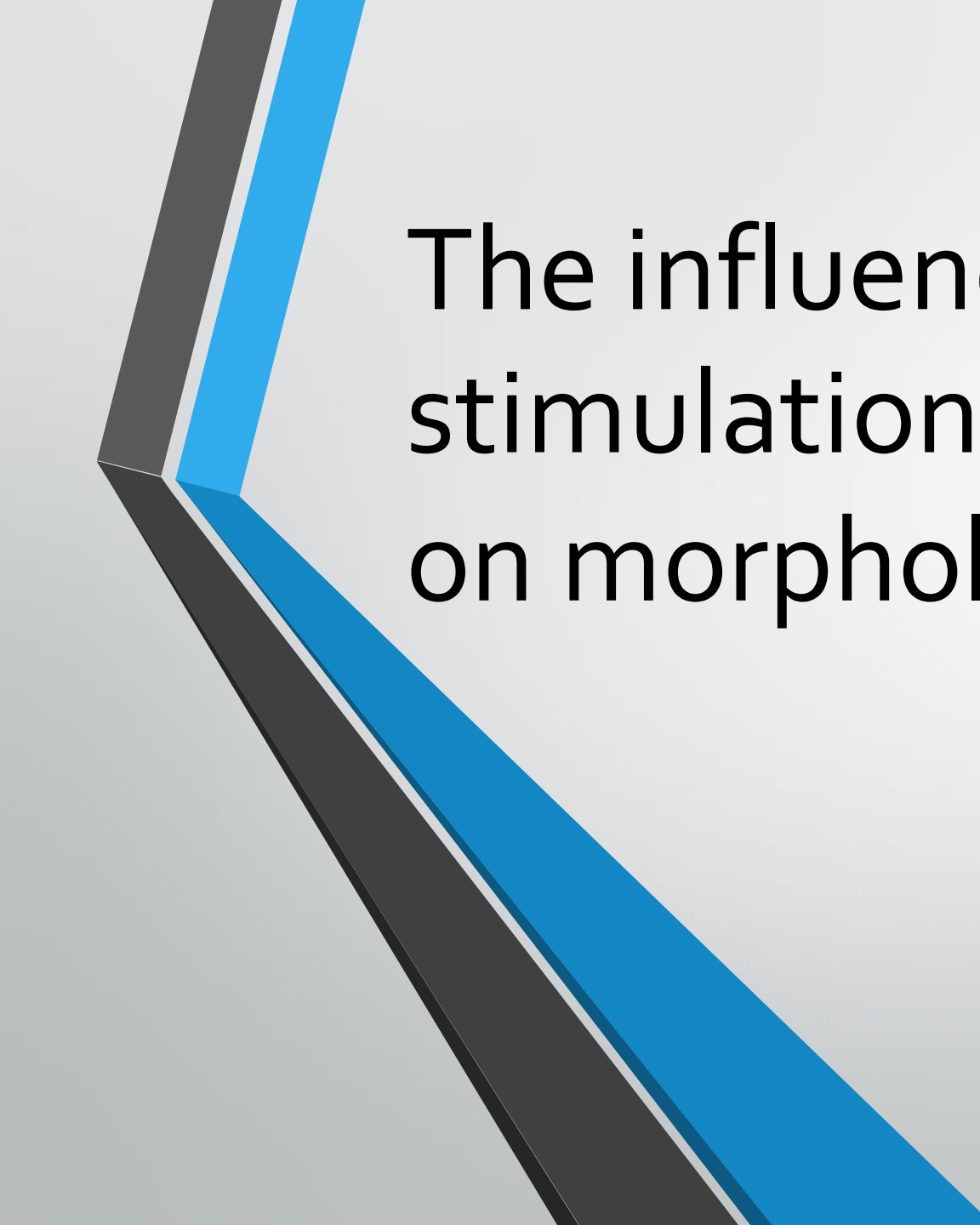




بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

BESMELLAH



The influence of ovarian stimulation drugs on morphology of human oocyte

By:

Dr. Sareh Karimi

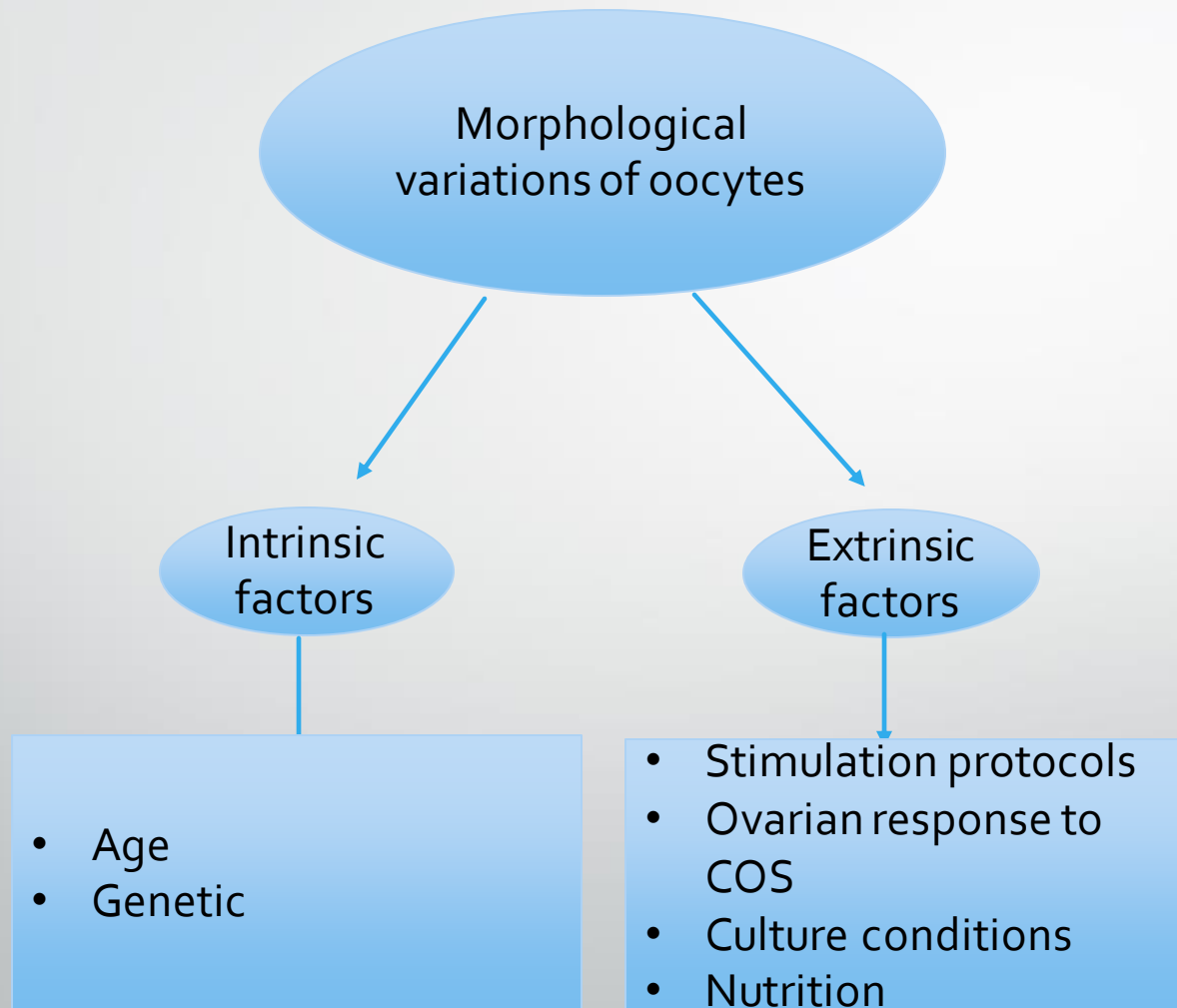
Embryologist

Armaghan infertility center

Headlines:

- Oocyte morphology
- Abnormal oocyte morphology & Cause of them
- Conclusion

Oocyte morphology

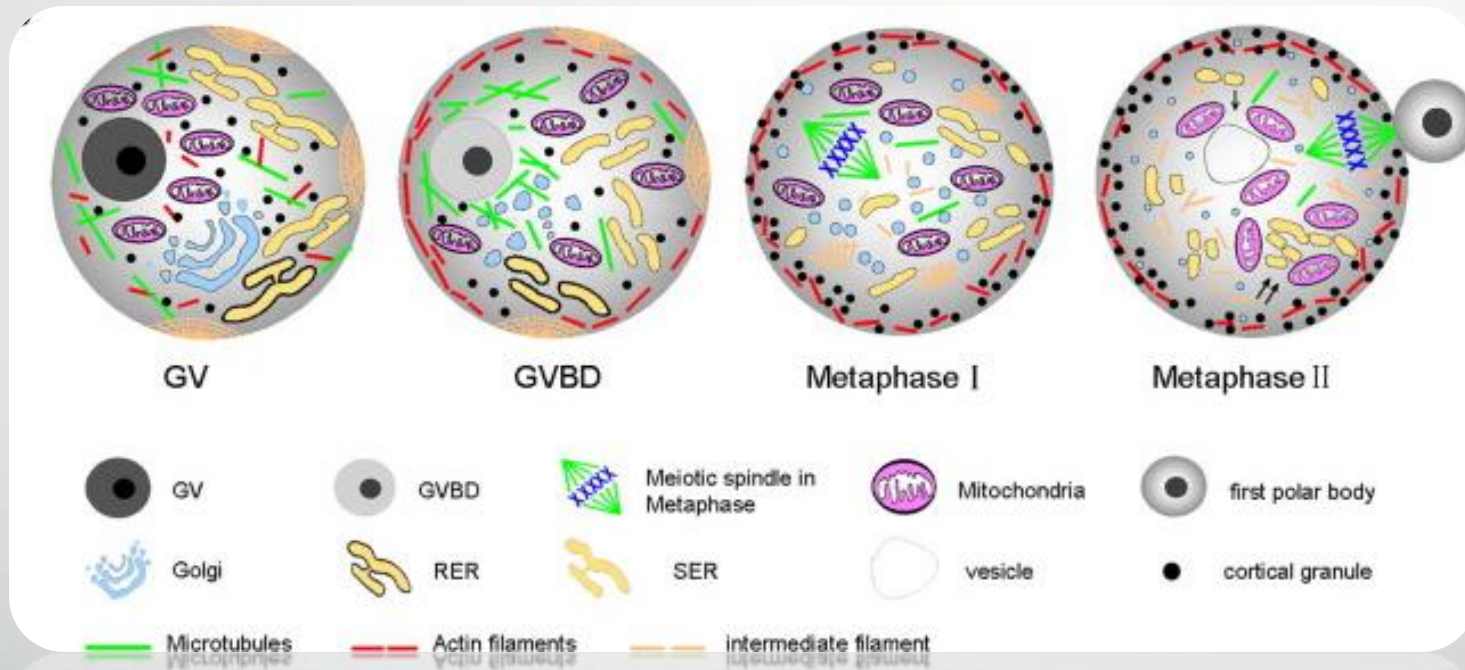


Normal oocyte

- Clear
- Moderately granular cytoplasm
- Small perivitelline space
- Ovoid or round first polar body with smooth surface
- Clear colourless zona pellucida
- Normal size:
 - Whole size of oocyte is almost 150 μm
 - Oocyte 115–120 μm
 - Zona pellucida 15–20 μm



Ultra-structure of oocyte



Is nuclear maturity alone enough to determine the competency of an oocyte??

✓ **Disturbance or asynchrony** of these two processes:

- Decline the oocyte quality
- Induce the different oocyte dysmorphisms

Abnormal oocyte morphology

➤ Cumulus-enclosed oocytes

- Compact cumulus–oocyte complex
- Sticky cumulus–oocyte complex

➤ Cytoplasmic features

- Granular cytoplasm
- Centrally located granular

➤ C

It has been reported that a majority (60% to 70%) of retrieved oocytes exhibit one or more of these abnormal morphologic characteristics

➤ Deficiency in cytoplasm fluidity

- Thick and dense ZP
- Perivitelline space

➤ Oocyte size and shape

- Small & large oocyte
- Elongated MII oocyte

- Polar body

When the first meiotic division takes place:



- Synchronous cytoplasmic maturation is characterized by:

- Ability to release calcium and cortical granules
- Mitochondrial changes
- Protein synthesis
- Cytoskeletal change

- ✓ Despite the presence of normal genetic material, ooplasmic factors play an important role in the fertilization process that could be compromised by cytoplasmic abnormalities.

Oocyte anomalies



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Oocytes recovered from stimulated IVF cycles:

Approximately **40%** incidence of numerical chromosomal abnormalities.
In addition, oocytes with abnormal cytoplasmic morphologies were found to have a high frequency of aneuploidy

Mature oocytes exhibiting severe cytoplasmic disorganization:

Lower intracytoplasmic pH
Lower adenosine triphosphate content
Increased incidence of aneuploidy and chromosomal scattering

Oocyte maturation stage

- Normal COS {
85% MII
10% MI
5% GV



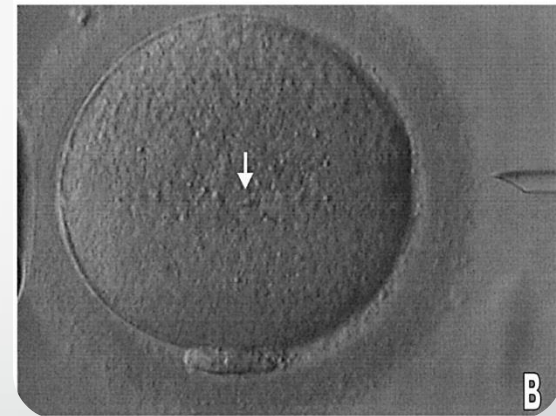
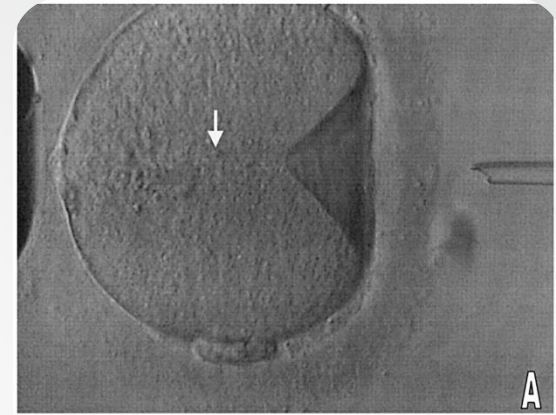
Oocyte maturation stage

High prevalence of immature oocytes:

- Using FSH alone rather than FSH and LH for ovarian stimulation
- GnRH antagonist rather than GnRH agonist
- Short GnRH agonist protocol rather than long GnRH agonist protocol
- HCG trigger increased immature oocyte in compare to dual trigger

Deficiency in cytoplasm fluidity

- Related to cytoskeletal function and MII meiotic spindle structure
- Viscosity of the cytoplasm may constrain cell organelles and pronuclei in their movement
- Impairing polarity and embryo development



Extracytoplasmic oocyte morphology



- Extracytoplasmic oocyte morphology induced by:
Total dose of administered FSH
Hyperstimulation protocols

Extracytoplasmic oocyte morphology



Large perivitelline space:

Could be related to oocyte overmaturity

Letrozole administration

Debris in perivitelline space:

High levels of gonadotropin during stimulation

Increased oocyte yield and

Perivitelline space granularity:

May be a sign of incomplete and premature exocytosis of cortical granules can occur

Affect 15% oocyte

May be a sign of gonadotropin overdose

High oocyte yield

Lower fertilization,
implantation, and clinical
pregnancy rates

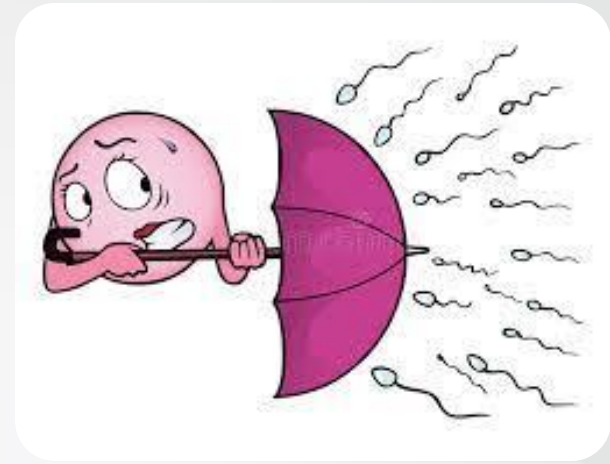
Defects of the ZP



- Defect
- Assoc

Agonist cycles
Letrozole administration
Elevated **basal FSH** concentrations
r/u FSH administration

Defects of the ZP



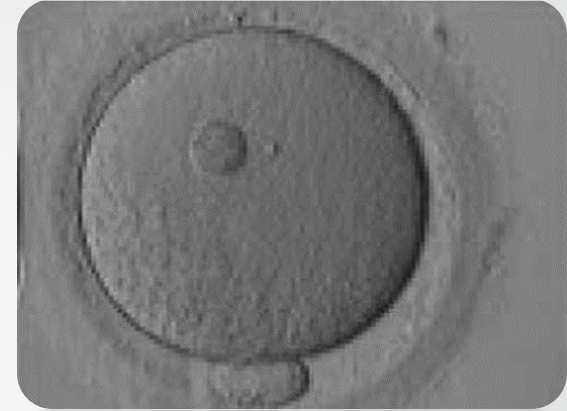
- hMG administration changes in paracrine in ovarian follicle caused by LH such as **cAMP** and **EGFR**

→
Increase the maturity of zona pellucida

- ✓ ICSI improves the likelihood of fertilization of oocytes with abnormal zona pellucida, but cannot improve the clinical outcome.

Decrease:
Fertilization rate until
60%
Embryo cleavage rate
Blastocyst development
when $>5 \mu\text{m}$

Refractile body



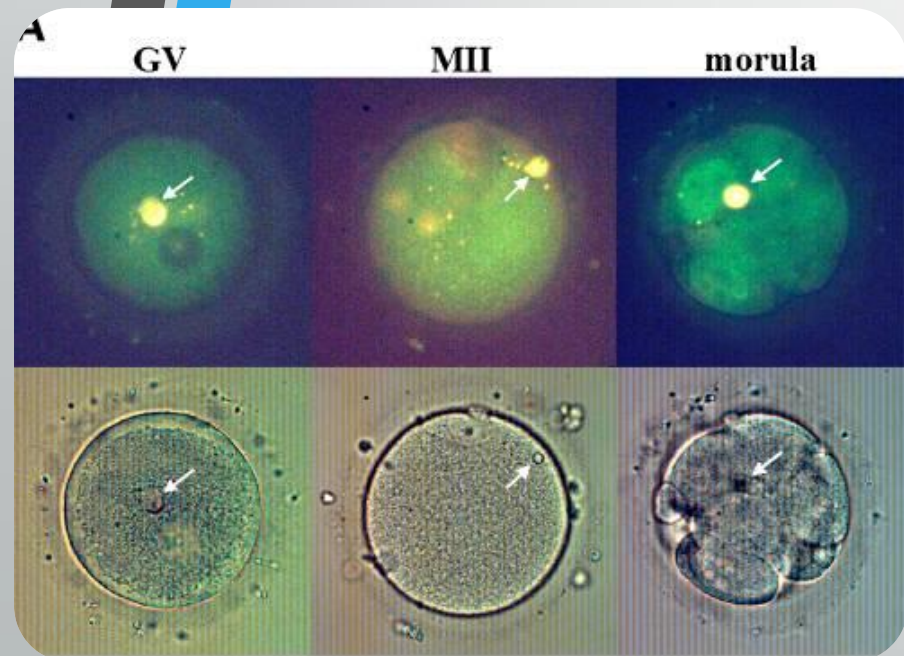
- Refractile bodies are a very common inclusion consisting:

Letrozole administration
hMG increase number of oocytes containing refractile body

○ Necrotic regions

Refractile body

- May occur during the growth phase of the oocytes when dominant follicles are being recruited into the preovulatory pathway.
- May be related to conditions of the developing ovarian follicles, such as perifollicular blood circulation and follicular fluid composition.
- Lipofuscin bodies in human oocytes were detected throughout meiotic maturation (GV, MI, MII stages)



Refractile body



- Recurrence in the same patient with repeated treatment cycles
- Usually associated with poor fertilization (2%) when IVF procedures were used.
- Oocytes containing refractile bodies were usually fertilized normally using ICSI

Oocyte granulation



- Sign of oocyte cytoplasmic immaturity
- 50% of risk of chromosomal abnormalities in the embryo
- Chromosomal abnormalities

Antagonist protocol with a **GnRH agonist trigger** may improve ooplasm granularity and embryo quality.

Letrozole administration increase ooplasm granularity

- ✓ **cytoplasmic**
 - The number of aspirated follicles
 - The number of retrieved oocytes

Lower implantation
Ongoing pregnancy rates
Higher embryo aneuploidy
rate

Central granulation



Letrozole administration increase dense central granulation
u/rFSH stimulation increase oocytes with dense central granulation in compare to hMG
r/FSH stimulation increase oocytes with dense central granulation in compare to **u/FSH**

Poorer rates of fertilization,
cleavage, and top quality
embryos.

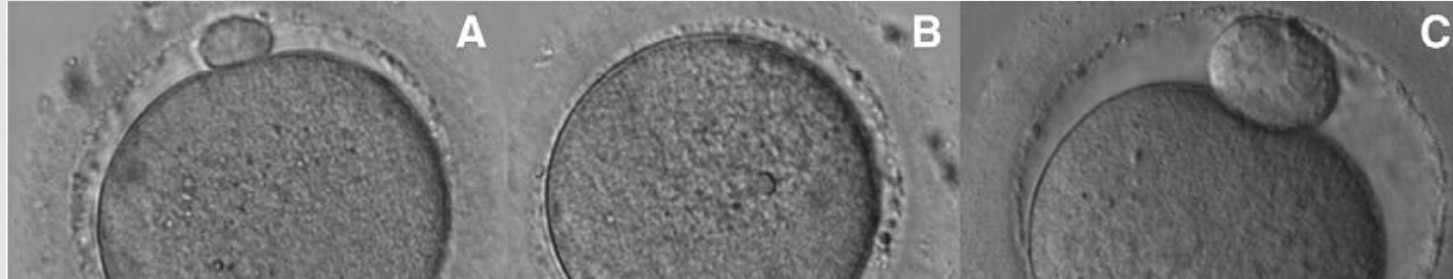
First polar body morphology

- The first polar body morphology is a prognostic indicator of the outcome
- A prognostic indicator of the outcome
- Distinguishing the post-ovulatory age of the oocyte

hMG administration decreased the PB abnormality

outcome

First polar body morphology



Intact first PB and of a normal perivitelline space:
 Better synchrony between the cytoplasmic and nuclear maturation
 Better oocyte quality

Fertilization rate, % ^g	70.8 ^a	69.7 ^a	20.7 ^d
Cleaved embryos	854	876	68
Cleavage rate, % ^h	62.5 ^c	60.5 ^c	18.7 ^d
Good quality embryos	260	248	18
Proportion of good quality embryos, %	19.0 ^e	17.1 ^e	5.0 ^f

Decreased the fertilization rate in multiple vacuoles or vacuoles >14

Vacuoles



SHORT COMMUNICATION

Human oocytes containing large cytoplasmic vacuoles can result in pregnancy and viable offspring

Birth of a newborn with a major malformation (double left kidney and ureter)

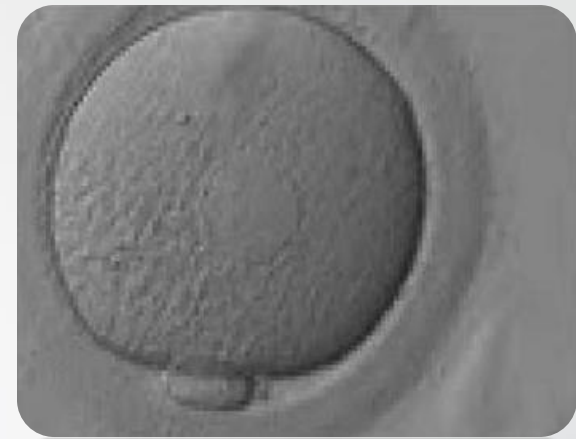
Péter Fancsovits *, Ákos Murber, Zsuzsa Tóthne Gilán, János Rigó Jr, János Urbancsek

Disturb the cytoskeleton resulting in fertilization failure

Lower blastocyst formation,
embryo quality, implantation and
pregnancy rate
Higher abortion rate, preterm
birth and neonatal death
Lower live birth delivery rate and

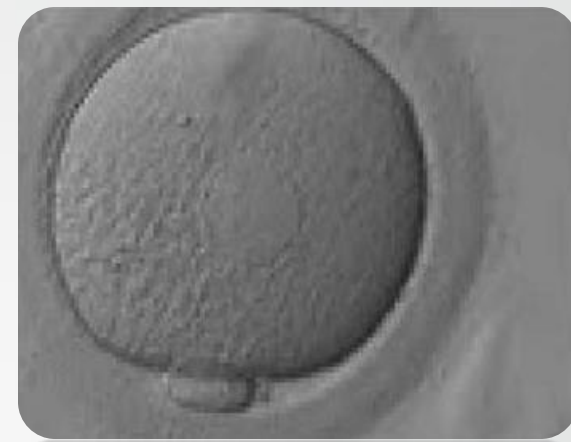
A

SER clusters



GnRH agonist cycles
Duration of the stimulation
The required dose of gonadotropins
Letrozole administration
rFSH increase in compare to HMG
Higher estradiol level on the hCG day in the GnRH agonist cycles

SER clusters



 Check for updates

CLINICAL: CASE REPORT

Complex chromosomal aberrations in a fetus originating from oocytes with smooth endoplasmic reticulum clusters

CASE REPORT

Ioannis A. Sfikakis
Katerina Anagnostou

^aEugonia Assisted
Reproduction, 1st
Thessaloniki, The

Normal karyotyping
holoprosencephaly, cyclopia, and single artery in umbilical cord
patent ductus arteriosus, dysgenesis of the cerebral sulcus, variation of septum pellucidum, and
closed external meatus of the right ear

*Cem Akarsu, M.D.,^a Gamze Çağlar, M.D.,^{a,b} Kubilay Vicdan, M.D., Ph.D.,^a Eran Sözen, M.D., Ph.D.,^a
and Kutay Biberoglu, M.D.^c*

In conclusion

In stimulated cycles, pharmacologic doses of gonadotropins create a supraphysiologic hormonal environment that induces the growth of a cohort of follicles, which, under natural conditions, would become atretic and regress

Oocyte characteristics	Total n:681	Group		P
		Agonist	Antagonist	
Cytoplasmic dysmorphism (presence of Diffuse granulation Central cytoplasmic granulation Refractile bodies SER aggregations Vacuoles Extracytoplasmic (alterati				0.483
Polar body shape				0.047
Perivitelline space	sERC (%)		4.57±11.62	0.046
Zona pellucida	Vacuoles (%)	15.76	6.50±15.00	0.746
Oocyte shape	Perivitelline granules (%)	45.36±32.31	51.24±34.32	0.213
	Large PVS (%)	24.08±29.21	24.51±29.24	0.917
	ZP dysmorphism (%)	11.84±25.13	6.05±14.76	0.049
	PB fragmentation (%)	33.10±26.30	32.30±27.75	0.677

In conclusion

rFSH and hMG have very little effects on oocyte morphometry!

hMG association were similarly effective in generating a clinical pregnancy when less than 8 oocytes were retrieved, but rFSH were significantly more effective when 8 or more oocytes were available.

Oocyte quality in women rece

Parameters		
Nuclear maturity (%)		
Metaphase II		
Metaphase I		
Germinal vesicle		
Atretic		
Zona morphology (%)		
Normal		
Abnormal		
Cytoplasmic appearance (%)		
Normal	56.9	65.4
Abnormal	43.1	34.6
Polar body morphology (%)		
Normal	66.7	68.2
Abnormal	33.3	31.8

0.402^a

			p level
			0.46
			0.45
			0.62
			0.64
			0.20
Rate of cytoplasmic vacuolisation (%)	7.1	4.1	0.09
Rate of cytoplasmic granularisation (%)	28.0	26.0	0.55
Fertilization rate (%)	68.9	59.9	0.01

The effect of HMG and recombinant human FSH on oocyte quality: a randomized single-blind clinical trial 2015
 Controlled Ovarian Stimulation with recombinant-FSH plus recombinant-LH vs. human Menopausal Gonadotropin based on the number of retrieved oocytes: results from a routine clinical practice in a real-life population 2015
 Impact of highly purified versus recombinant follicle stimulating hormone on oocyte quality and embryo development in intracytoplasmic sperm injection cycles 2011

In conclusion

Comparisons of morphological parameters of oocytes in different groups.

Parameters	Group I (rFSH)	Group II (HMG)	P
Number of oocytes	212	147	—
Normal oocyte ^a	23.58	37.41	.000
Single defect ^a	7.9	5.6	.783
Double defects ^a	19	13.5	.347
Multi			.006

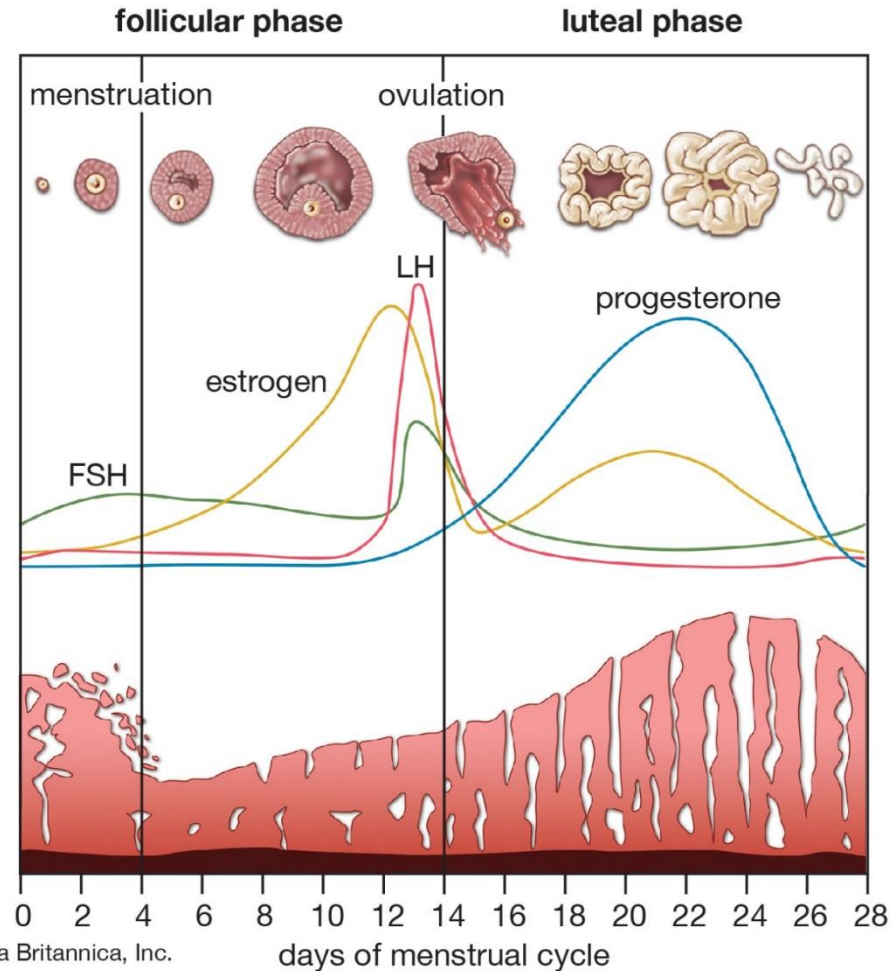
	Group A (n = 109)	Group B (n = 111)	p
No. of oocytes M2 (%)	625/751 (83.2%)	545/701 (77.7%)	<0.01
No. of oocytes M1 (%)	88/751 (11.7%)	105/701 (15%)	n.s.
No. of GV (%)	38/751 (5.1%)	51/701 (7.3%)	n.s.

- The normal morphology of oocytes were significantly enhanced in hMG group.
- Sequential protocol using u-FSH and subsequently r-FSH may improve the IVF outcome in patients of advanced reproductive age.
- Patients of advanced reproductive age exhibited a better response to uFSH than to rFSH

Recombinant FSH (n=132)		HMG + recombinant FSH (n=141)		Urinary HMG (98)	
Mean	SD	Mean	SD	Mean	SD
0.76	0.2	0.42	0.1	0.62	0.3
0.66	0.1	0.08	0.001	0.08	0.02
0.27	0.1	0.28	0.12	0.26	0.01
0.09	0.01	0.007	0.001	0	0
0.53	0.01	0.03	0.02	0.07	0.02
0.1	0.01	0.101	0.01	0	0
0.0075	0.001	0	0	0	0
0.16	0.01	0	0	0.04	0.2
30.9		11.4		13.1	

In conclusion

The menstrual cycle



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Gonadotropin-releasing hormone agonist (GnRHa) trigger 2017

Gonadotrophin-releasing hormone agonist triggering may improve central oocyte granularity and embryo quality 2020

- Possible advantage of GnRH triggering over hCG triggering:
- Simultaneous induction of an FSH surge, comparable with the surge of the **natural cycle**
 - FSH has been shown to induce LH receptor formation in luteinizing granulosa cells
 - Optimizing the function of the corpus luteum
 - FSH has been reported to specifically promote oocyte nuclear maturation

کسری نغمه خود خواند و از صحرای رود!

همه میزنند ما ست!
 صحرای یکتاک

زندگی

صحرای بیگانه است!

بسیارند بیاد!
 صحرای نغمه مردم

