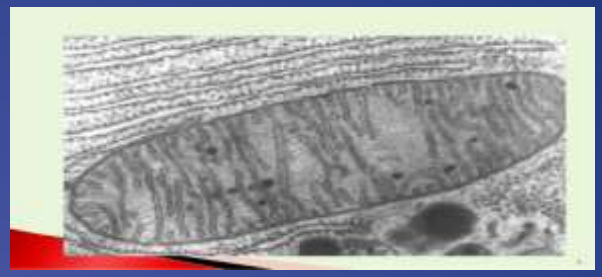

CHANGES IN MITOCHONDRIAL DISTRIBUTION DURING DIFFERENT STAGES OF HUMAN OOCYTE MATURATION.

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РАРЧ

9 – 12 сентября 2015

Сочи

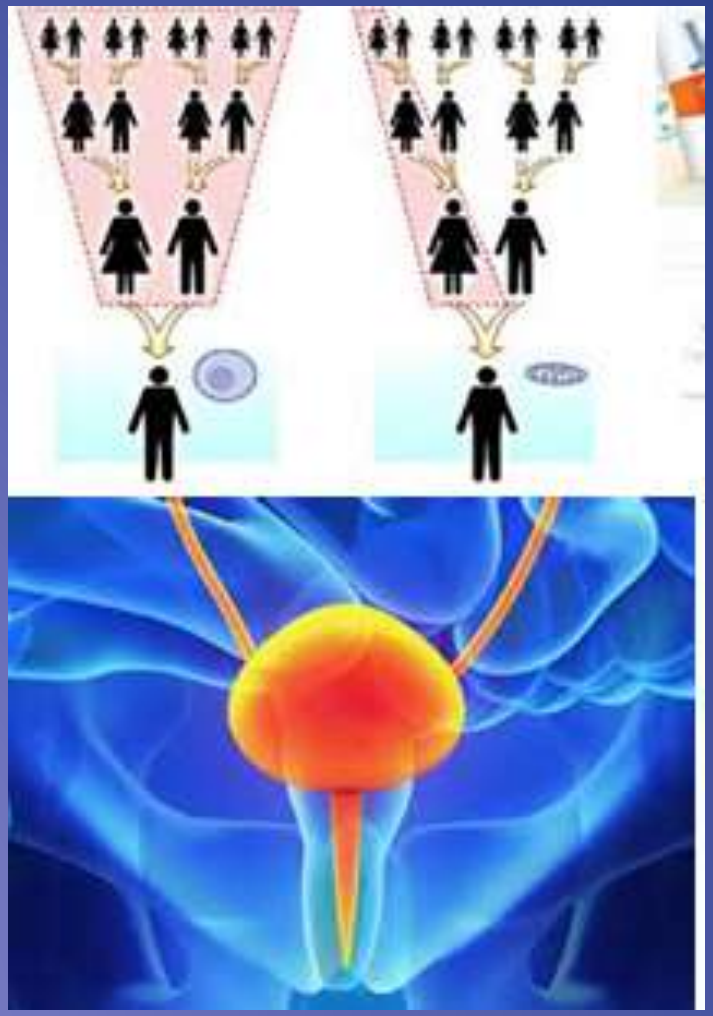
- **Mitochondrion** (plural **mitochondria**) is a membrane enclosed organelle range from 0.5 to 1.0 µm in diameter.

- **Mitochondria** are found in the cytoplasm of most cells and produces enzymes for the metabolic conversion of food to energy.


MOM

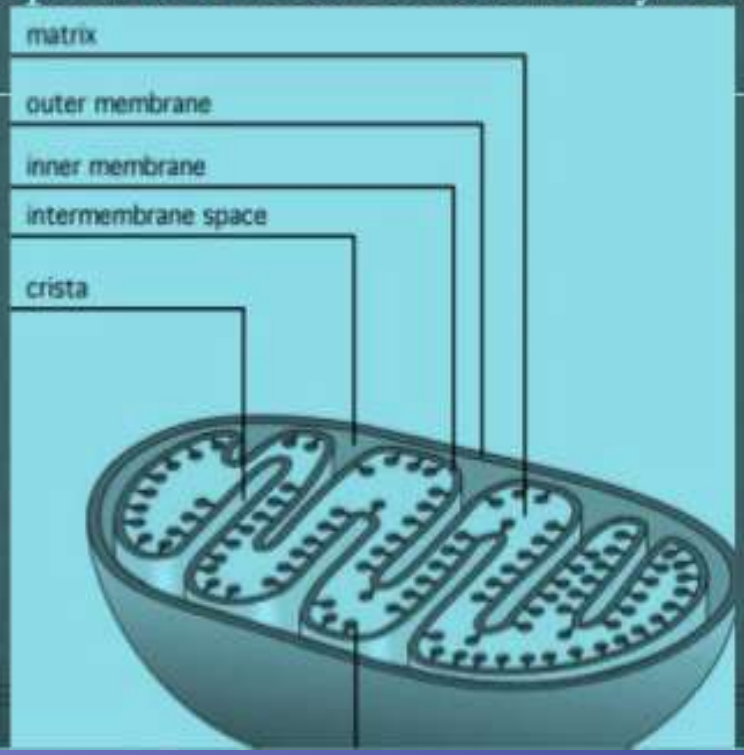
Nuclear DNA is inherited from all ancestors

Mitochondrial DNA is inherited from a single lineage



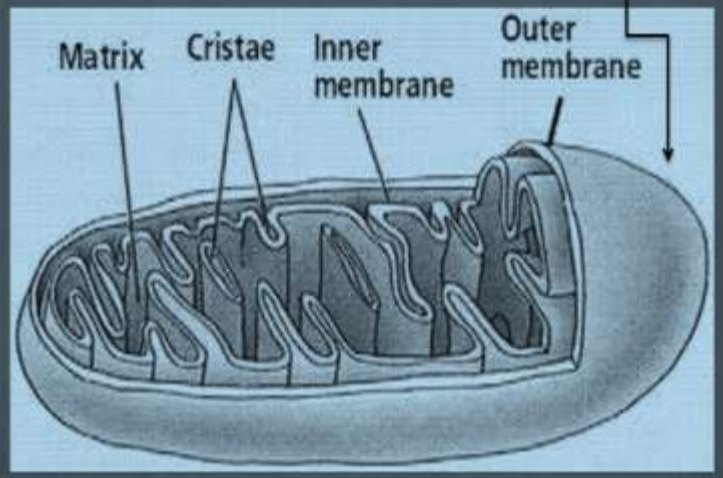
STRUCTURE

A mitochondrion contains outer and inner membranes composed of phospholipid bilayers and proteins. The two membranes have different properties. Because of this double-membrane organization, there are five distinct parts to a mitochondrion. They are:

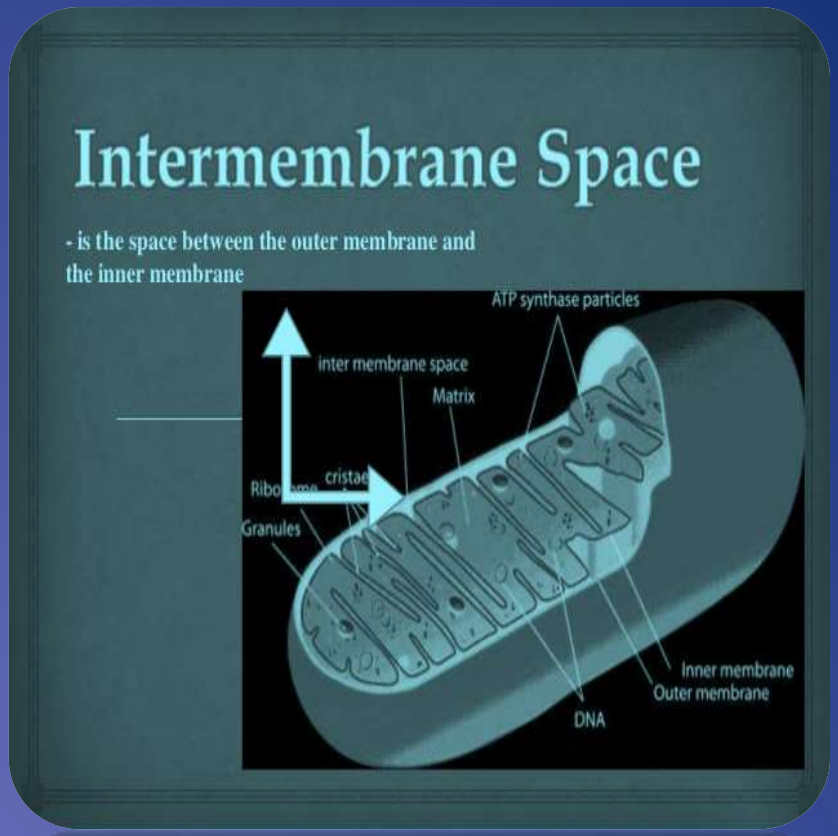


Outer Membrane

- encloses the entire organelle, has a protein-to-phospholipid ratio similar to that of the eukaryotic plasma membrane

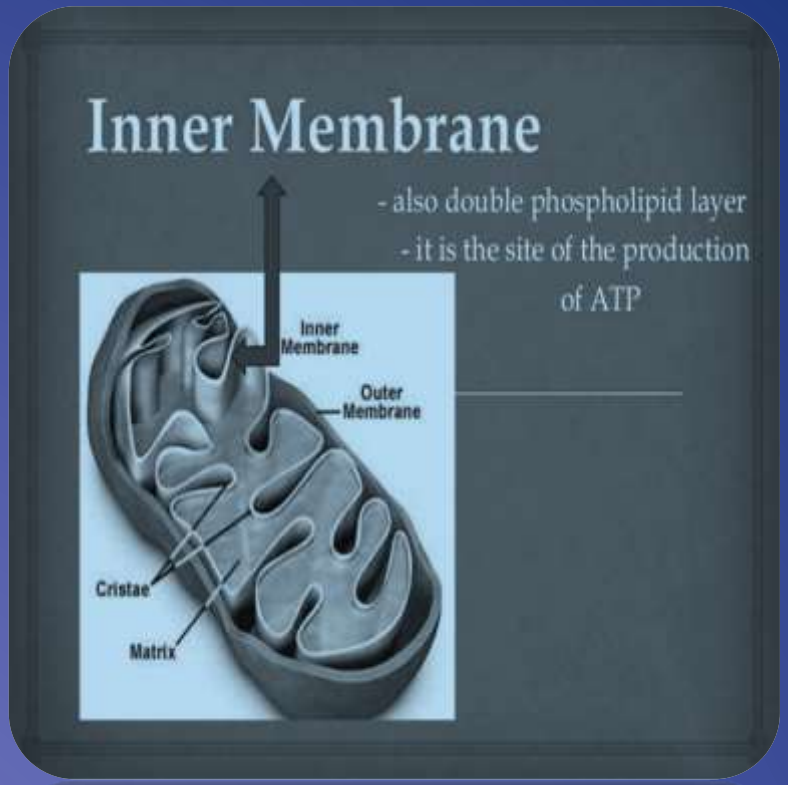


- Contains large numbers of integral proteins called porins.
- They allow molecules to freely diffuse from other side of the membrane to the other



- Known as Perimitochondrial space

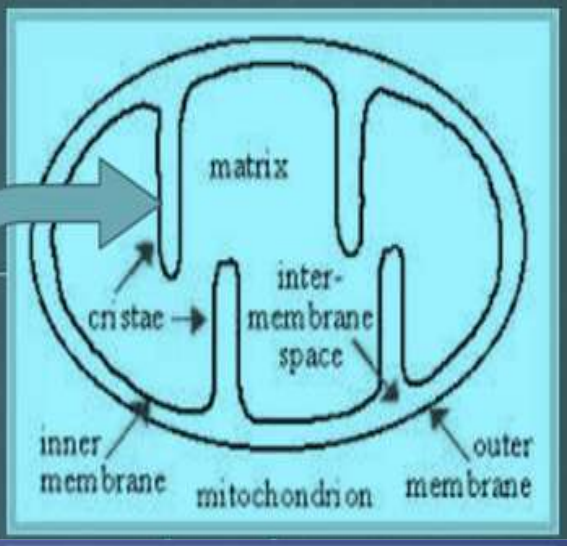
- Is freely permeable to small molecules, therefore the concentration (sugars,=) is the same as the cytosol



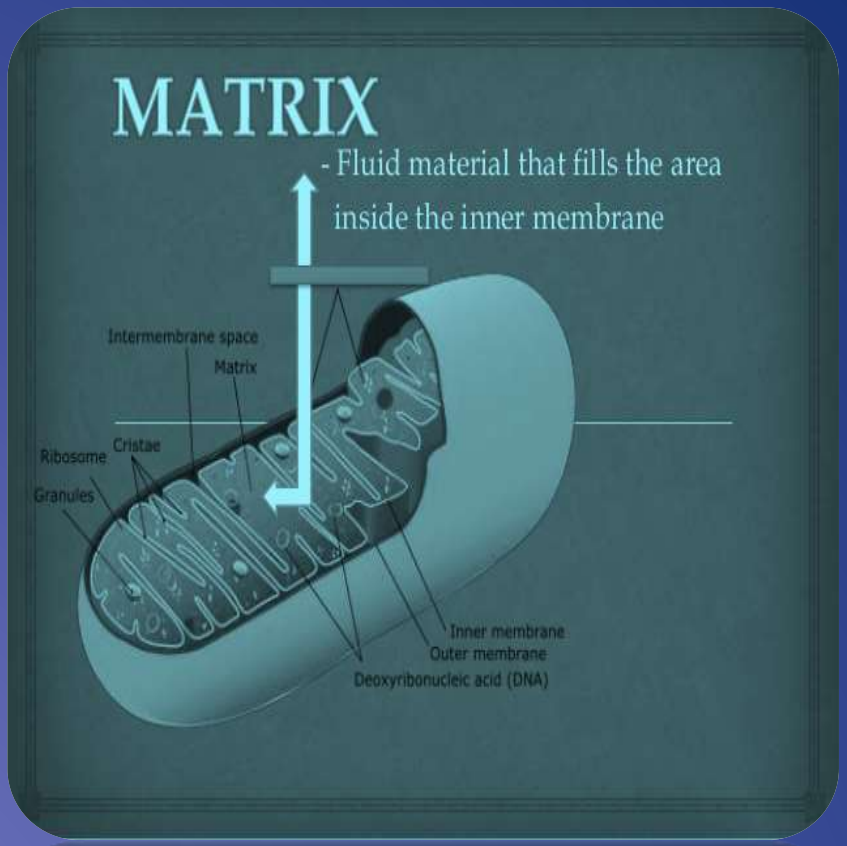
Functions:

- perform the reactions of oxidative phosphorylation.
- specific transport of proteins that regulate metabolite passage into and out of the matrix.
- protein transport machinery.
- - mitochondria fusion.

CRISTAE



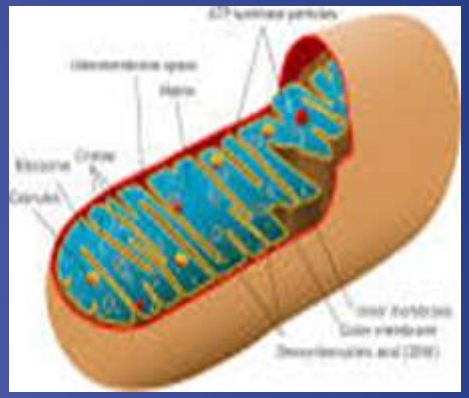
- The folding of the inner membrane that allows more surface area, enhancing its ability to produce ATP



- Is the space enclosed by the inner membrane
- It contains about 2/3 of the total protein in the mitochondrium
- Is important in the production of ATP with the aid of the ATP synthase contained in the inner membrane

- Mitochondria are responsible for converting nutrients into energy yielding molecule adenosine triphosphate (ATP) to fuel the cell activity.

- This function, known as aerobic respiration, is the reason mitochondria are frequently referred to as powerhouse of the cell.



In addition mitochondria are involved in other tasks such as signaling , cell death, cellular differentiation, as well as the **control of the cell cycle and cell growth.**

- The maturation of mammalian oocytes is a process involving nuclear and cytoplasmatic maturation.
- Nuclear changes during oocytes maturation are coordinated with movements of organelles in the cytoplasm to ensure normal fertilization.
- Mitochondria, being the most abundant organelles in the cytoplasm and the source of ATP for the oocytes may have an essential role in cytoplasmatic maturation.

The aim of the study :

To elucidate the relationship between mitochondrial distribution and pattern of spreading through the cytoplasm during different stages of human nuclear oocyte maturation.

Material and Methods

Prospective observational study of 69 oocytes in different maturation stage:

germinal vesicle (GV)=19

meiosis(MI)=19,

M II=31 oocytes,

21 patients aged 34±3.2 years that underwent *in vitro* fertilization (IVF) treatment with ICSI between 1.2.2014-30.11.2014.

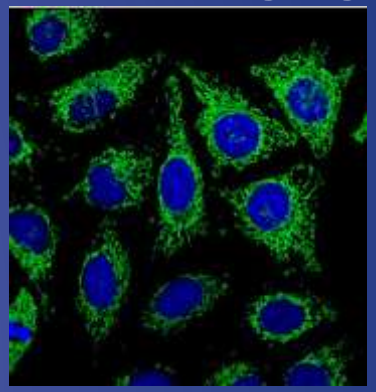
- After denudation, Meiosis (M)II oocytes were injected for the routine cycle. Immature germinal vesicle (GV) and MI oocytes were separated and kept in different wells.

- Immediately after embryo transfer, those immature oocytes (*GV=19*, *MI=19*) (as well as non fertilized mature oocytes-(*MII=31*) were allocated for study.

- All oocytes were stained by Mito-Tracker Green FM and observed at X600 magnification with a scanning confocal microscope (Nikon) by a laser ray to point out the Mito-Tracker Green .

- MitoTracker® dyes are cell permeable probes that contain a mildly thiol-reactive chloromethyl moiety for **mitochondrial labeling.**

- To label mitochondria, cells are simply incubated with MitoTracker® probes, which passively diffuse across the plasma membrane and accumulate in active mitochondria.
- MitoTracker® Green FM is recommended for live cell imaging only



- The distribution of mitochondria was observed by confocal fluorescent microscopy.

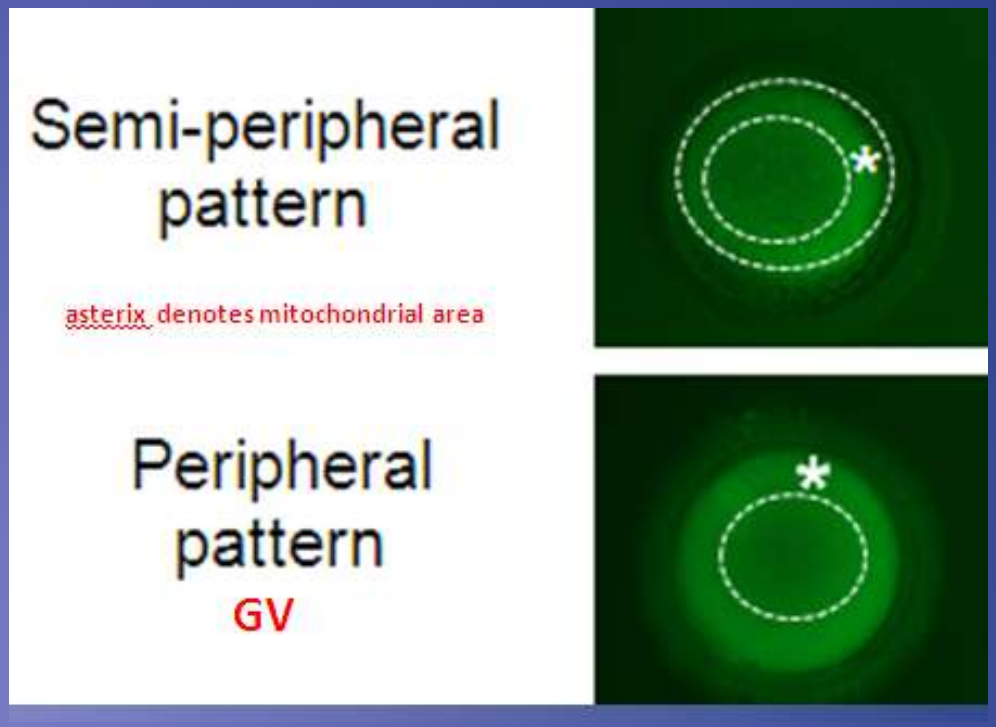
- Laser scanning confocal microscopy represents one of the most significant advances in optical microscopy, primarily because the technique enables visualization deep within both living and fixed cells and tissues and affords the ability to collect sharply defined optical sections from which three-dimensional renderings can be created

The area of the cytoplasm and the percentage of cytoplasm occupied by mitochondria was analyzed using ImageJ software (NIH).

Results:

The distribution of mitochondria through the oocyte as cytoplasm was as follows:

Peripheral/semi- peripheral (GV).



Central located (MI)

Homogenously diffuse (MII)

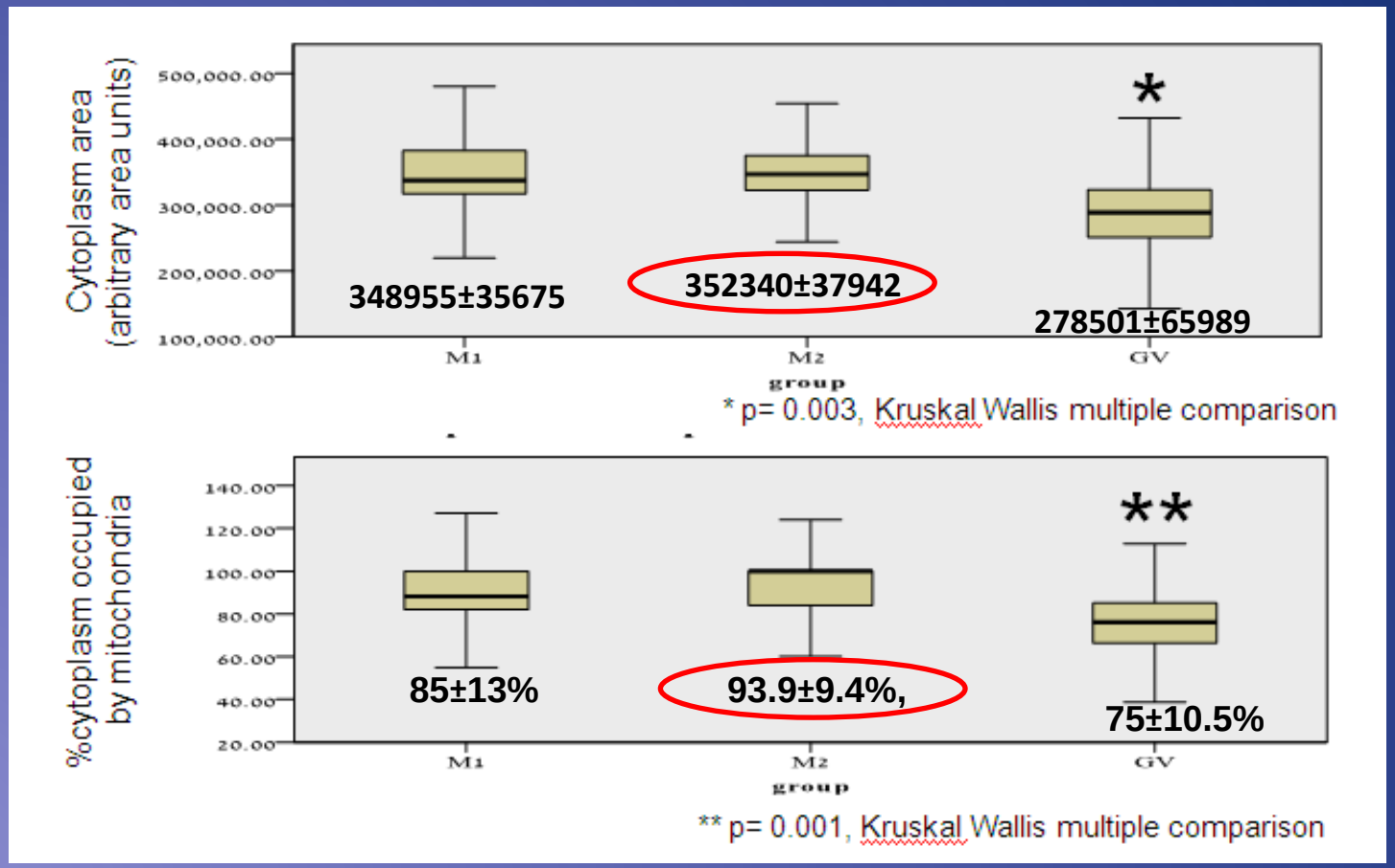
Table 1

Distribution pattern

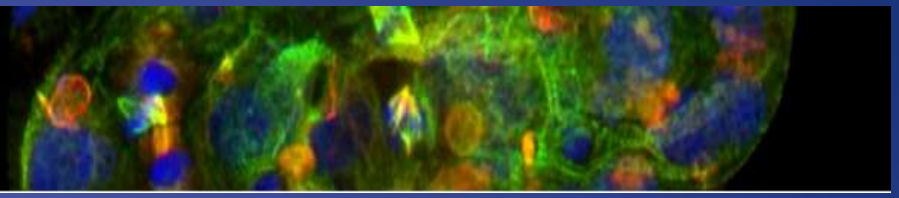
			Homogenous	Central	Peripheral	Total
Group	**GV**	**Count** % within the group	4 20	5 26.6	10 **53.3***	19 100
	M I	**Count** **% within the group**	6 31.2	9 **43.7**	4 25	19 100
	M II	**Count** **% within the group**	21 **74***	5 13	5 13	31 100
TOTAL		**Count** **% within the group**	31 45	19 27.5	19 27.5	69 100

*** P=0.009; Fisher exact test with multiple comparisons**

Fig. 2. Area and percentage of cytoplasm occupied by mitochondria



Zygote 2015 Jul 14:1-9



A stereological study on organelle distribution in human oocytes at prophase I

Ana Sílvia Pires-Luís **et al**

Seven immature GV oocytes were processed for transmission electron microscopy and a classical manual stereological technique

Mitochondria (C: 3.6% versus SC: 6.0%, IC: 7.2%, P = 0.005) were preferentially located in the subcortex and inner cytoplasm

Mitochondrial morphology in human fetal and adult female germ cells

Pietro M.Motta[1,3], Stefania A.Nottola[1], Sayoko Makabe[2], Rosemarie Heyn[1]

2000; 15,129-147

The aim of this study has been to observe, by electron microscopy, the morphological changes affecting mitochondria and associated organelles in the human female germ cell during oogenesis, maturation and fertilization

Mitochondrial morphology in human fetal and adult female germ cells

Pietro M.Motta[1,3], Stefania A.Nottola[1], Sayoko Makabe[2], Rosemarie Heyn[1]

2000:15,129-147

- In **the primordial germ cell** (PGC), rounded mitochondria **are disposed near the nucleus** and significantly increase in number during PGC migration and settlement in the gonadal ridge, where they differentiate into oogonia
- In oocytes **at early prophase stage**, mitochondria proliferate while aligned along the **outer surface of the nuclear membrane**, contain a more dense matrix than before, and have lamellar crista
- Oocytes of primordial and primary follicles mostly contain round or irregular mitochondria, typical parallel, arched cristae, and are **clustered near the nucleus**
- **When follicles grow**, the mitochondria of the oocytes become even more numerous and are **dispersed in the ooplasm.**

- By ovulation, mitochondria are the most prominent organelles in the ooplasm.
- They form voluminous aggregates with smooth endoplasmic

reticulum (SER) tubules and vesicles.

These mitochondrial-SER aggregates (M-SER) and the mitochondrial-vesicle complexes (MV) could be involved in the production of a reservoir of substances or membranes anticipating subsequent fertilization and early embryogenesis

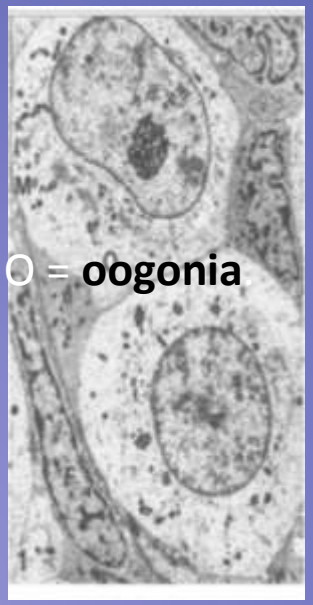
Mitochondrial morphology during preimplantational human embryogenesis

A.H.Sathananthan[1] and A.O.Trounson

2000;15:148-159

Oogonia show a sparse and even distribution of mitochondria, which are oval or elongated

Except around nuclei, growing oocytes from small antral follicles have more dense rounded or oval mitochondria, associated with the rough endoplastic reticulum.



Oogonia from fetal ova
Mitochondria (M) are sparse and variable in form.

Growing primary oocyte in an early antral follicle from adult ovary.
The mitochondria (M) are mostly located in peripheral ooplasm .
N = nucleus; Z = zona

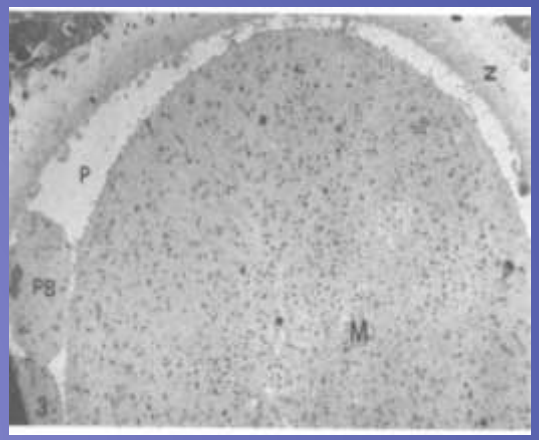
Mitochondrial morphology during preimplantational human embryogenesis

A.H.Sathananthan[1] and A.O.Trounson

2000;15:148-159

Mitochondria in fully grown **GV oocytes** are **usually absent from the cortical part of the cytoplasm and show a perinuclear distribution.**

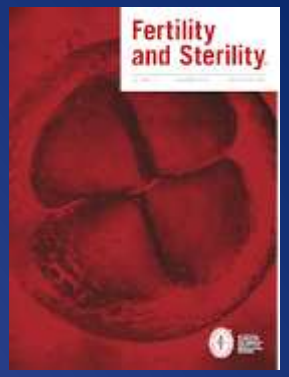
Mitochondria **in metaphase I and II oocytes**, including fertilized oocytes, present a similar structure, but they are numerous and **evenly spread in the ooplasm**, associating closely with vesicles or aggregates of tubular smooth endoplasmic reticulum.



In mature (metaphase II) oocytes and in oocytes soon after fertilization, mitochondria are usually evenly distributed.

Changes in the distribution of mitochondria before and after in vitro maturation of human oocytes and the effect of in vitro maturation on mitochondria distribution

Shan Liu, M.D., Ph.D., Yuan Li, M.D., Ph.D., Xuan Gao, M.Sc., Jun-Hao Yan, M.D., Ph.D., and Zi-Jiang Chen, M.D., Ph.D.



2010;93:1550-5

3 mitochondria distribution patterns were identified: **peripheral**, semiperipheral, and **evenly diffused**.

- A peripheral distribution of mitochondria was presented by 64.1% of the germinal vesicle (GV) oocytes;

- 45.2% (28/62) of the meiosis I oocytes maintained the peripheral distribution; and 38.7% (24/62) presented a diffused status.

ATP synthase promotes germ cell differentiation independent of oxidative phosphorylation

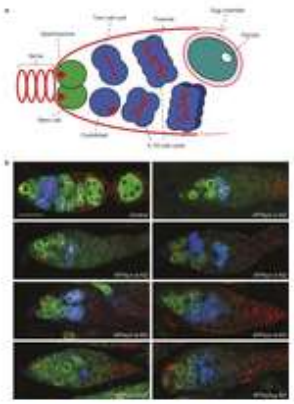
Felipe K. Teixeira, Carlos G. Sanchez, Thomas R. Hurd, Jessica R. K. Seifert, Benjamin Czech, Jonathan B. Preall, Gregory J. Hannon & Ruth Lehmann

nature cell biology

2015, 17:689-696

- Analysing genes required for germline stem cell differentiation in the *Drosophila* ovary, = the mitochondrial ATP synthase plays a critical role in this process.
- ATP synthase acted to promote the maturation of mitochondrial cristae during differentiation through dimerization and specific upregulation of the ATP synthase complex.
- Taken together, the results suggest that ATP synthase-dependent crista maturation is a key developmental process required for oocyte differentiation

Figure 1: The ATP synthase has an essential role during stem cell differentiation.



Conclusions:

1. There are evident changes in the distribution and pattern of spread of mitochondria during the maturation of human oocytes.

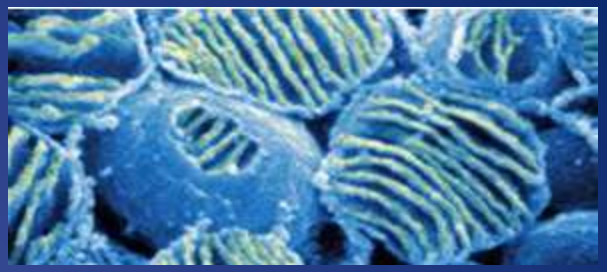
2. Mature oocytes were larger and the mitochondria occupied a significant part of their cytoplasm in a homogenous fashion.

- Our results demonstrated that mitochondria were distributed mainly in the peripheral region in immature oocytes and homogenously in M II oocytes, revealing qualitative information of spreading through the cytoplasm.

- We obtained also quantitative information about mitochondrial localization within the ooplasm. This pattern of distribution may play an important role in the cytoplasmic maturation of human oocytes and their further development.

- Although the specific mechanisms between mitochondria and the cell cycle regulation is not well understood, studies have shown that low energy cell cycle checkpoints monitor the energy capability before committing to another round of cell division

- The variation in ATP levels at different stages of the cell cycle support the hypothesis that mitochondria plays an important role in cell cycle regulation.



Studies on the redistribution of mitichondria before and after maturation could contribute to elucidation of the mechanism of cytoplasmic maturation and promote the development of ART technology including IVM.

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