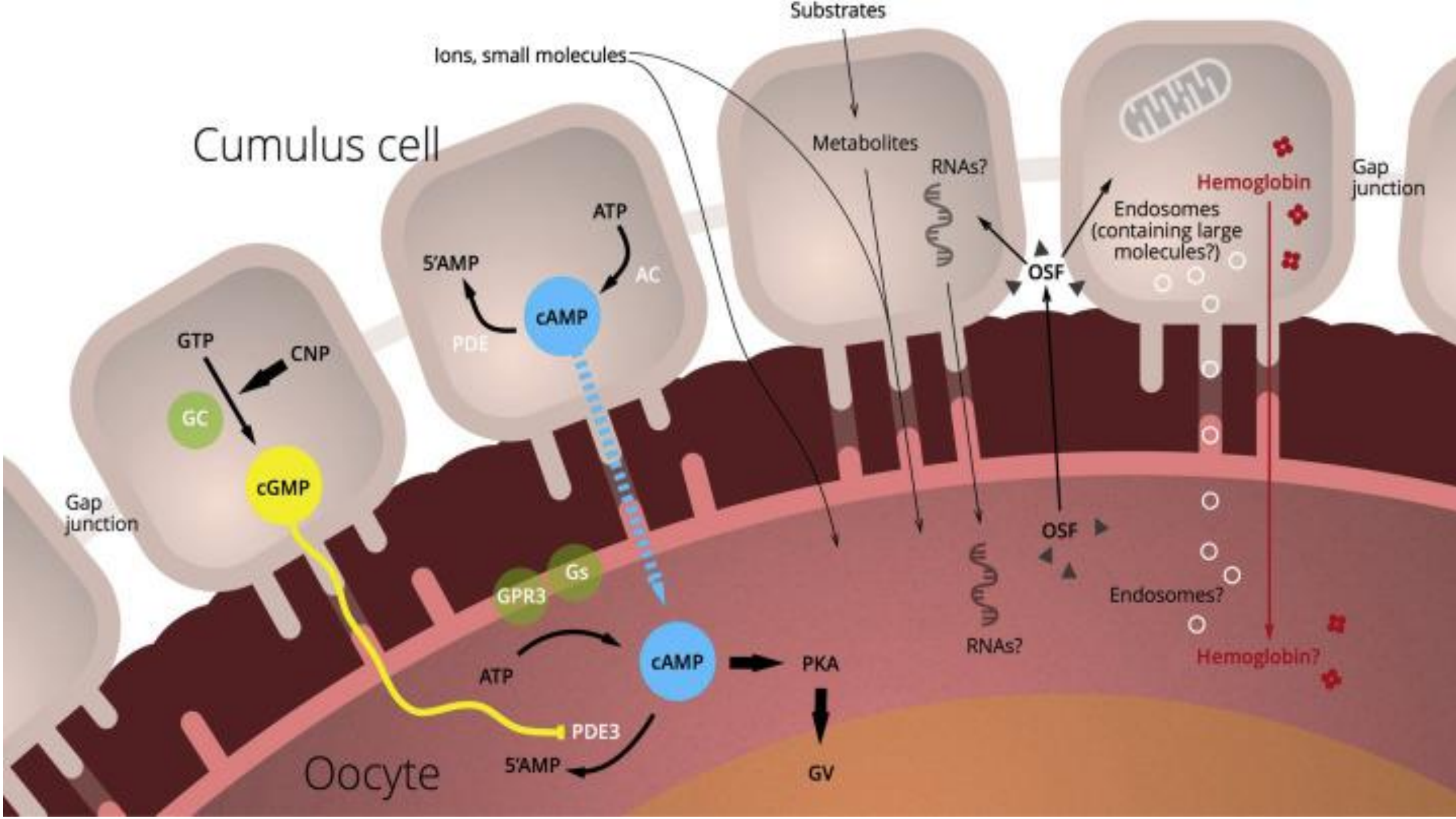
Objectives:

- Increase awareness of possible pitfalls associated with denuding
- Review relevant data on timing of denuding and outcomes
- Provoke a lively discussion

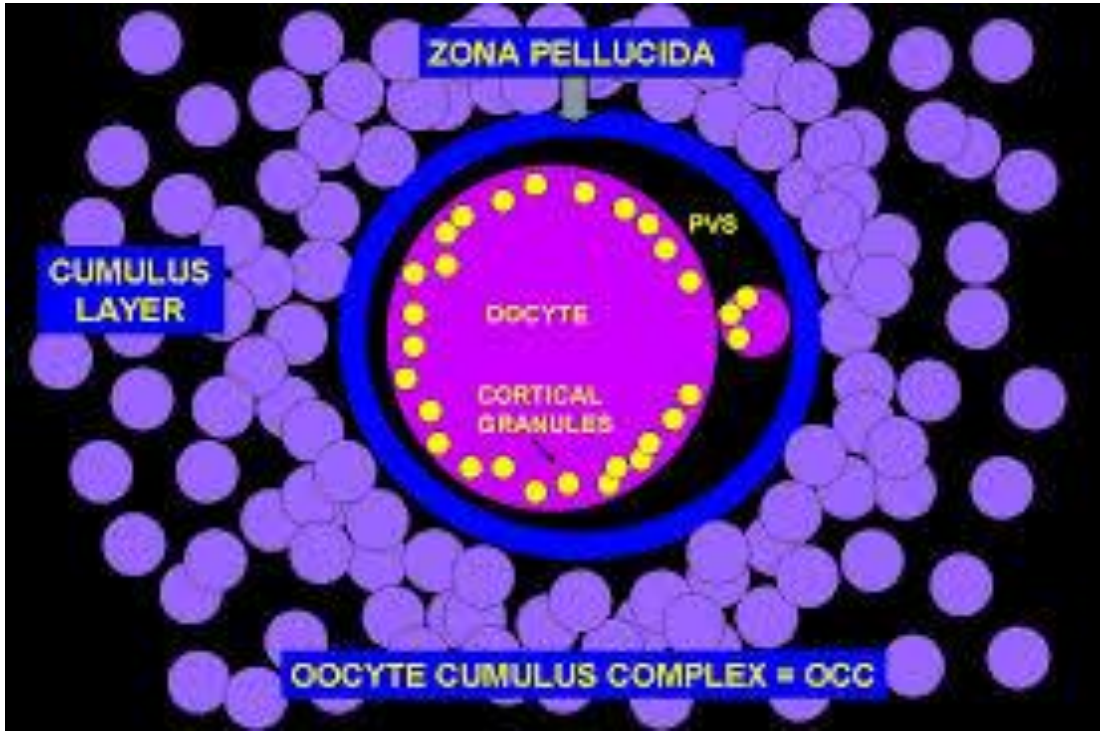
History

- ICSI was first reported in 1992 as a treatment for male factor infertility and has become a very common procedure over the last 30+ years
- More than 2 million ICSI babies have been born
- >60% of all IVF cycles worldwide were ICSI
- >70% of US IVF cycles utilize ICSI


Primordial follicle
Primary follicle
Secondary follicle
Antral follicle
Preovulatory follicle
Oocyte
Flattened granulosa cells
Flattened granulosa cells
Theca cells
Antrum
Mural granulosa cells
Cumulus cells


Substrates
Ions, small molecules
Cumulus cell
Metabolites
RNAs?
Hemoglobin
Gap junction
Endosomes (containing large molecules?)
ATP
5'AMP
AC
cAMP
PDE
OSF
GTP
CNP
GC
cGMP
Gap junction
Gs
GPR3
OSF
Endosomes?
RNAs?
ATP
cAMP
PKA
Hemoglobin?
PDE3
5'AMP
Oocyte
GV

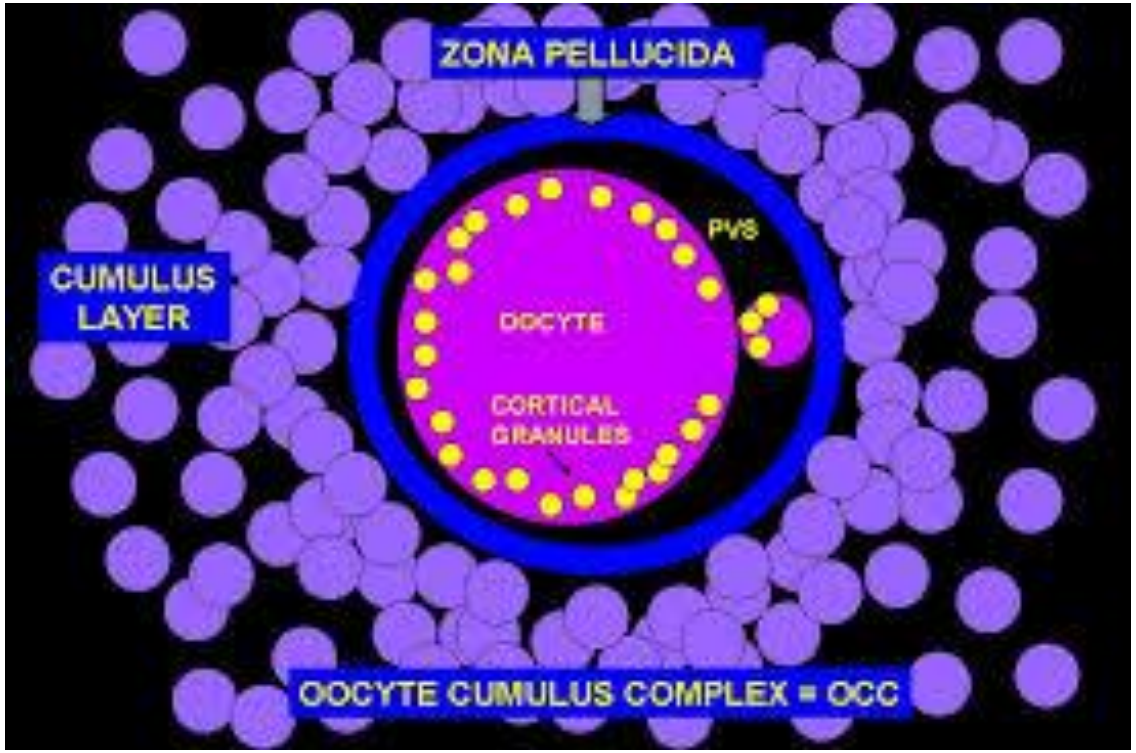
Cumulus Corona Complex



- Metabolically active mediator of oocyte maturation
- Gap junctions penetrate the ZP and touch the oolemma
- Gap Junctions into the oocyte mostly withdrawn after LH surge and the resumption of meiosis
- Paracrine communication compensates for the loss of GAP junctions
- May provide protections against oxidative stress

Cumulus Corona Complex – Post Ovulation

- Maintaining oocyte viability
- Sperm guidance
- Acrosome reaction trigger
- Preventing pre-mature fertilization
- Maintenance of meiotic arrest
- Transcription mediation



Maturation:

Nuclear Maturation

- Synchronous in vivo
- Recovery from the first meiosis
- Germinal vesicle breakdown
- First polar body formation

Cytoplasmic Maturation

- Helps prepare the oocyte for fertilization and subsequent embryonic development
- Provide enough energy, enzymes, and protein synthesis reserves to meet the needs of new functional protein synthesis during embryonic development
- Unlike the nucleus, there is no clear standard for defining and detecting cytoplasmic maturation

Why do we Denude?

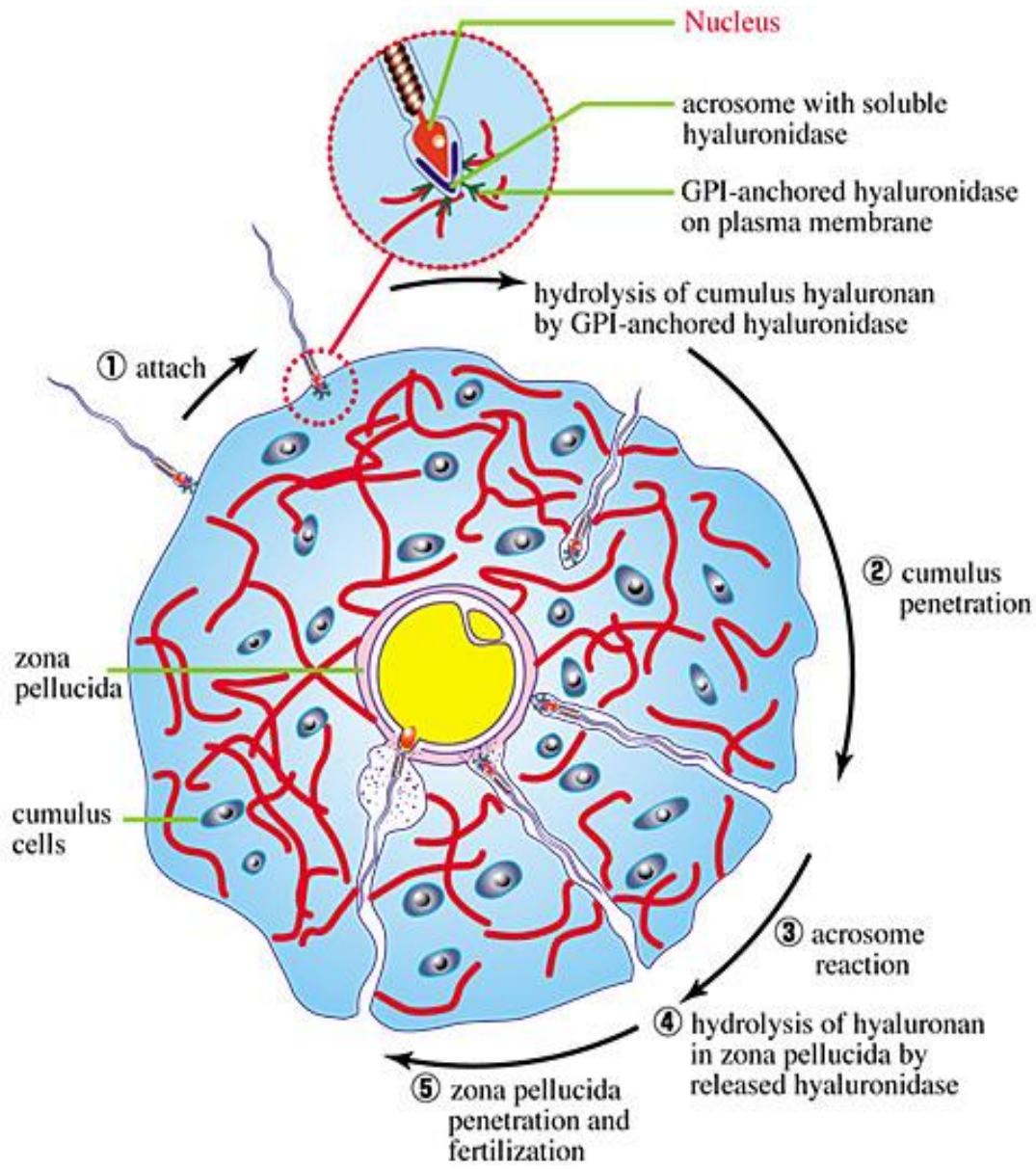
- To access nuclear maturity
- To remove cells that could potentially clog the ICSI pipette
- To align the oocyte so ICSI avoids the spindle apparatus

- Do we have to Strip Oocytes Naked of can some C/C be left???

Fun Reproductive Fact!

- The male seahorse gives birth to offspring
  - Allows the females to keep laying eggs
  - Males are more aggressive at keeping predators at bay

- Water by the expanded domain of this large, polyanionic molecule is most likely responsible for the expansion of the preovulatory COC
- Hyaluronidase (naturally occurring in the sperm) breaks the bonds between hyaluronan molecules allowing dispersion of the matrix



Hyaluronidase

Bovine/Porcine

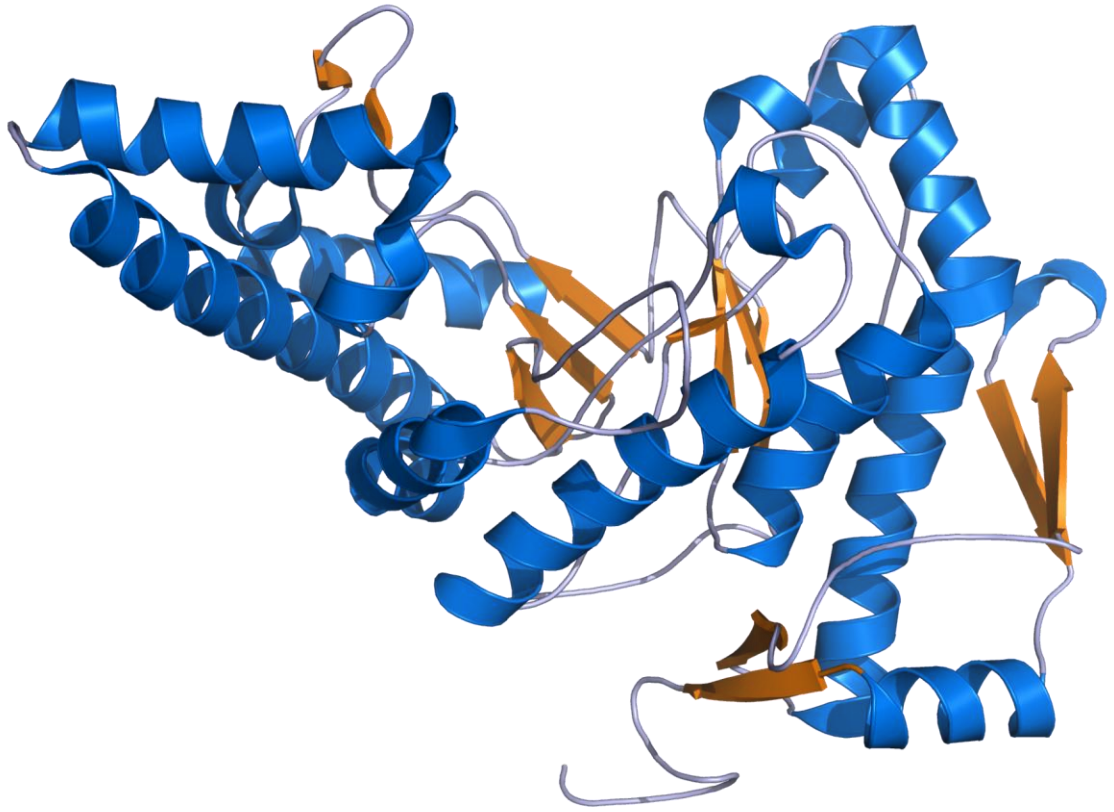
- Derived from animal testes
- Purity rates <50%
- Toxic to oocytes so short exposure necessary
- Animal: human- theoretical allergic response
- Genuine risk of disease transmission

Recombinant

- Recombinant Enzyme rHuPH20
- Increase purity (99%)
- Non-toxic to oocytes – exposure time not critical
- Human: human - decreases risk of allergic response
- No risk of disease transmission
- 40-120 U/mL

Recombinant versus bovine hyaluronidase for oocyte denudation before intracytoplasmic sperm injection: a systematic review and meta-analysis

- 3 high quality RCTs
- Equal results between the use of recombinant and bovine
- No difference in the length of time required for denudation
- No difference in M2s, fertilization, embryo quality, CIUPs and LB



Craciunas, Laurentiu & Tsampras, Nikolaos & Kollmann, Martina & Raine-Fenning, Nick. (2015).

Manufacturer	Concentration	Exposure Time (s)	Source	MEA Tested
Cooper - Sage	80 IU/mL	30 - 45	Bovine	Yes
Cooper - Synvitro		5 - 10*	Non-bovine	Yes
Cooper - Cumulase	40 - 120 IU/mL	60	Recombinant	Yes
Irvine	80 IU/mL	30	Bovine	Yes
VitroLife	800 IU/mL	30	Not clear	No
Planar GM 501	80 IU/mL	30	Bovine	Yes

Steps for Cumulus-Corona Removal

- Make a dish containing hyaluronidase solution and multiple wash drops (+/- oil overlay)
- Take several (2 – 12) oocytes at a time with a large bore pipette (>300 µm)
- Expose to hyaluronidase solution for 30 seconds while pipetting using the large bore pipette
- Remove from hyaluronidase solution and place into wash drop
- Using a 200 – 300 µm pipette move the oocytes into a different wash drop
- Drop to no smaller than 135 µm pipette to finish cleaning up the oocytes
- Wash steps

Other Schemes for Cumulus Removal

- Short term exposure to hyaluronidase
- Followed by quick rinse
- Culture at 37^{o}C for some period
- Then perform mechanical cell removal


PHILIPS

Fun Reproductive Fact!

The largest and smallest cell of the human body are the egg and sperm

- Immediately after OPU
- 1 – 2 hours post OPU
- 3 – 4 hours post OPU
- > 4 hours post OPU

When to Denude??

Early

- Oocyte aging is associated with changes in the M-phase promoting factor (MPF) and mitogen-activated protein kinase (MAPK) activity from C/C
- Intact cumulus cells may induce apoptosis by releasing soluble FAS ligand
- ROS creation
- Prevent parthenogenic activation

Later

- Improves cytoplasmic maturity ?
- C/C provides some protection against ROS in culture
- Some GAP junctions remain after ovulatory surge providing support for the oocyte

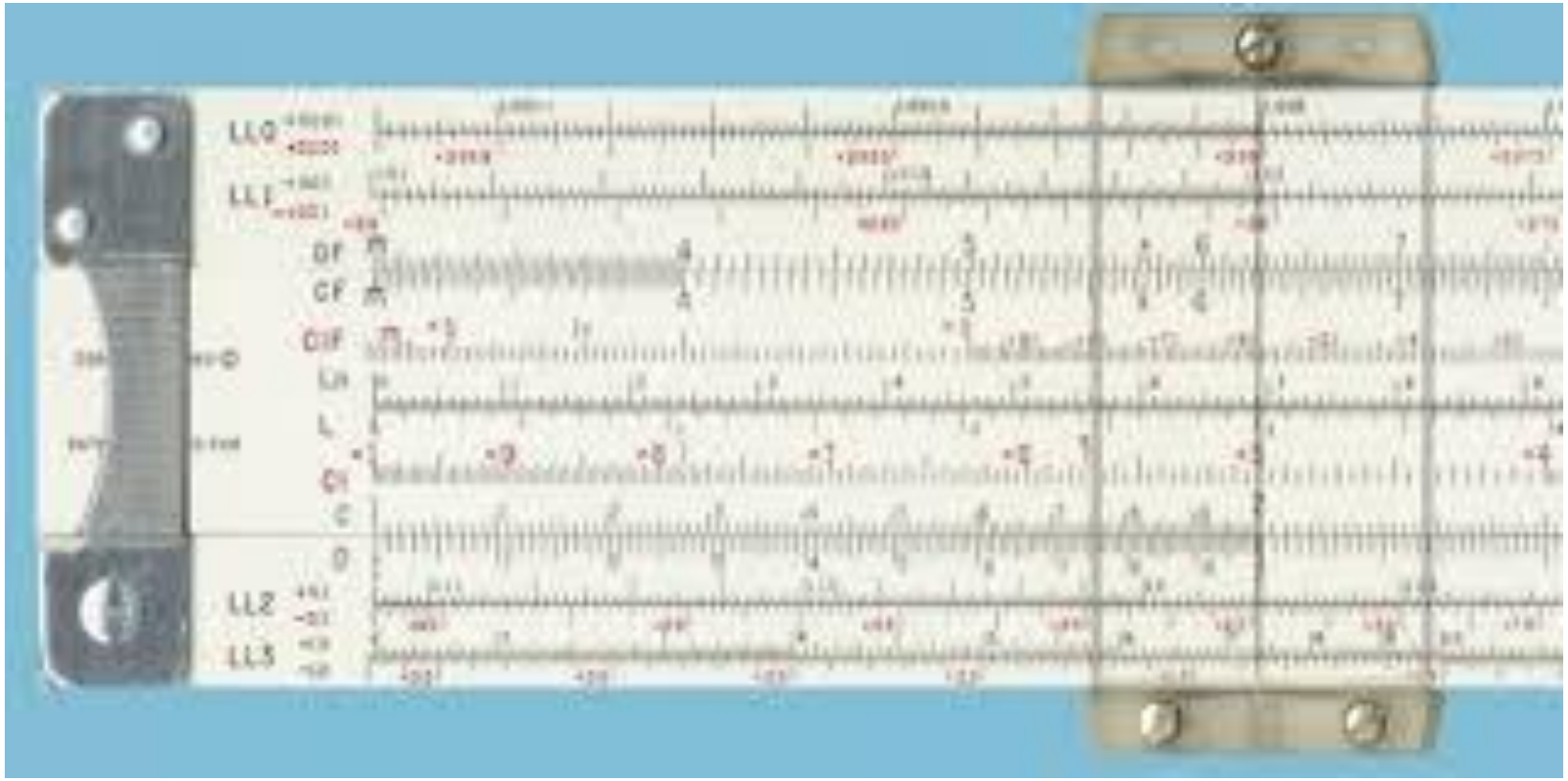
Some providers get more immature oocytes than others

ICSI < 9 hours from OPU is best practice

Measured Outcomes vary by laboratory practice (D3 Vs D5; Volume; Strict timing; Workload)

Variability in Studies

- Inclusion criteria
- Size of study
- Differing laboratory routines
- Timing of trigger shot
- Outcomes measured – maturity, fertilization, PR, LBR
- Study length (cleavage stage or blastocyst stage)
- Randomization method


LL0
LL1
DF
CF
CIF
LN
L
CI
C
D
LL2
LL3

Trigger	• 34 • 39
DN	• Preincubation > 4 hours • Preincubation < 4 hours • No preincubation
ICSI	• Immediately post denuding • >1 hour after denuding

AUTHOR	DESIGN	HCG-OPU	OPU_DEN	DEN-ICSI	OPU - ICSI
Velde, et al	PS	36	1 to 6	0 to 4	1 to 6
Yanagida	PS	35	1 to 11	0	1 to 11
Renzi	RP	36	2 to 12	0	2 to 12
Andrews	RP	36	0 to 1	?	0 to 5
Hassan	PS	?	0 to 4	0 to 4	0 to 4
Jacobs	RS	36	0 to 4	0	0 to 4
Ho	RS	34	1 to 8	0	0 to 4
Isiklar	PS	36	0 to 4	0	0 to 4
Falcone	RS	36	2	0 - 10	2 to 12
Boldi	RS	?	?	0 to 3	?
Aletebi	PS	36	0 to 2	0 to 2	0 to 4
Patrat	RS	36.5	0 to 3	0 to 3	0 to 6

AUTHOR	DESIGN	HCG-OPU	OPU_DEN	DEN-ICSI	OPU - ICSI
Esbert	RS	?	2 OR 4	?	45 mins
Garor	RS	34 to 38	1 to 7	1 to 5	?
Terasawa	RS	?	0 to 3	?	?
Ishikawa	PS	?	0 to 2	?	?
Pereira	RS	35 to 37	2 to 3	?	?
Barcena	RS	36	0.5 to 3	0.6 to 10	1.4 to 12
Pujol	RS	36	0.4 to 2	0.26 to 11	1 to 12
Mizuno	PS	36 to 38	0 or 2	2.5 to 4	2.5 to 4.5
Naji	RS	36	0 to 5	?	?
Zhang	RS	36	1.5 to 3	?	?
Azizi	PS	35 to 39	0.5 to 6	0 to 4.8	?
Maggiuli	RS	34 to 37	2 to 7	0	2 to 7

Study Conclusions:

- ICSI should be delayed until the noon hour (H. Van de Velde)
- ICSI at anytime between 1 and 9 hours post-retrieval (Yanagida)
- The optimum time range between 3 and 12 hours following oocyte retrieval can improve the fertilization rate and embryo quality (Renzi)
- < 3 hour incubation better than 5 hours (Andrews)
- Maturation is improved with cumulus on preincubation (Hassan)
- Incubation durations of 30 min to 6 h prior to ICSI did not improve the ICSI results(Jacobs)

Study Conclusions:

- Incubate for 2.5 hours to improve maturity (Ho)
- Pre-incubation with cumulus improves maturation, fertilization and outcome (Isiklar)
- Incubate 5 to 6 hours before ICSI (Jacobs)
- Perform ICSI immediately after denuding (Boldi)
- Optimal timing of ICSI is within 4 hours of denudation (Zhang)
- It is preferable to allow an interval between oocyte retrieval and sperm injection (Aletebi)
- Incubation of oocytes around 2 hours between retrieval and denudation lead to the optimal combination of FR and IR (Patrat)

Study Conclusions:

- Delaying denuding does not improve outcomes (Esbert)
- Delaying denuding does not improve outcomes (Garor)
- Denude soon after OPU (Terawawa)
- Pre-incubate with cumulus cells for 2 hours (Ishikawa)
- Modulating time intervals between OPU, DN and ICSI is feasible (Pereira)
- High quality oocytes may withstand ageing to a certain extent to fall into laboratory workflow with adverse events (Barcena)
- ICSI should not be delayed whenever possible but PR decreased as OPU – to ICSI increased (Pujol)


WHOOP DEE DOO BASIL
WHAT DOES IT ALL MEAN?

Conclusions

- Good quality oocytes are not influenced by pre-incubation with cumulus corona cells vs no pre-incubation
- Fertilization and good embryo development can occur across a span of time
- Each laboratory has to establish guidelines that adapt to their workflow
- There is no right or wrong way to do this

References available upon request