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PLATELET-ACTIVATING FACTOR IN BOVINE, MURINE AND HUMAN OOCYTE MATURATION AND EMBRYO DEVELOPMENT

Lynn Vandenberghe

6/9/2019

FACULTY OF VETERINARY MEDICINE
accredited by EAEVE

INTRODUCTION


Evening News

Meet Louise, the world's first test-tube arrival

SUPERBABE

Wide-eyed Louise Brown pictured in hospital 18 hours after she was born. Today she's doing well. See Page Three

Back to the future

2000's

1990's

1980's

1970's

1950's

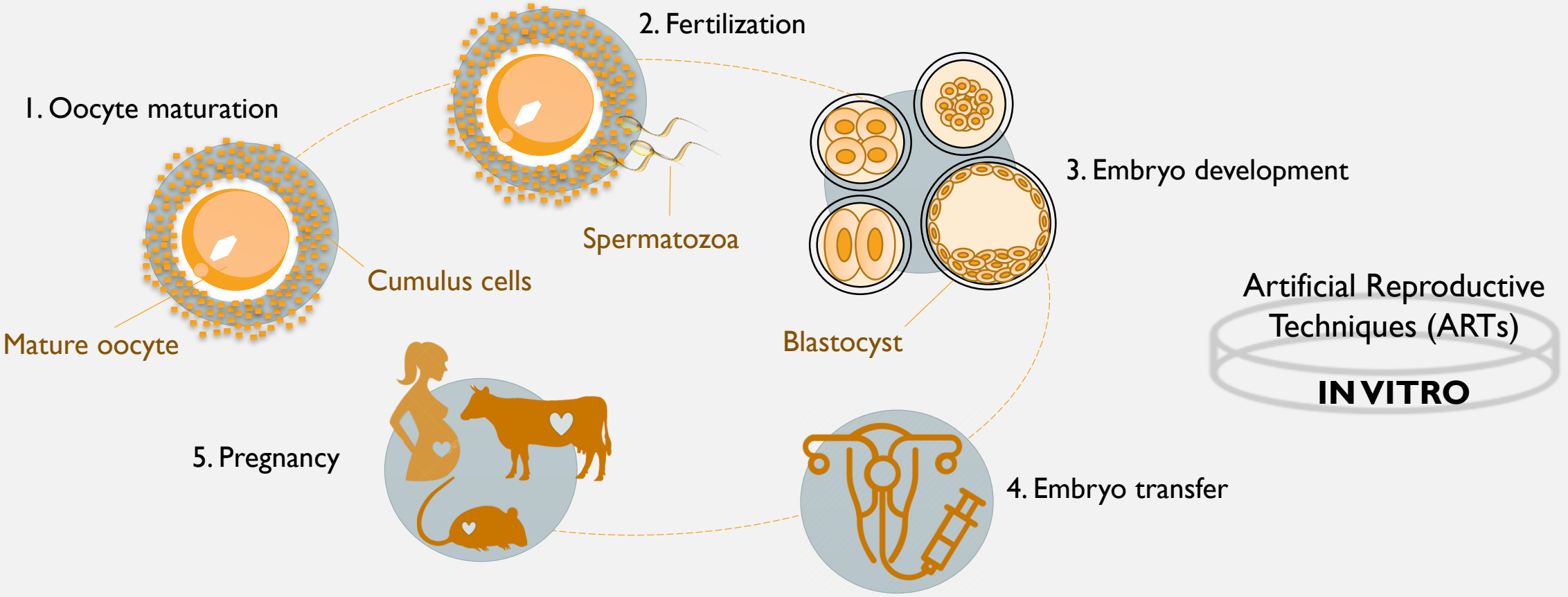
World's **5-millionth** IVF baby (2012)…	Genomic testing & selection
First pregnancies after introduction of Intracytoplasmic Sperm Injection	Optimization of culture conditions
First delivery of a baby from egg donation and after thawing of frozen embryos	**World's first IVF calf (1982)**

25/7/1978: **world's first IVF baby** was born: Louise Brown

Discovery of sperm capacitation: an elementary process necessary for fertilizing oocytes (hamster and rabbit)

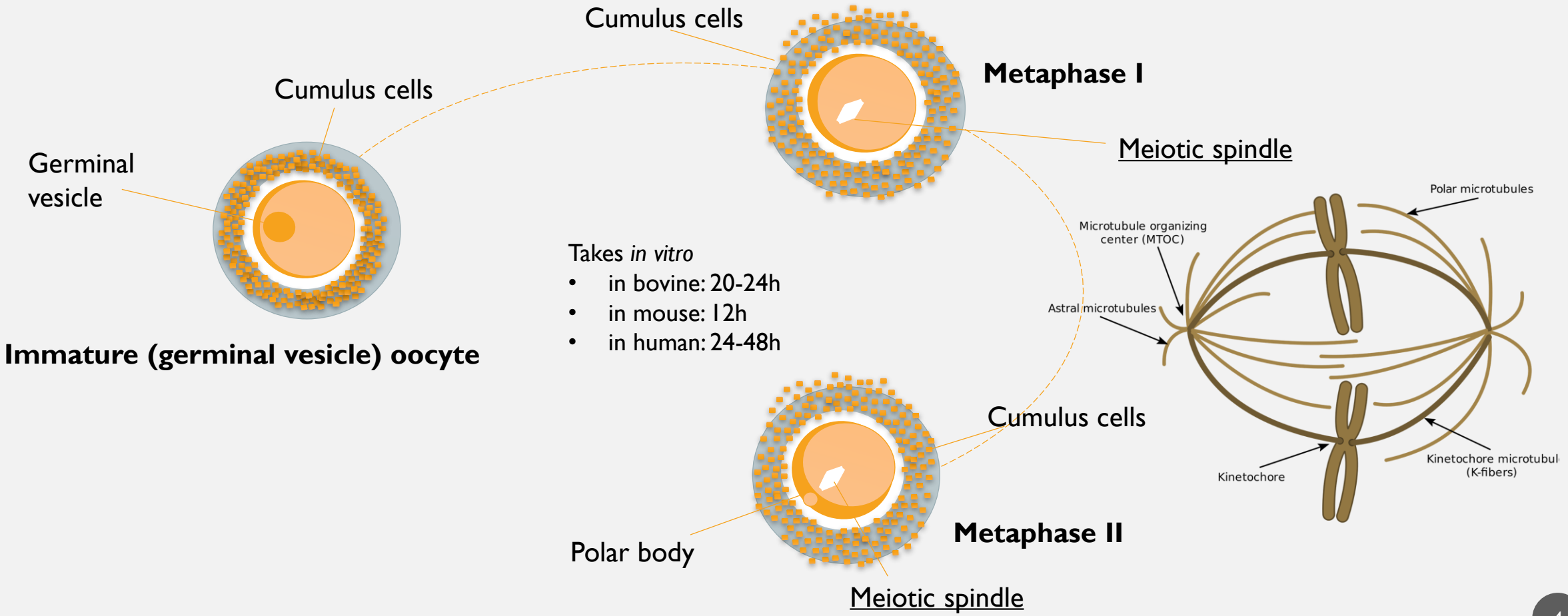
INTRODUCTION

From oocyte to baby (cow)



INTRODUCTION

Oocyte maturation



INTRODUCTION

ARTs: are we fooling mother nature?

IN VIVO

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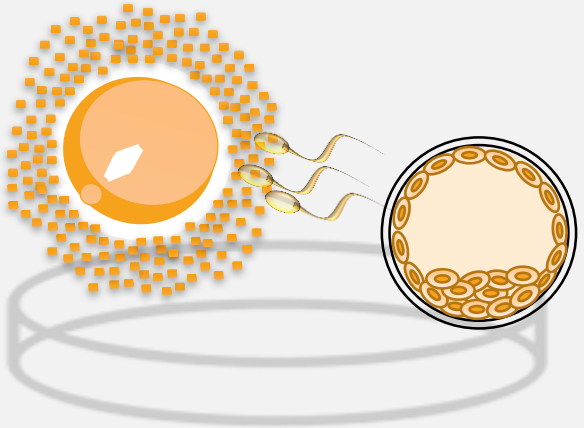
IN VITRO



Culture conditions are **suboptimal**,
ARTs are related to certain abnormalities in the offspring

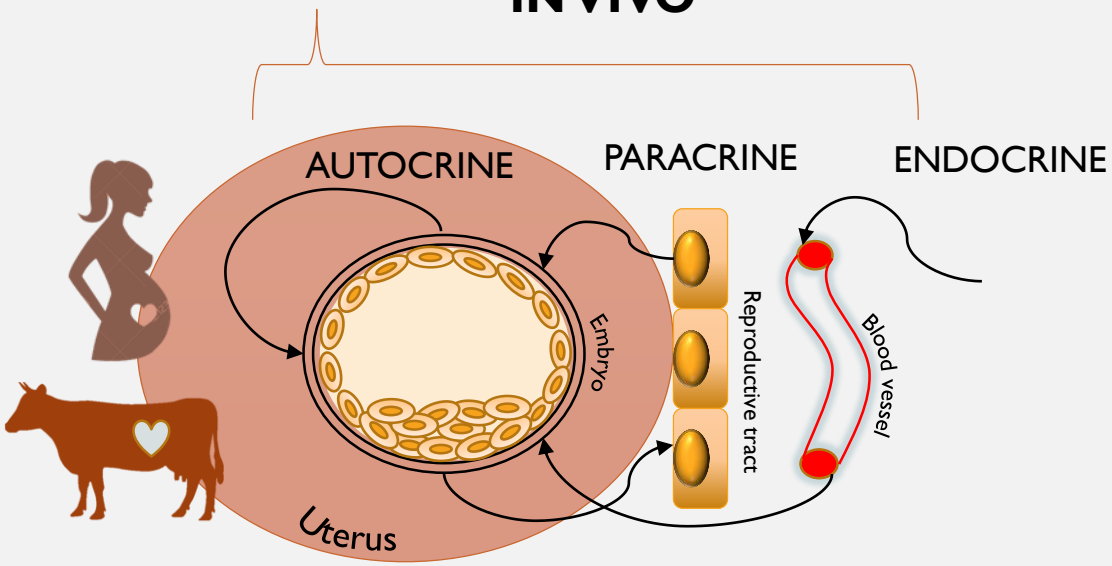
Large Offspring Syndrome
(Young et al., 1998)

INTRODUCTION

Embryo development:
Sharing is caring!

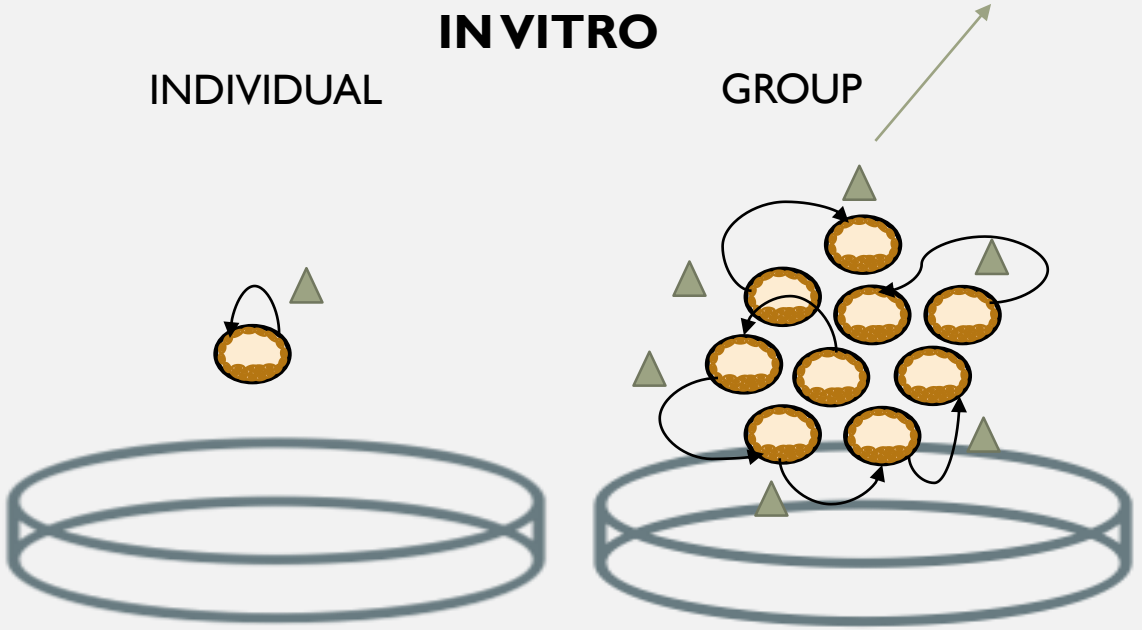
Signaling molecules
e.g. *platelet-activating factor*

IN VIVO



IN VITRO

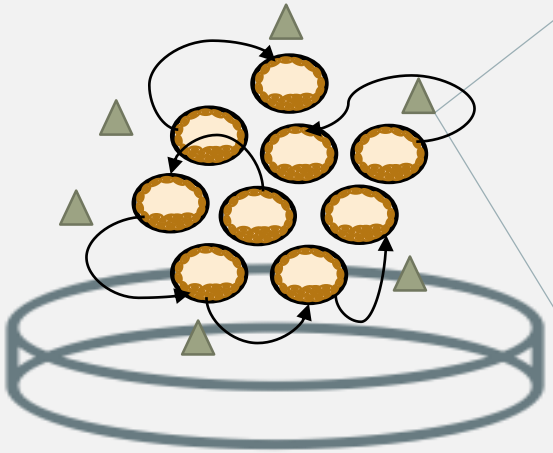
Signaling molecules
e.g. *platelet-activating factor*

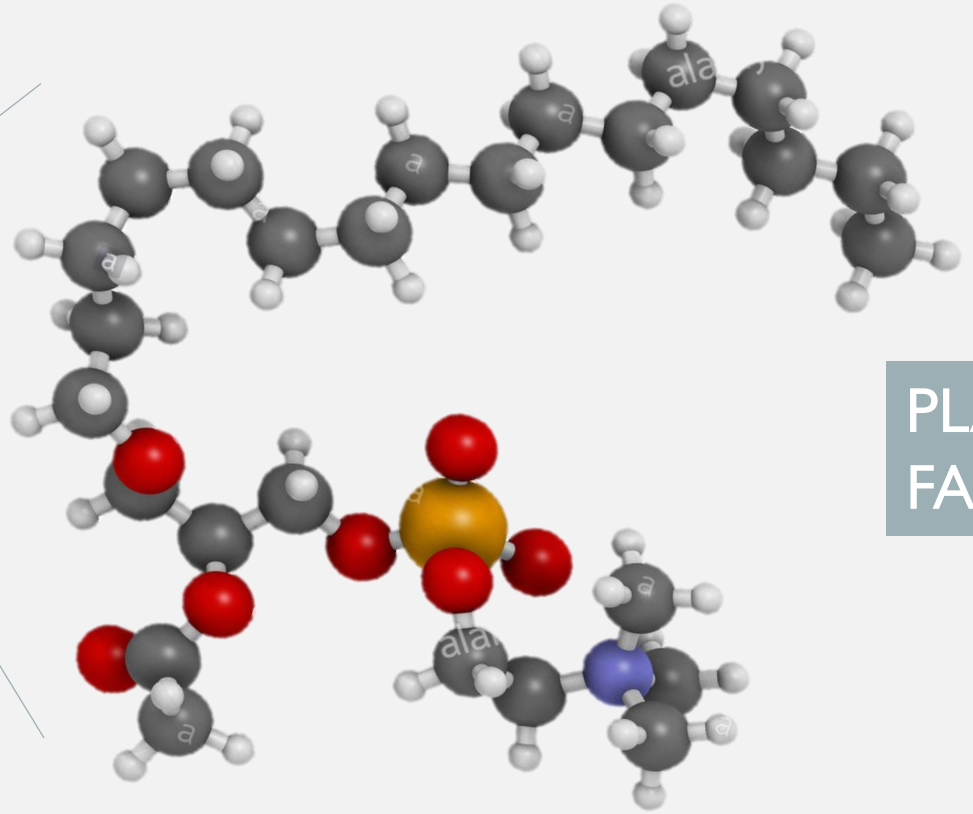


Embryos cultured in **group** are of higher quality and
have a higher chance to implant and result in a pregnancy

Ebner et al., 2010;
Kelley and Gardner, 2017

INTRODUCTION





PLATELET-ACTIVATING FACTOR (PAF)

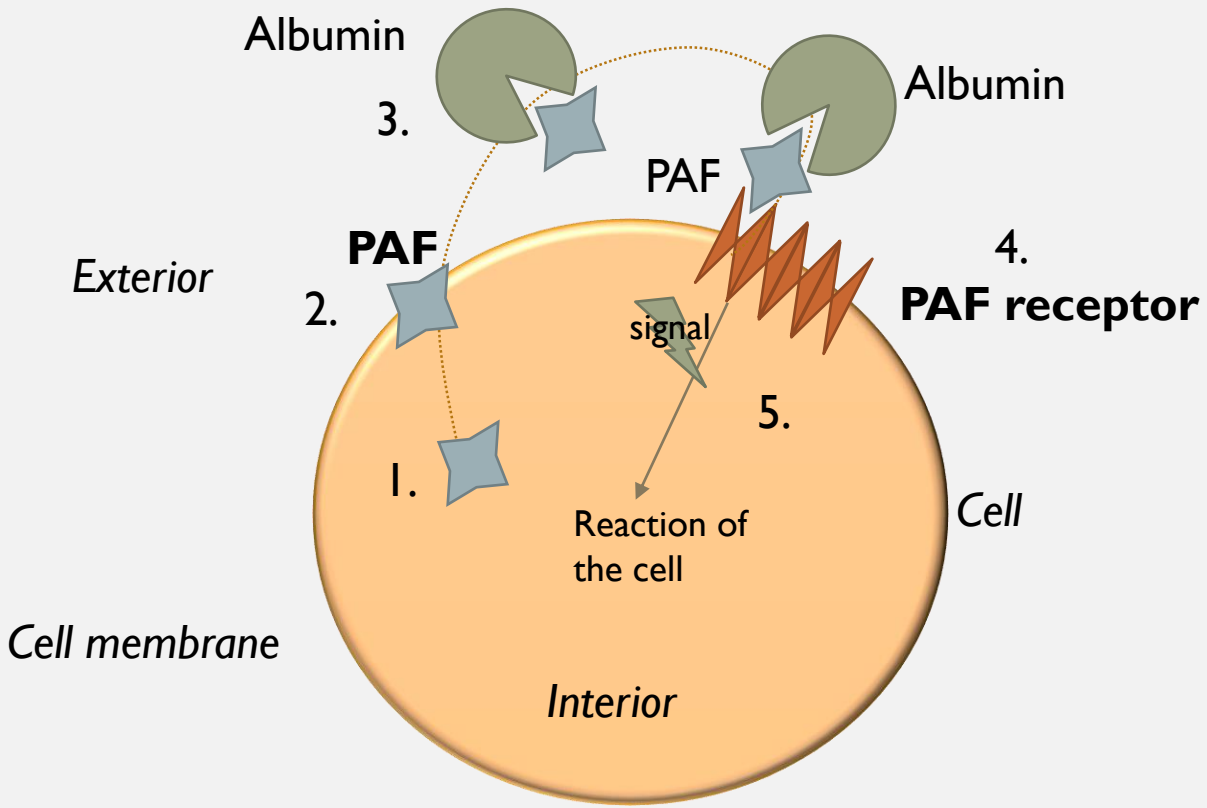
1-*O*-alkyl-2 acetyl-sn-glycero-3-phosphocholine

Role of PAF in **ovulation**, detection of **pregnancy**, stimulation of **embryo development**, embryo **quality** and **viability**

Narahara et al., 1996; O'Neill, 1997; Roudebush et al., 2002; Gopichandran & Leese, 2006

INTRODUCTION

PAF signaling: current model

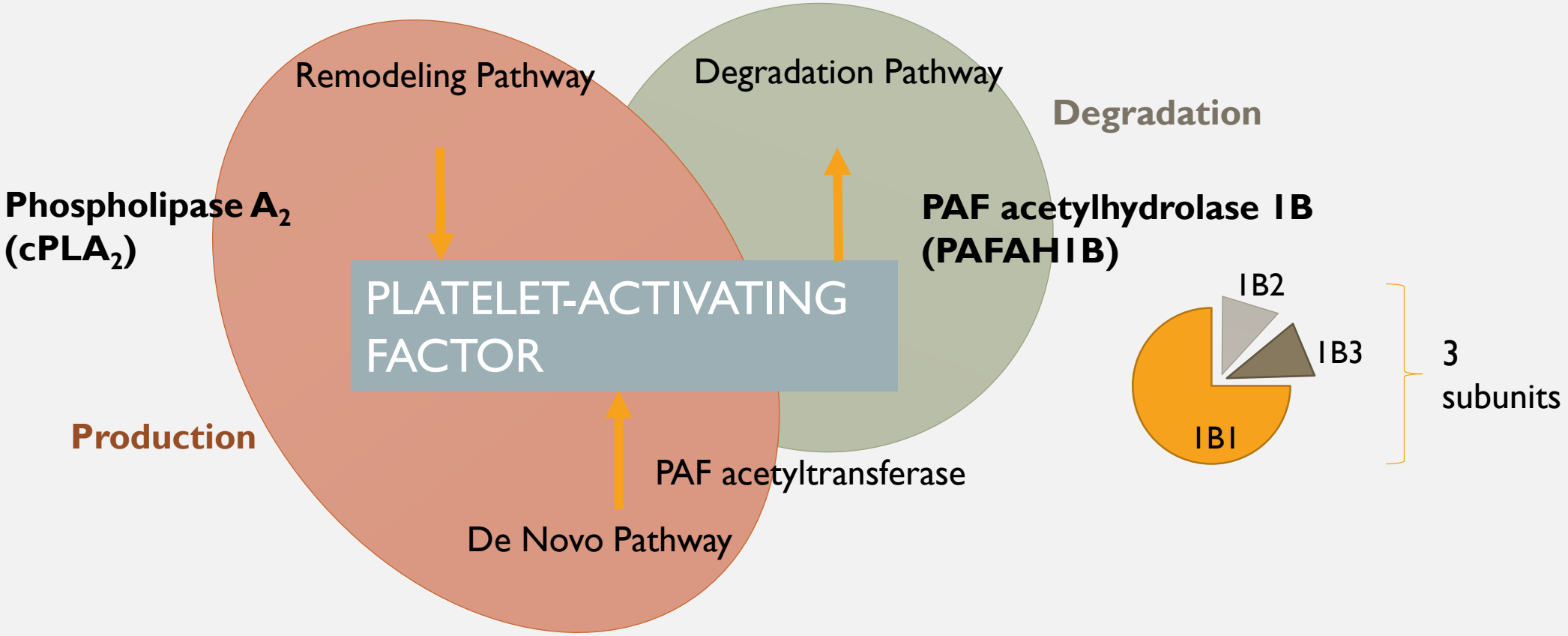


1. PAF is produced by the cell,
2. Travels to the plasma membrane,
3. Picked-up by albumin for transport,
4. PAF binds to its receptor,
5. PAF binding to the receptor activates an intracellular signaling cascade and evokes an intracellular reaction

O'Neill, 2005

INTRODUCTION

PAF metabolism



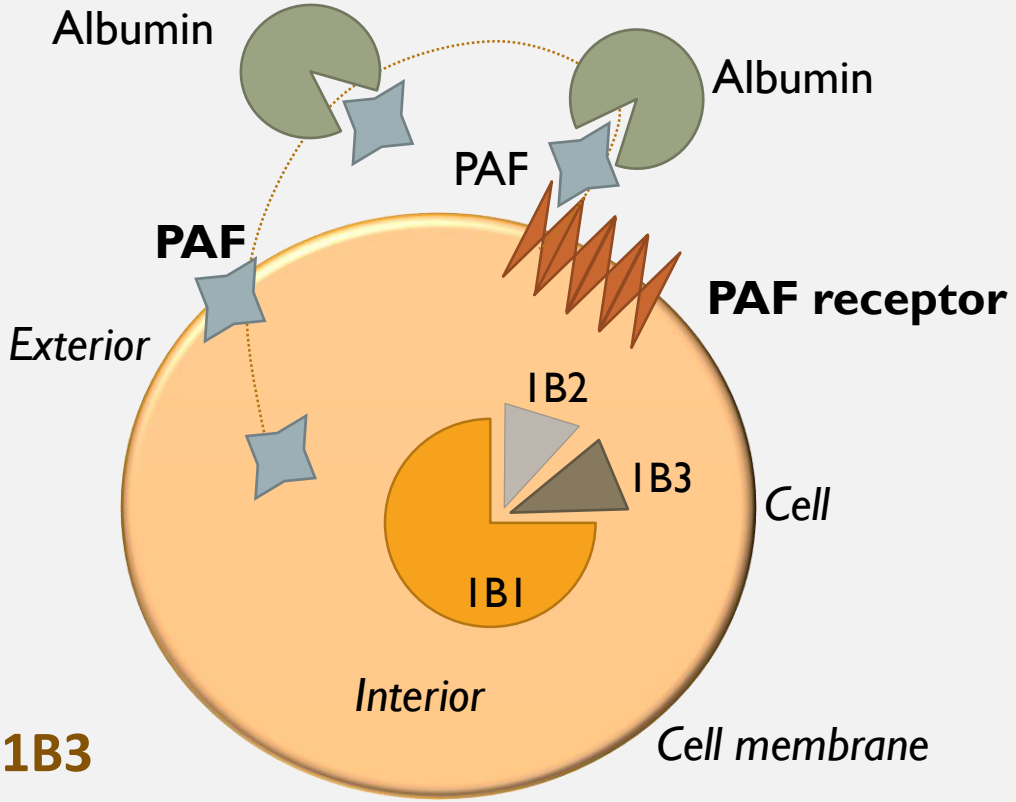
Hattori et al., 1996; Liu et al., 2016

AIMS

Oocyte maturation and embryo development

The **presence** and **localization** of:

- **PAF**
- **PAF receptor (PTAFR)**
- the specific intracellular enzyme **PAFAH1B**

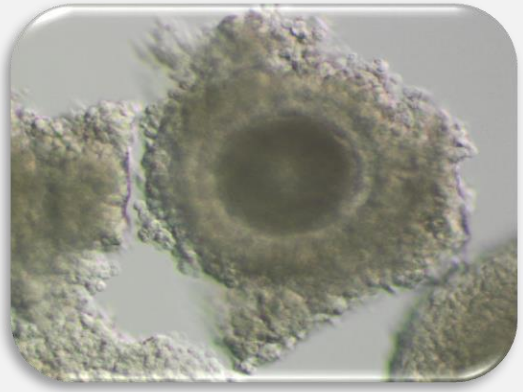


Oocyte maturation:

- Evaluate the **function** of the catalytic subunit **PAFAH1B3**

MATERIALS & METHODS

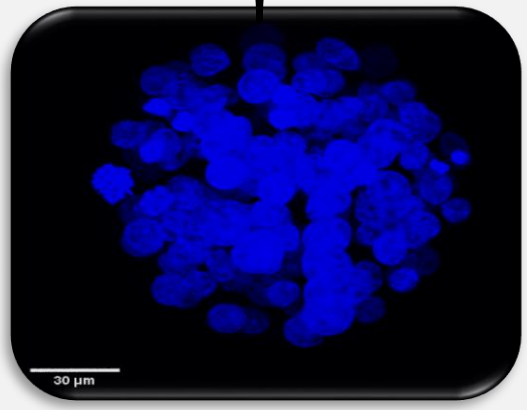
1. *In vitro* oocyte maturation



Artificial Reproductive Techniques (ARTs)

IN VITRO

2. *In vitro* embryo development

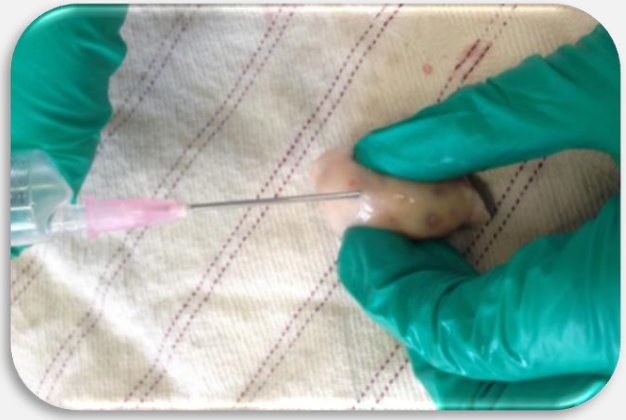


Detection using Immunofluorescent microscopy

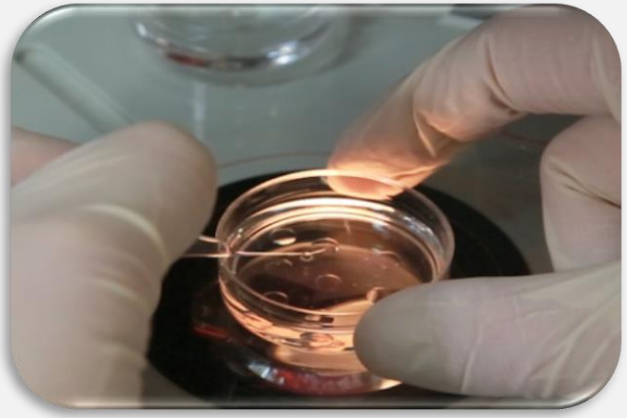
MATERIALS & METHODS

Oocyte maturation

BOVINE



MOUSE



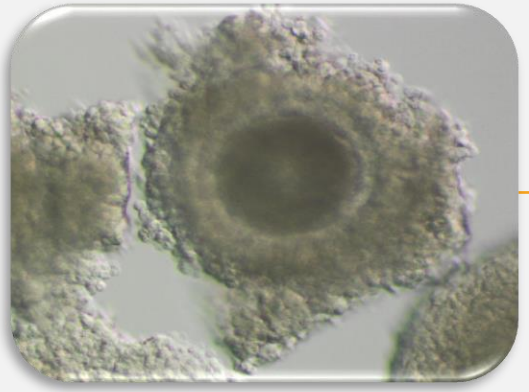
Oocytes of good quality

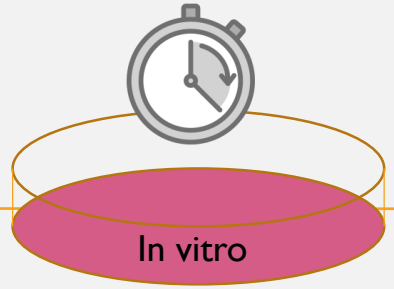
≠

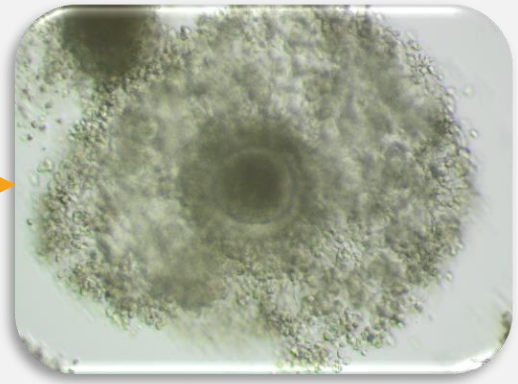
HUMAN

Oocytes of "inferior" quality

IMMATURE OOCYTE





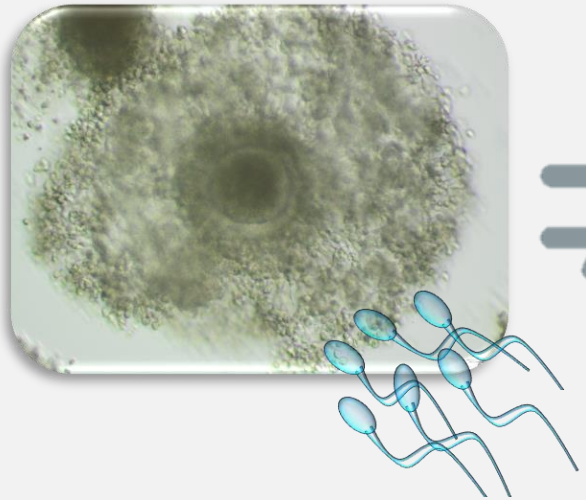


MATURE OOCYTE

MATERIALS & METHODS

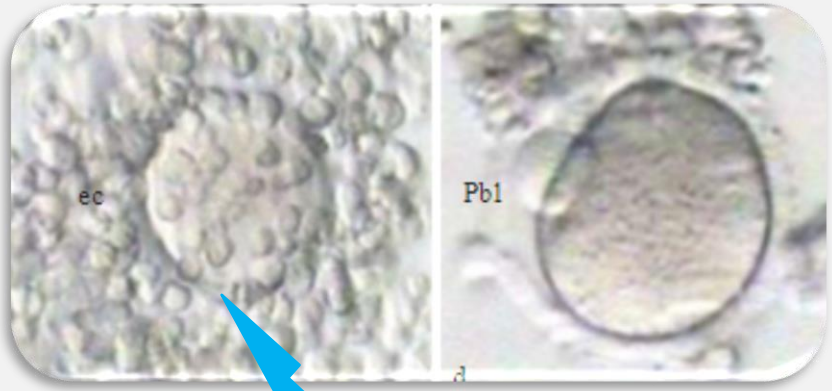
Embryo development

BOVINE



***In vitro* maturation and fertilization**

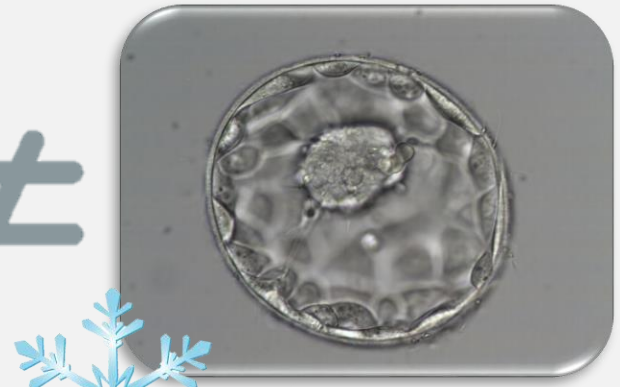
MOUSE



Nurbariah et al., 2011

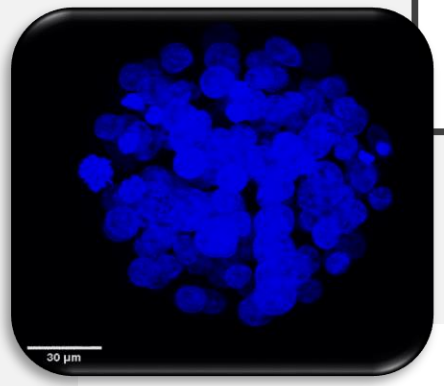
Parthenogenesis
(asexual reproduction)

HUMAN



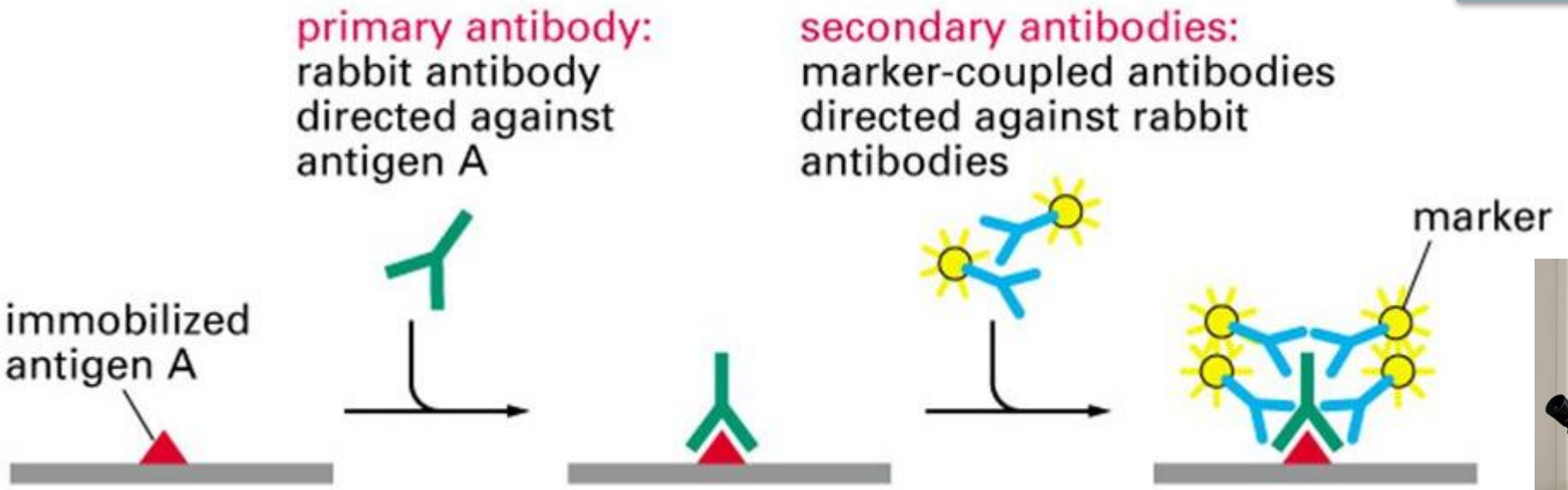
***In vitro* fertilization and cryopreservation**

MATERIALS & METHODS

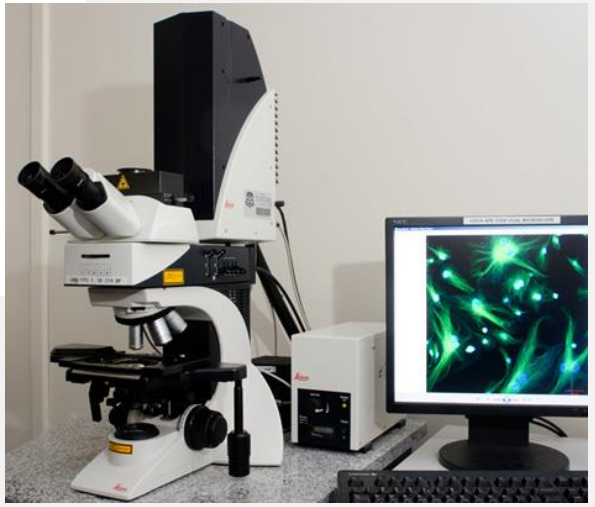


Immunofluorescent staining

Antigen A=
- PAF
- PTAFR
- Three subunits of PAFAH1B



Molecular Biology of the Cell, 4th Edition



RESULTS

PART I: Oocyte maturation

- PAF
- PTAFR – PAFAH1B
- PAFAH1B3 in spindle formation

PART II: Embryo development

- PAF
- PTAFR – PAFAH1B

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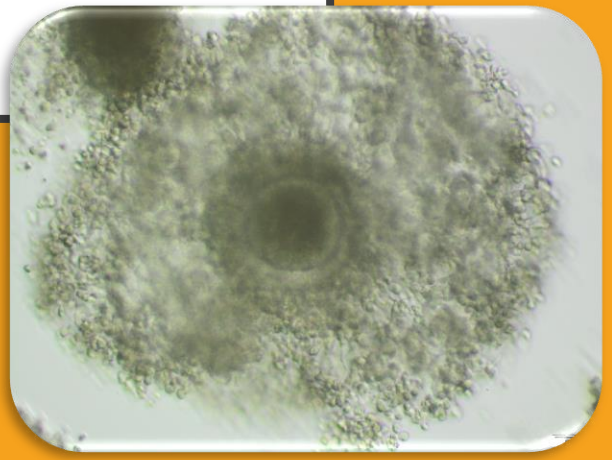
RESULTS – PART I: OOCYTE MATURATION



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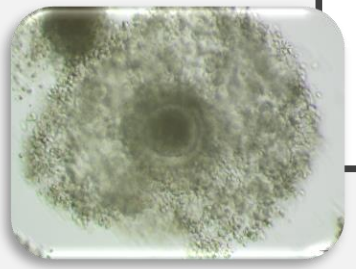
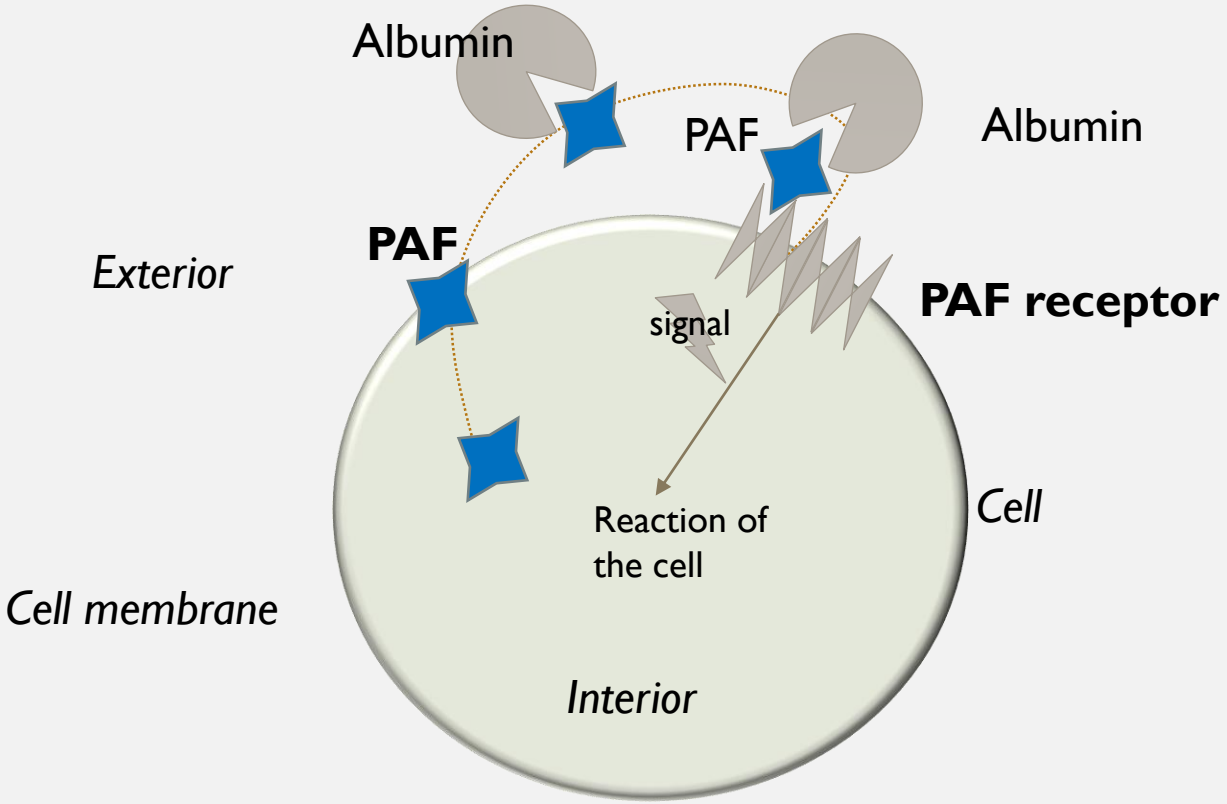


RESULTS

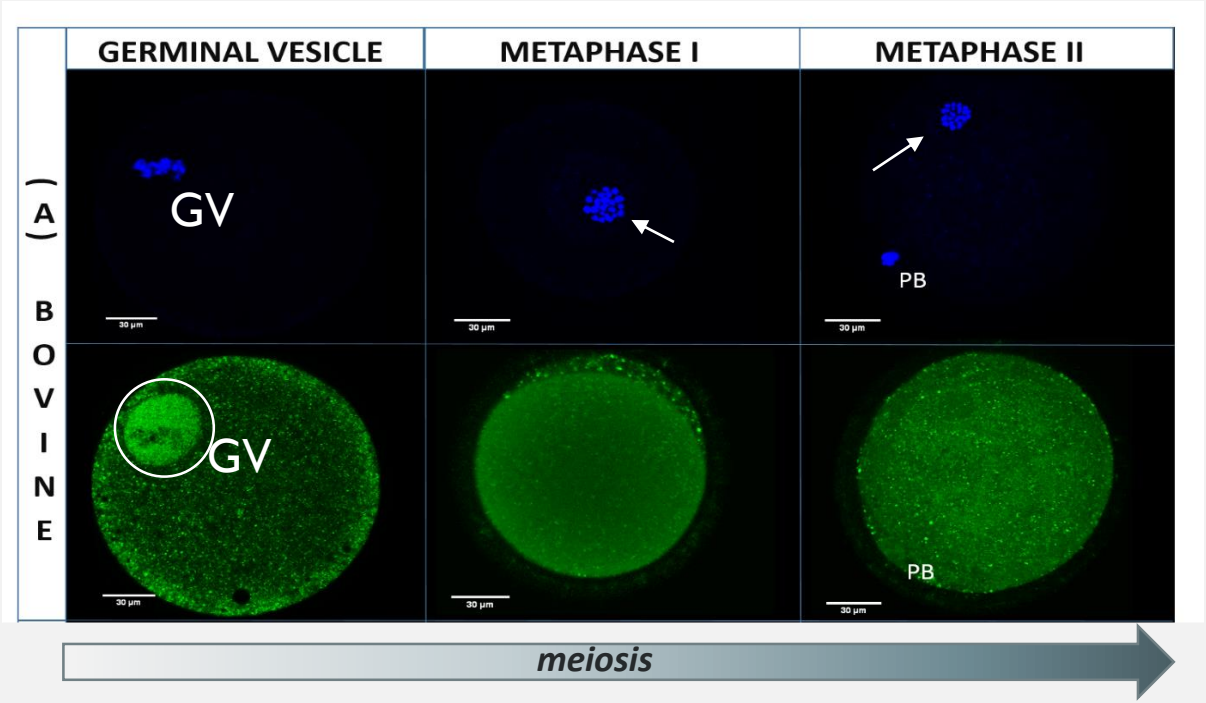
PAF in oocyte maturation



Aim: The **presence** and **localization** of **PAF**

- Suggested to locate on the **cell membrane**
- PAF is necessary for the induction of ovulation

O'Neill, 2005

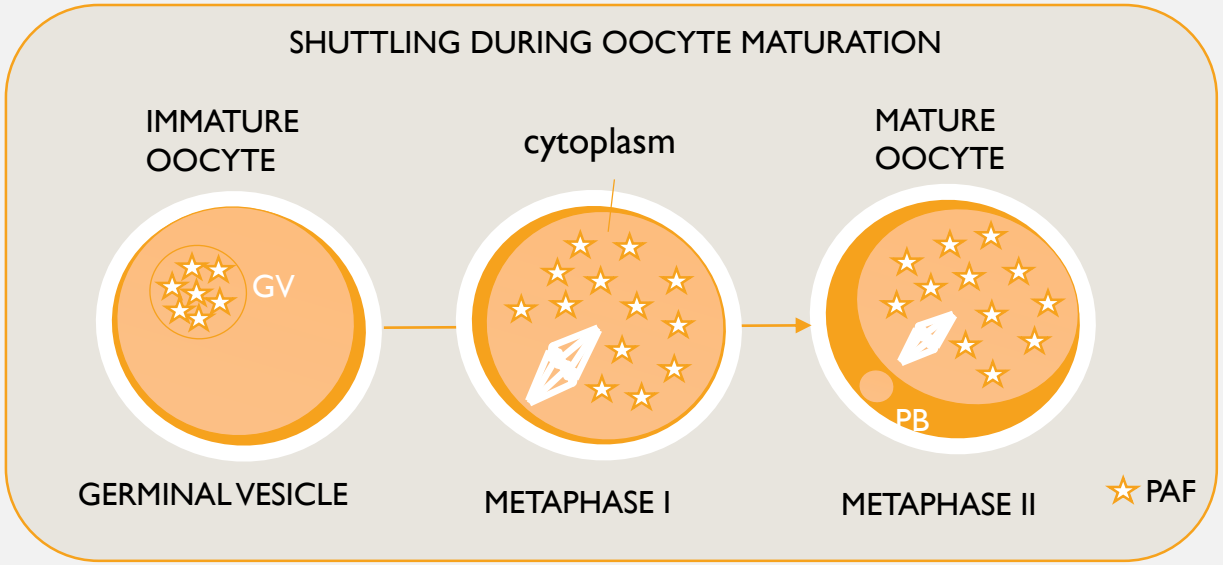


DNA

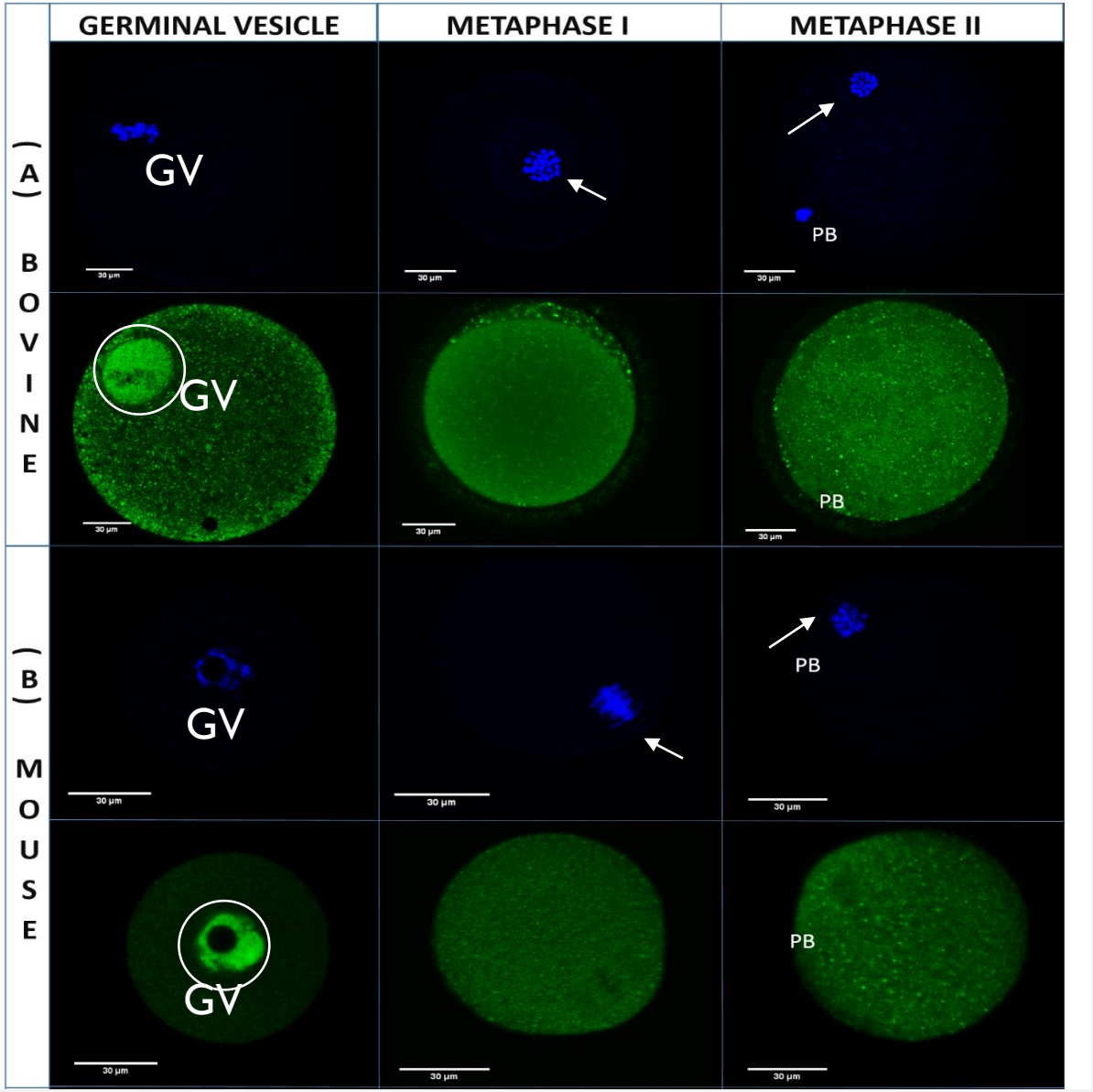
PAF

Spindle

GV = nucleus of the oocyte



1. Accumulation of PAF in the GV
2. Spreading of PAF to the cytoplasm



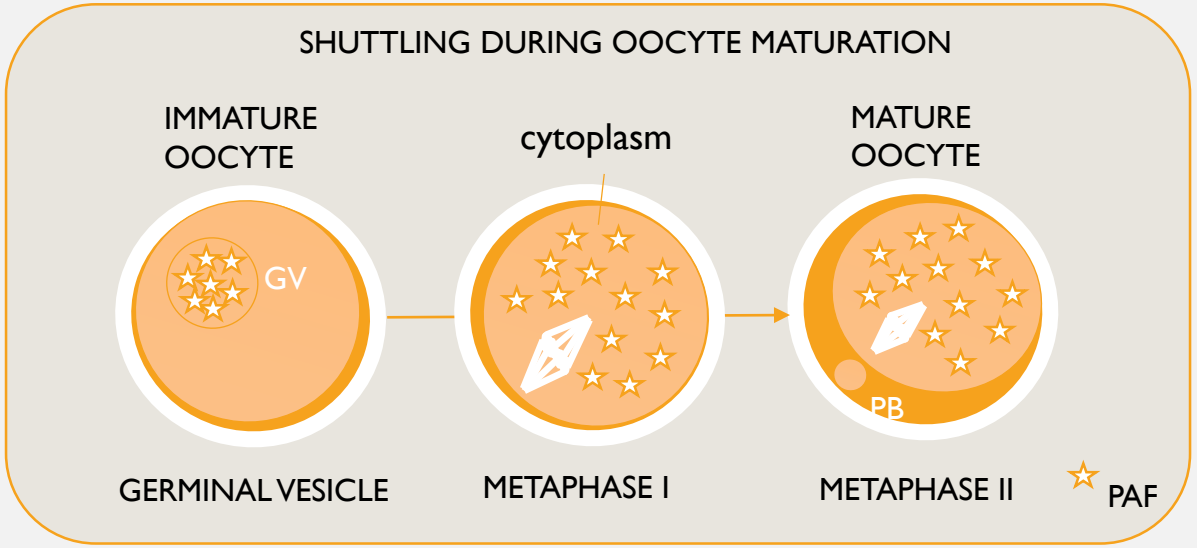
meiosis

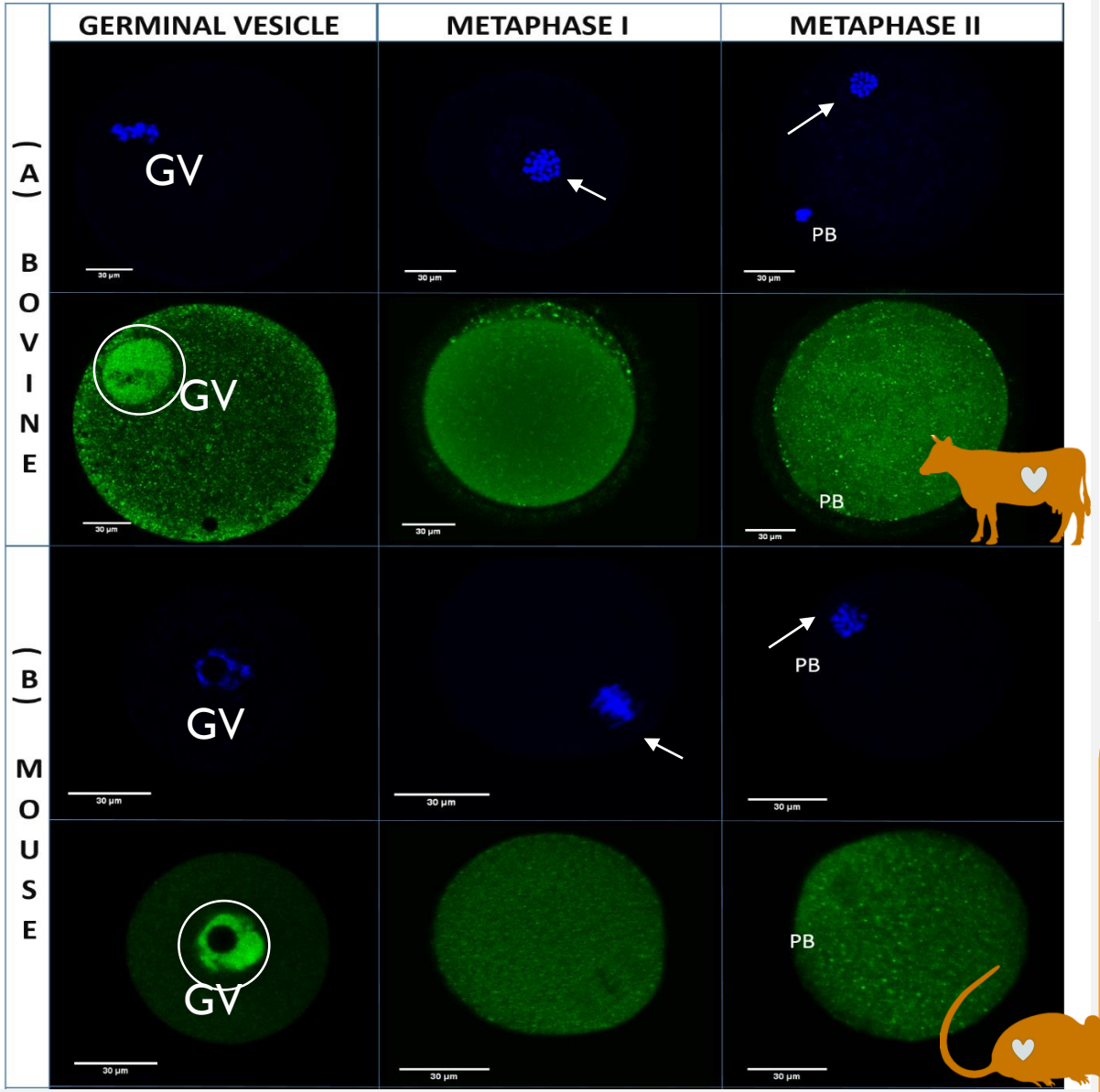
DNA

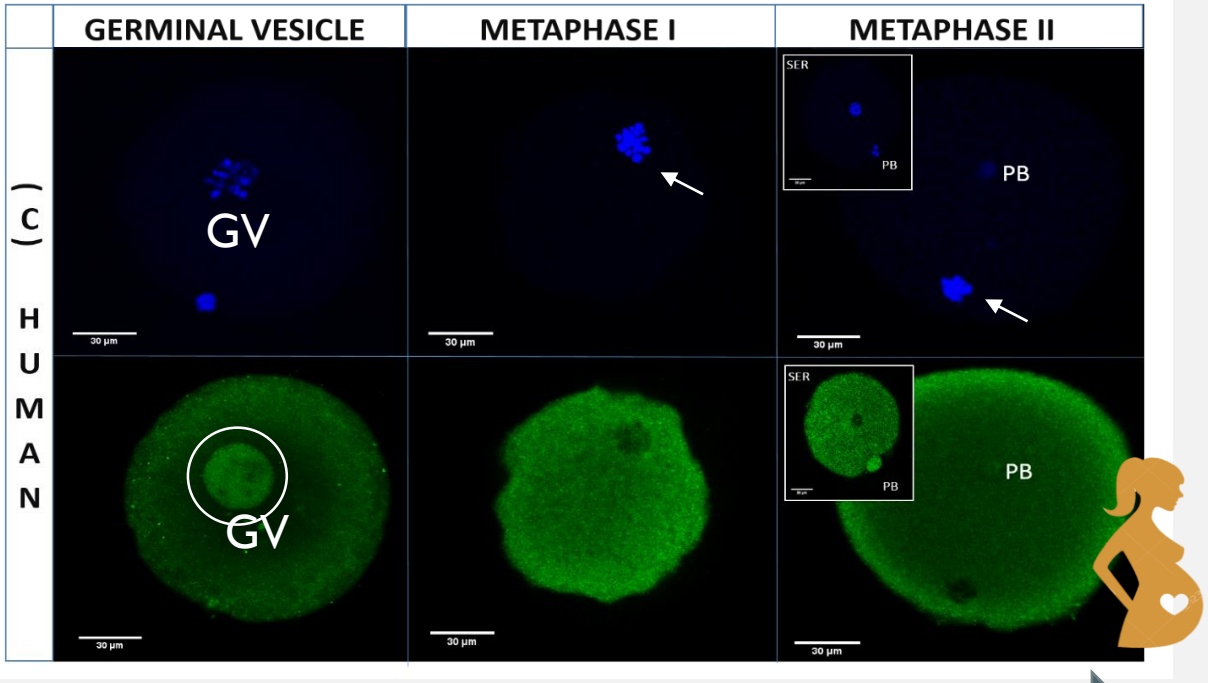
PAF

Spindle

1. Accumulation of PAF in the GV
2. Spreading of PAF to the cytoplasm

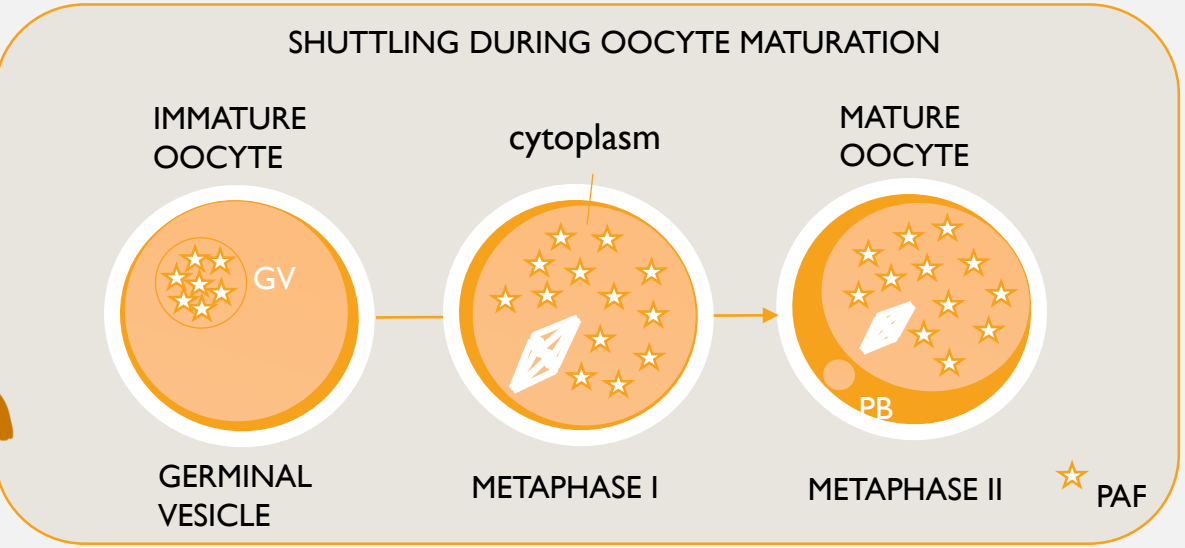






meiosis

meiosis



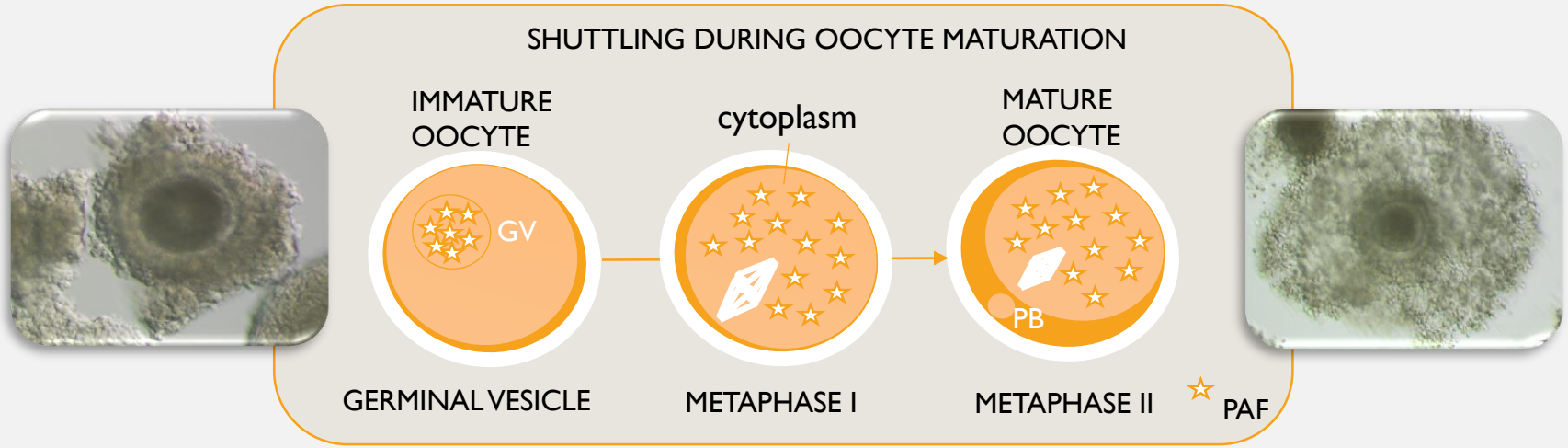
RESULTS

PAF in oocyte maturation

Aim: Investigate the **presence** and **localization** of **PAF**

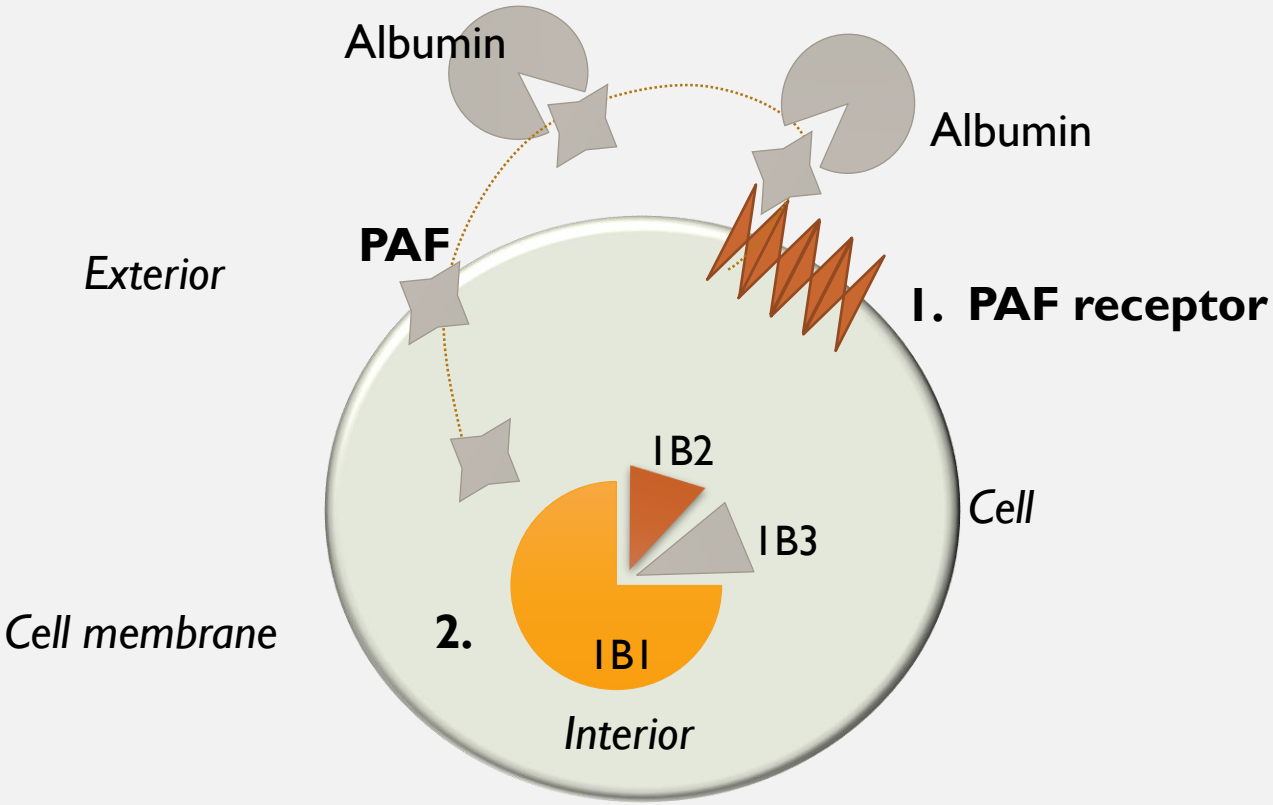
Conclusions

- PAF is not present on the cell membrane but in the **nucleus** (=germinal vesicle)
- PAF relocates to the **cytoplasm** when oocyte maturation (=meiosis) proceeds



RESULTS

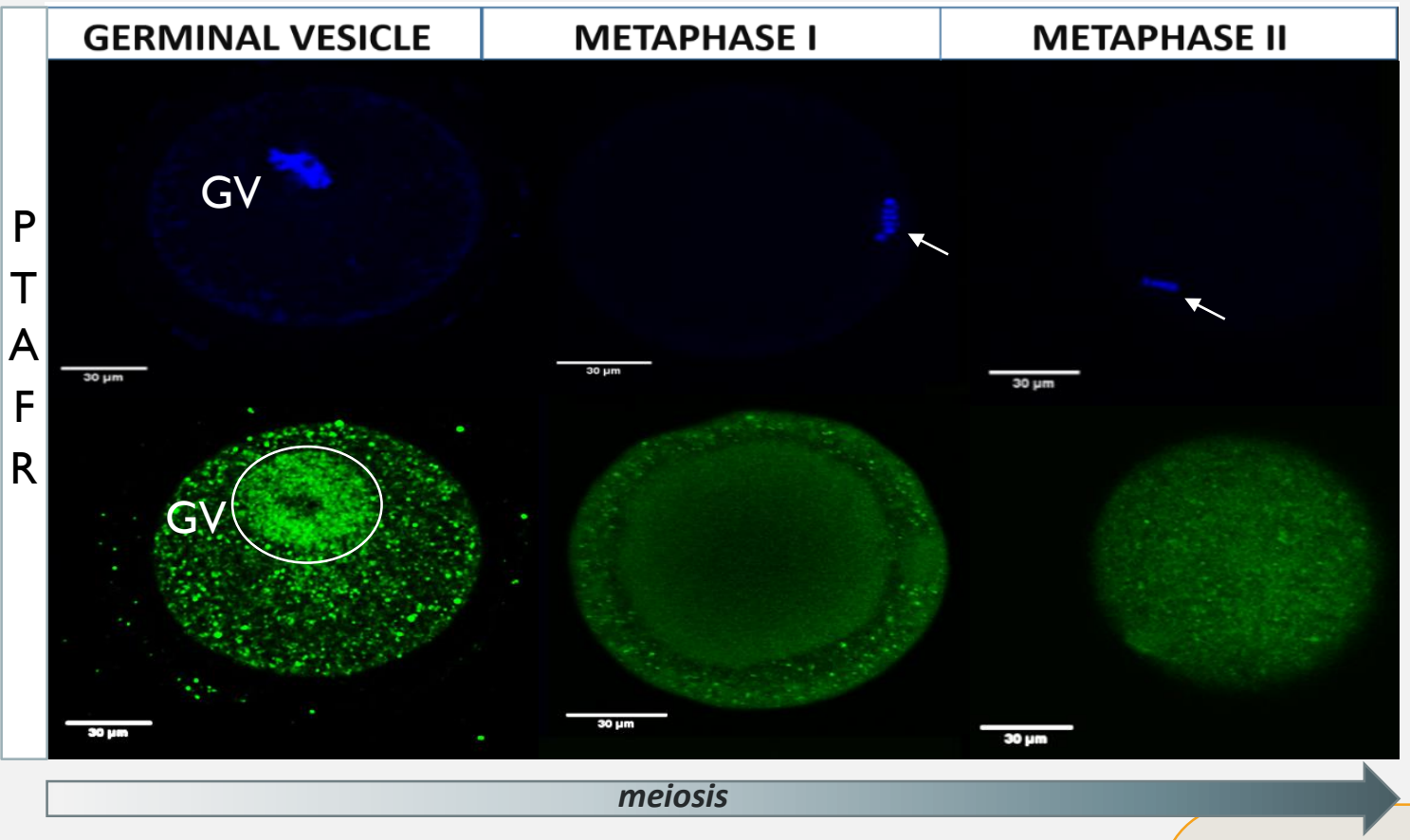
PTAFR & PAFAH1B in oocyte maturation

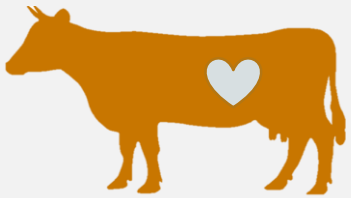


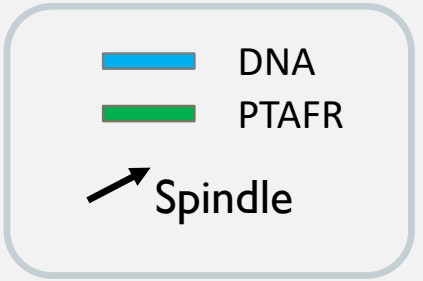
Aim: The **presence** and **localization** of the **PAF receptor (PTAFR)** and the specific intracellular enzyme **PAFAH1B**

1. PTAFR: Suggested to locate on the **cell membrane**
2. PAFAH1B: located **intracellularly** in mouse oocytes

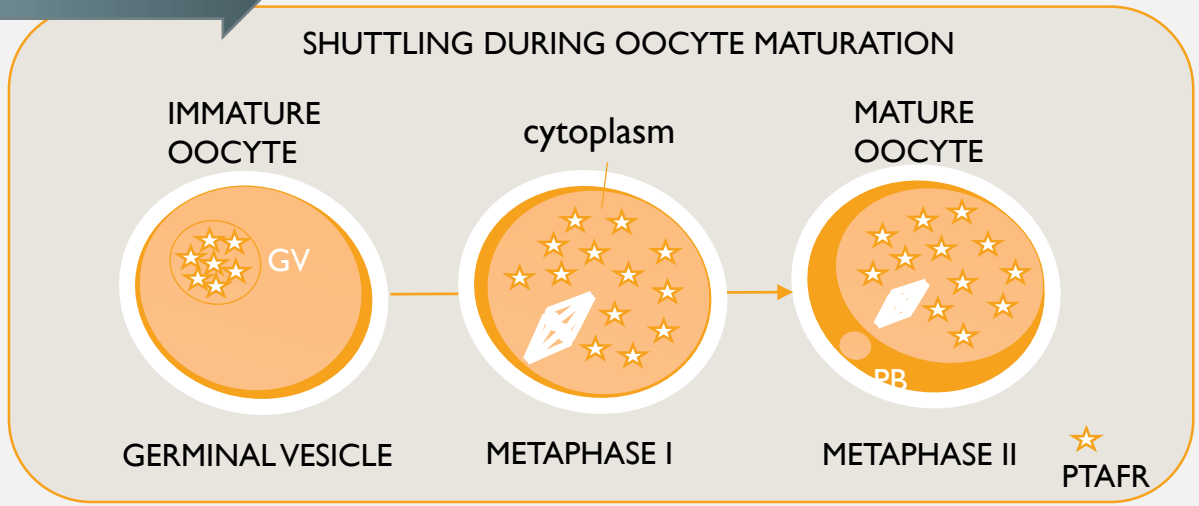
O'Neill, 2005; Yao et al., 2015

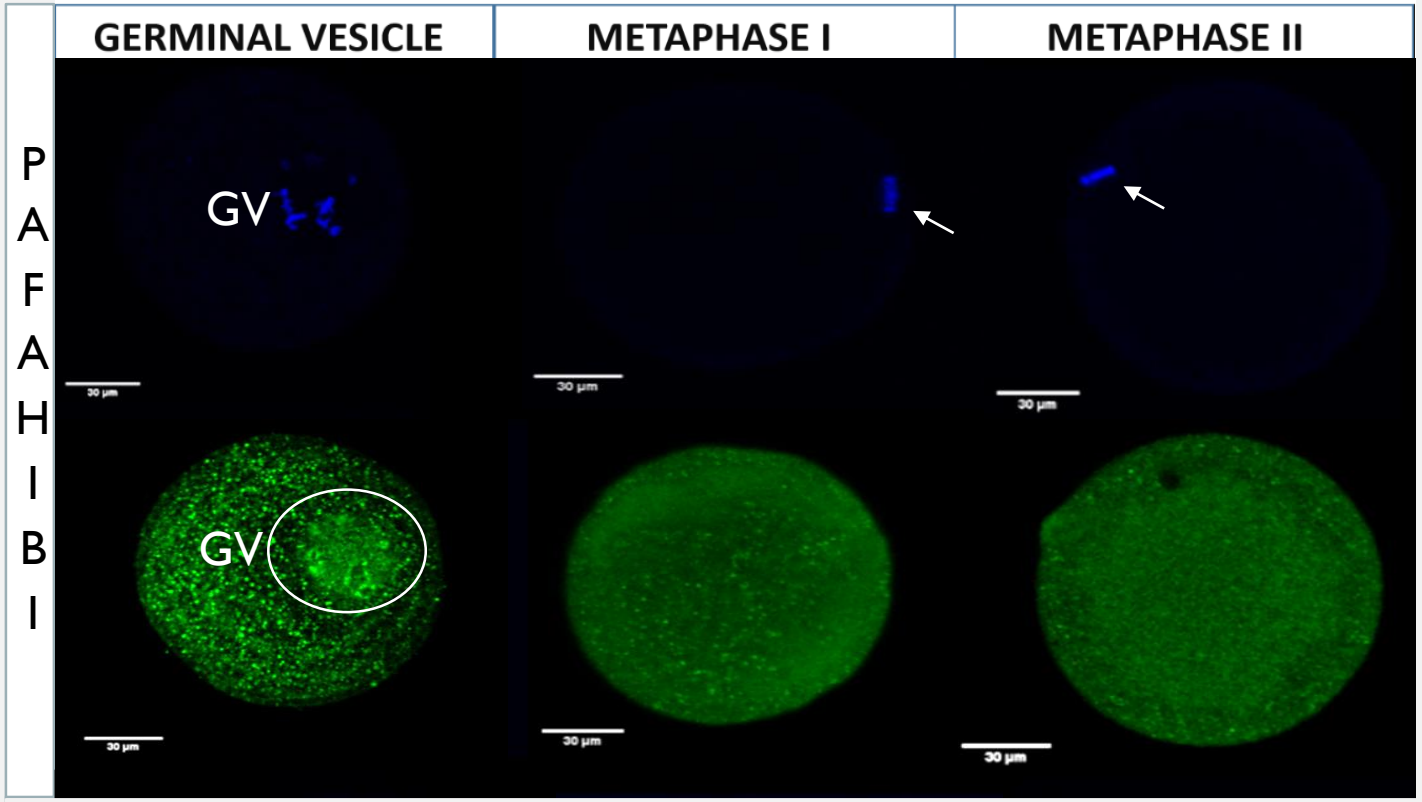


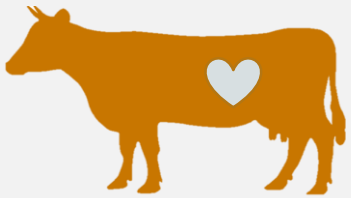


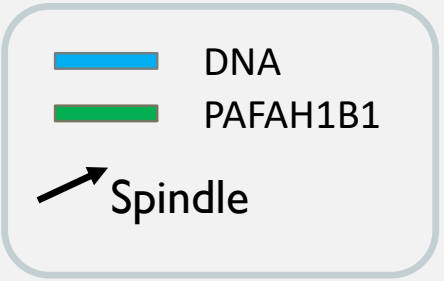


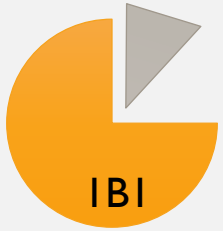
1. Accumulation of PTAFR in the GV
2. Spreading of PTAFR to the cytoplasm



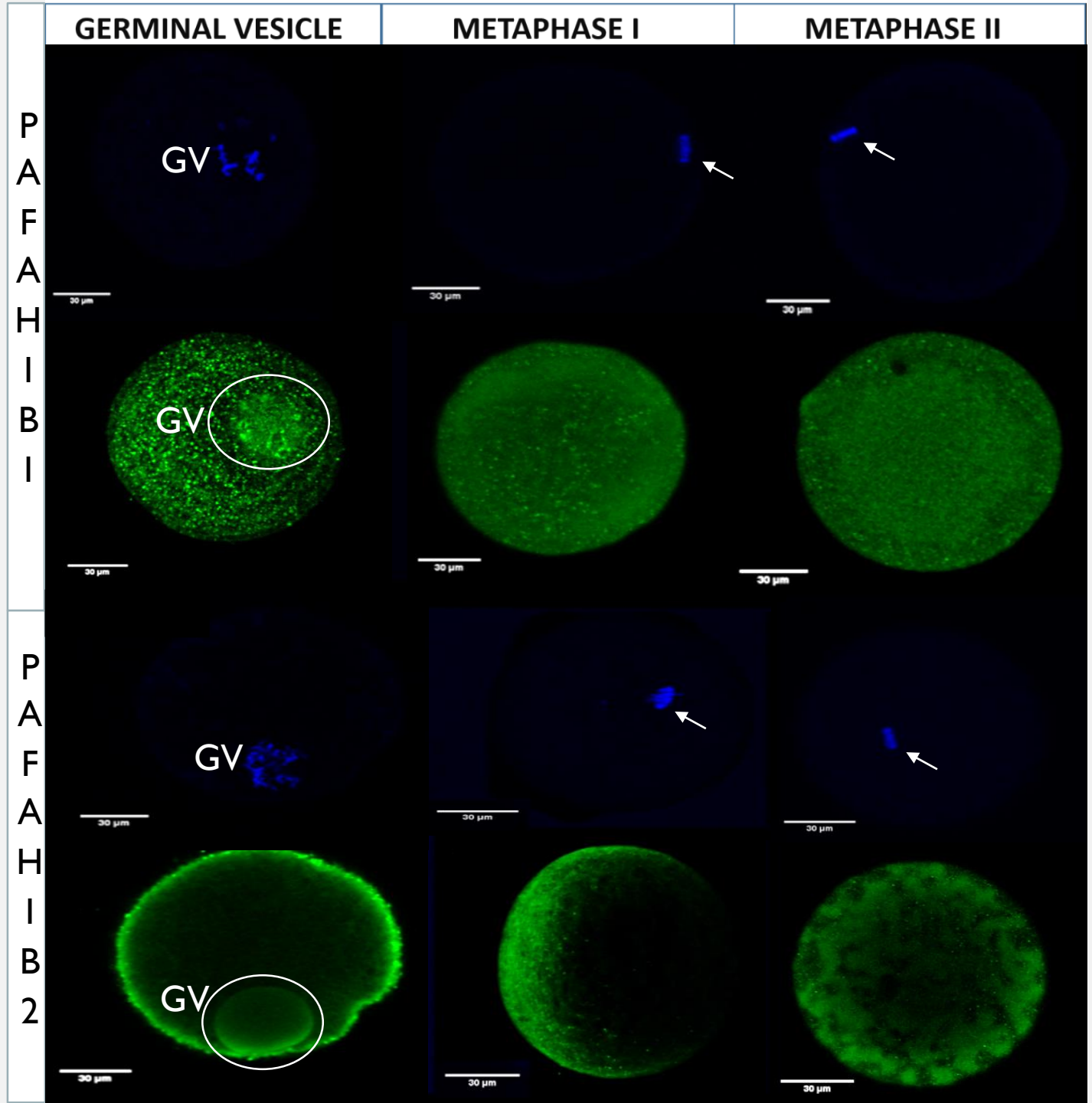


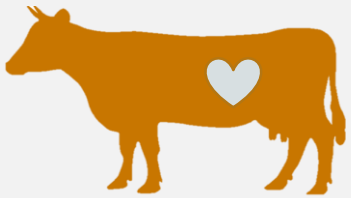




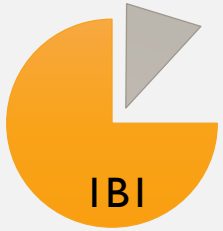


1. Accumulation of PAFAH1B in the GV
2. Spreading of PAFAH1B to the cytoplasm

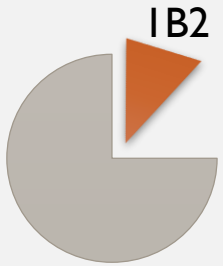




DNA
PAFAH1B1/2
Spindle



1. Accumulation of PAFAH1B in the GV
2. Spreading of PAFAH1B to the cytoplasm



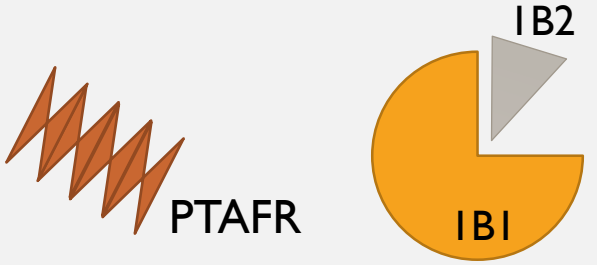
RESULTS

PTAFR in oocyte maturation

Aim: The **presence** and **localization** of the **PAF receptor (PTAFR)** and the specific intracellular enzyme **PAFAH1B**

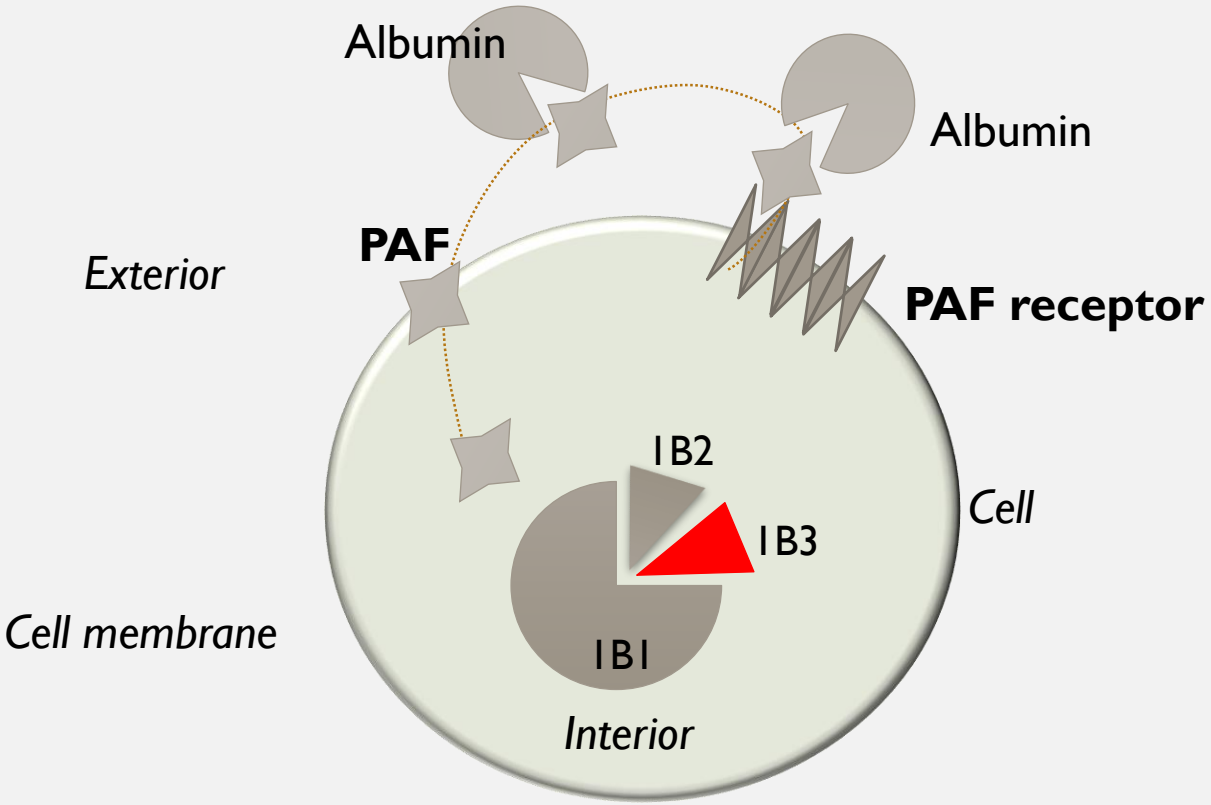
Conclusions

- PTAFR and PAFAH1B1/2 are not present on the cell membrane but in the **nucleus (germinal vesicle)**
- PTAFR and PAFAH1B1 and 1B2 relocate to the **cytoplasm** when maturation (=meiosis) proceeds
- Same cellular dynamics as for PAF



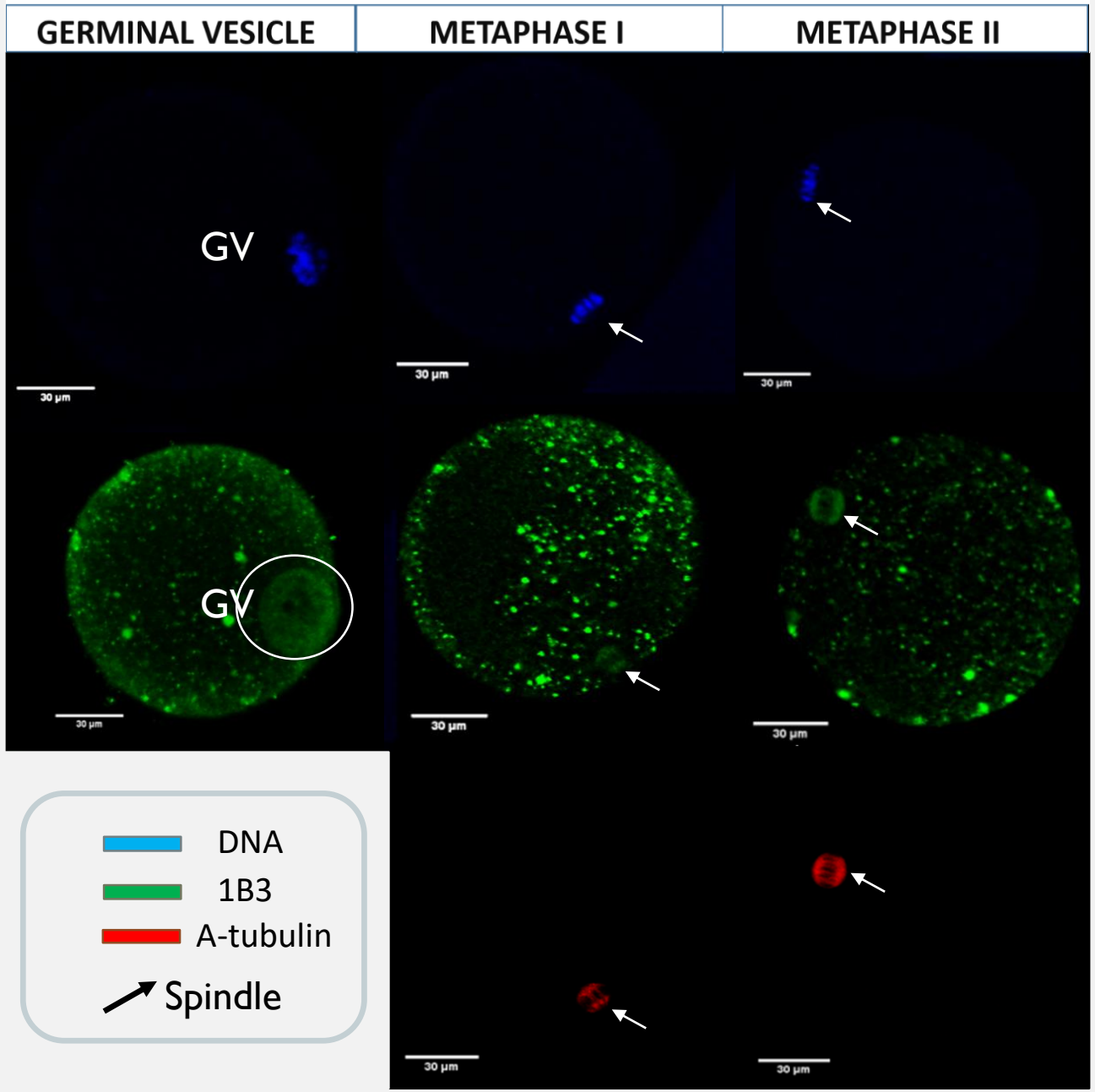
RESULTS

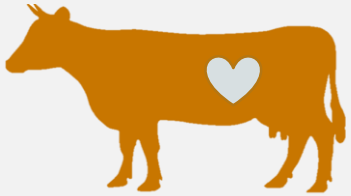
PAFAH1B3 in oocyte maturation



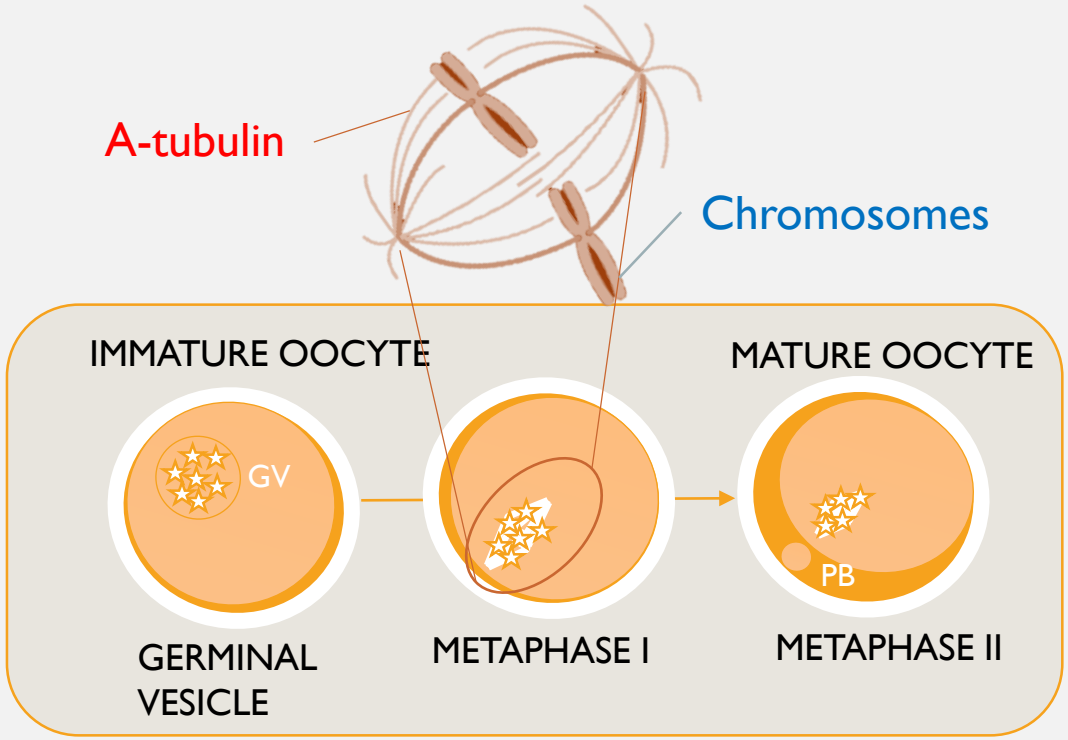
Aim: Evaluate the functional role of the catalytic subunit **PAFAH1B3** in **spindle formation** and **meiotic progression**.

- No literature on PAFAH1B3 in oocytes



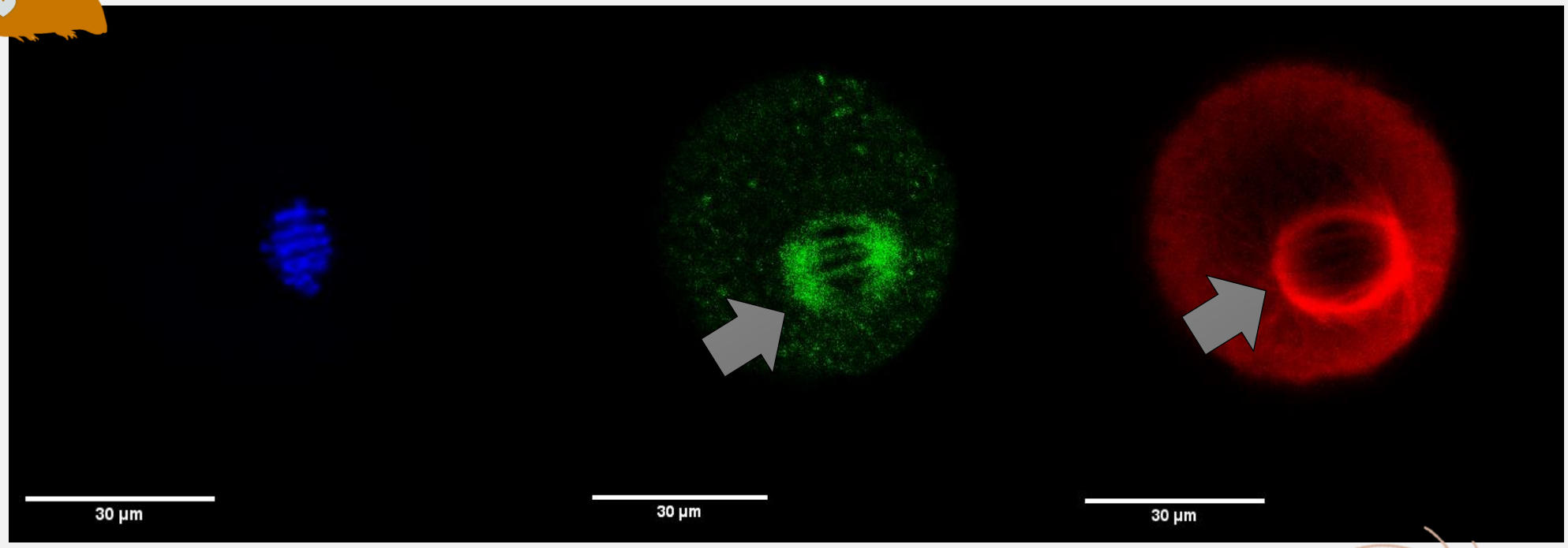


PAFAH1B3 does not relocate to the cytoplasm during oocyte maturation but associates with the **meiotic spindle**

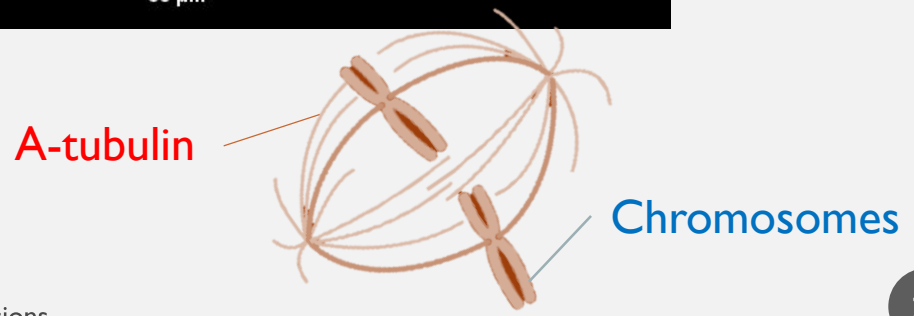


RESULTS

PAFAH1B3 in oocyte maturation

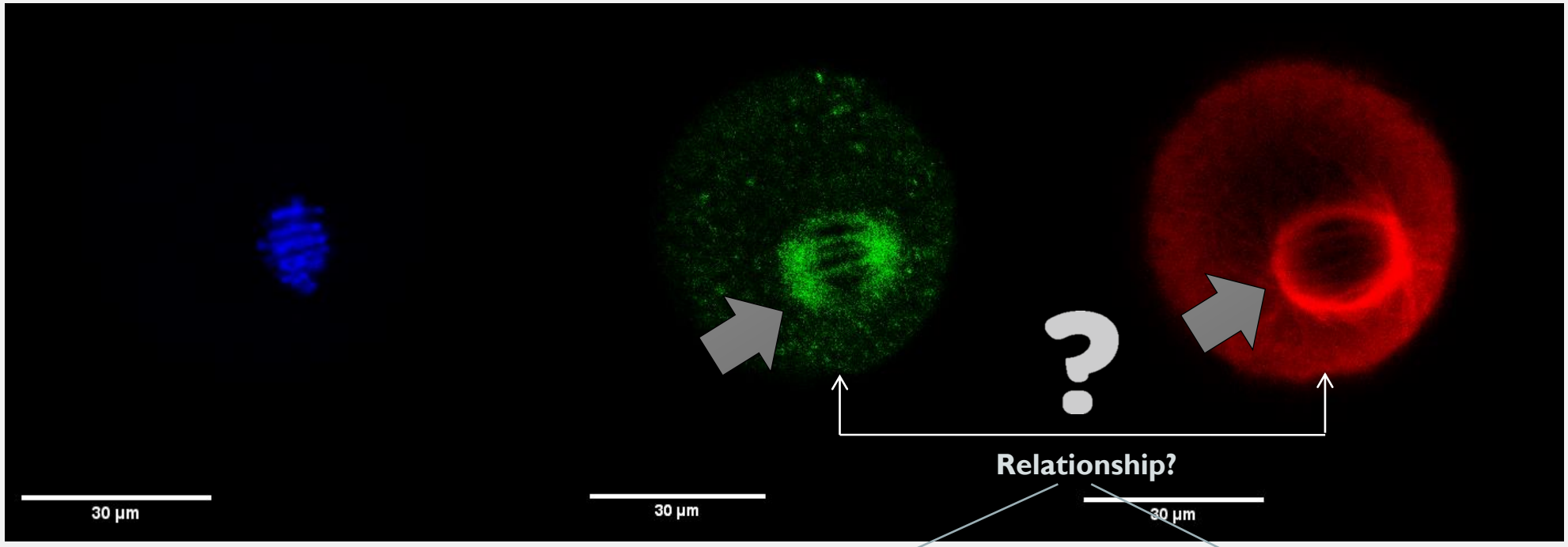


DNA
1B3
A-tubulin



RESULTS

PAFAH1B3 in oocyte maturation



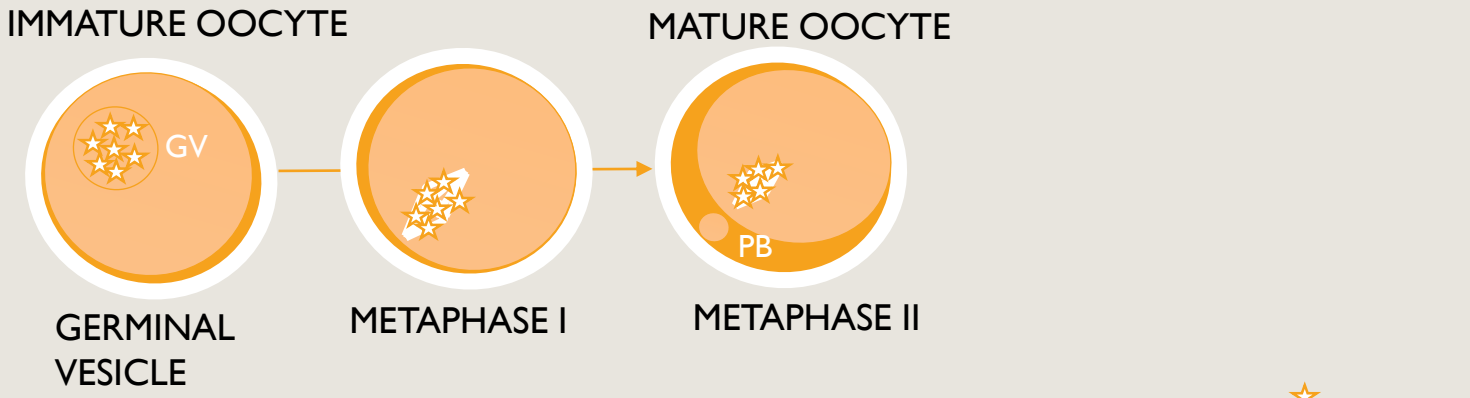
B. Disruption of the **meiotic spindle**

C. Disruption of **PAFAH1B3**

DNA
1B3
A-tubulin

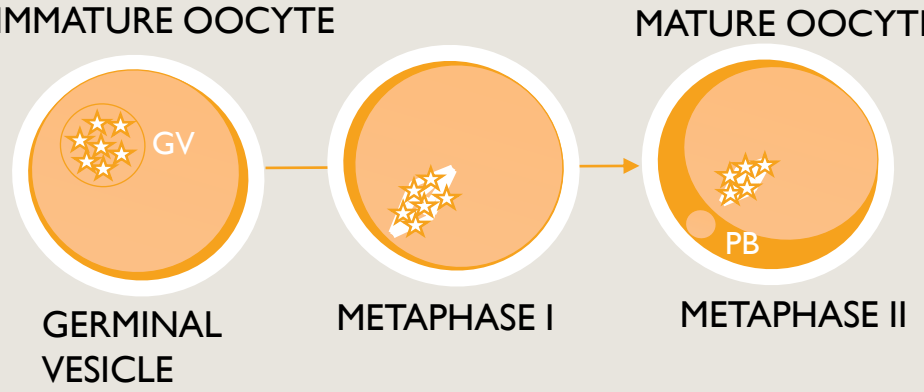
THE RELATIONSHIP BETWEEN PAFAH1B3 AND THE MEIOTIC SPINDLE

A. NORMAL



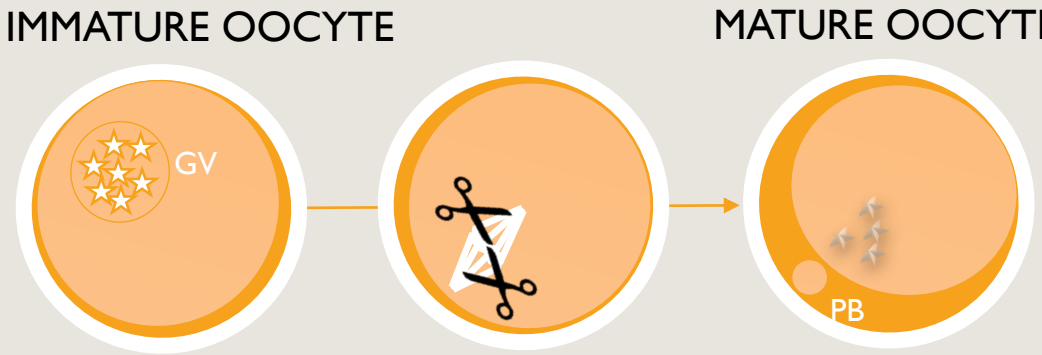
THE RELATIONSHIP BETWEEN PAFAH1B3 AND THE MEIOTIC SPINDLE

A. NORMAL



B. DISRUPTION OF THE <u>MEIOTIC SPINDLE</u>

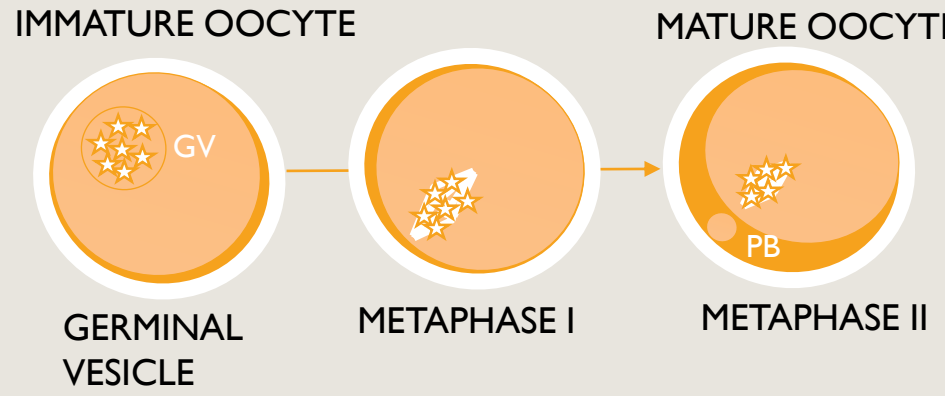
B.1 Nocodazole



☆ PAFAH1B3

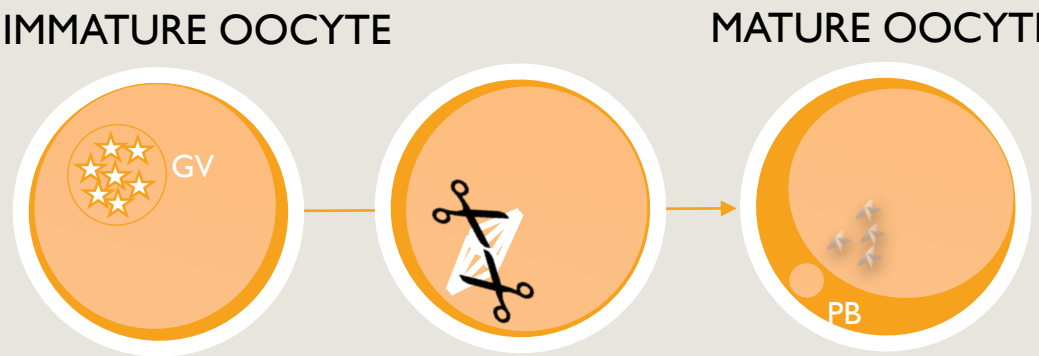
THE RELATIONSHIP BETWEEN PAFAH1B3 AND THE MEIOTIC SPINDLE

A. NORMAL



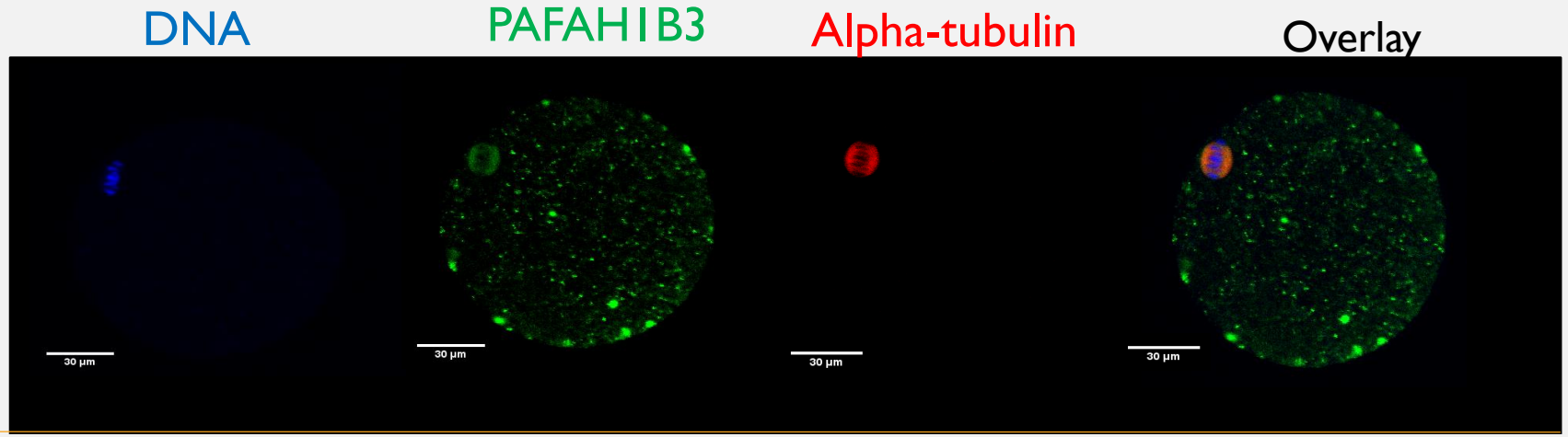
B. DISRUPTION OF THE <u>MEIOTIC SPINDLE</u>

B.1 Nocodazole



B.2 Taxol

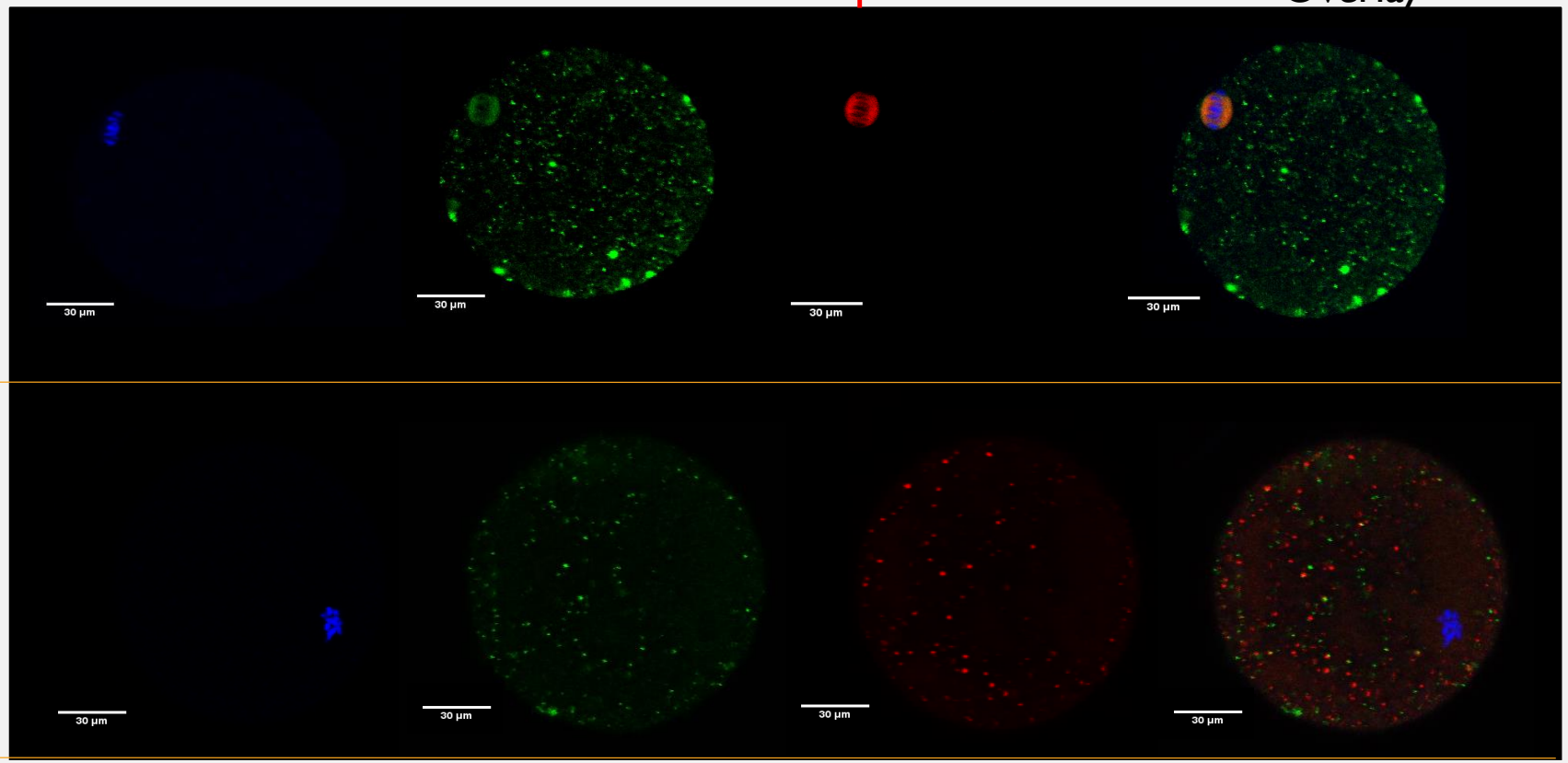
A. Normal

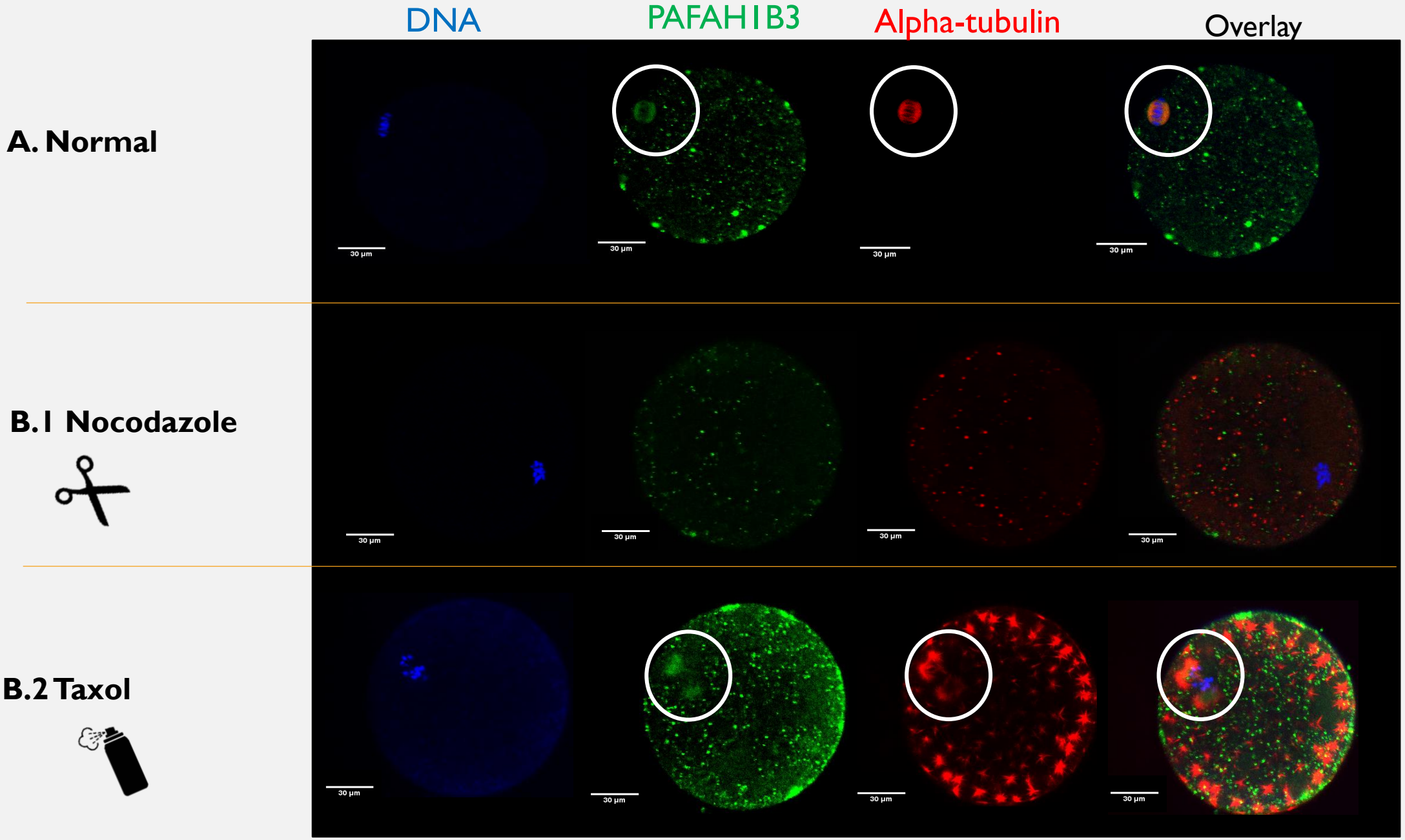


DNA PAFAH1B3 Alpha-tubulin Overlay

A. Normal

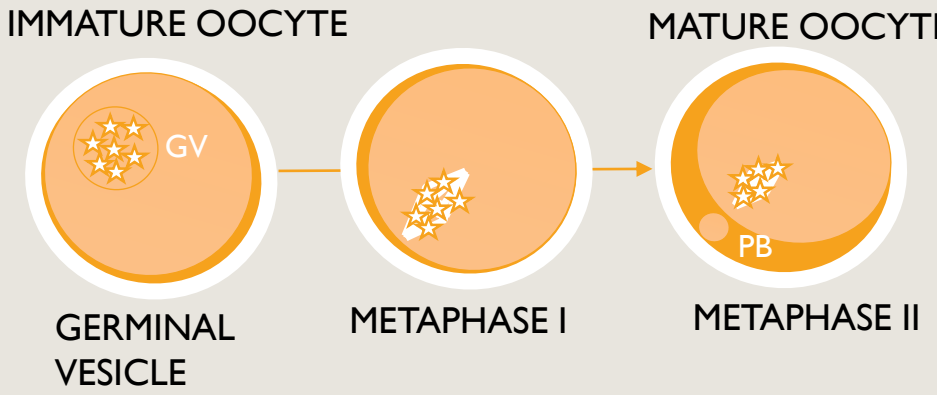
B.1 Nocodazole




DNA
PAFAH1B3
Alpha-tubulin
Overlay
A. Normal
B.1 Nocodazole
B.2 Taxol
30 µm

THE RELATIONSHIP BETWEEN PAFAH1B3 AND THE MEIOTIC SPINDLE

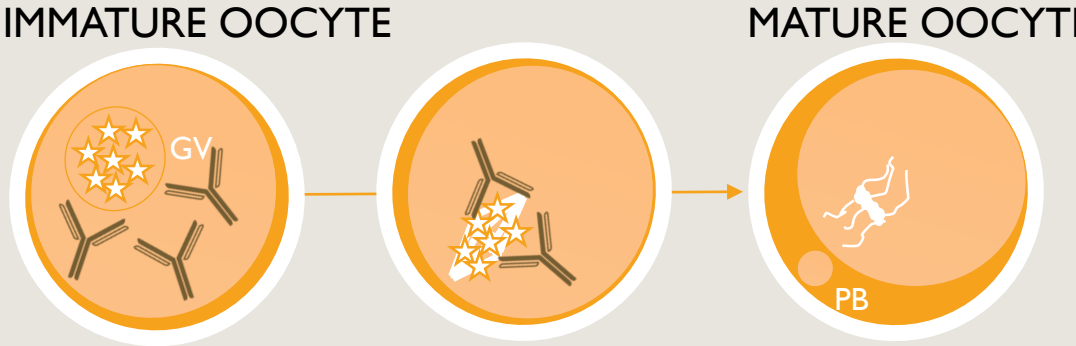
A. NORMAL

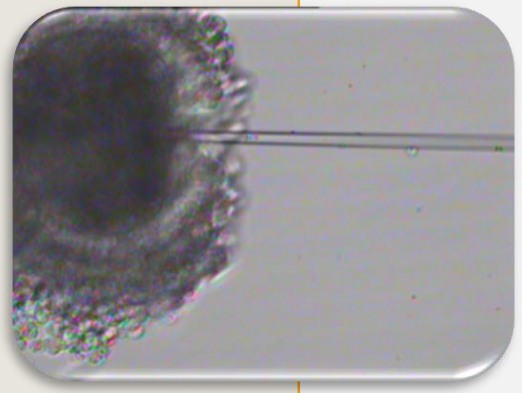


☆ PAFAH1B3

C. DISRUPTION OF <u>PAFAH1B3</u>

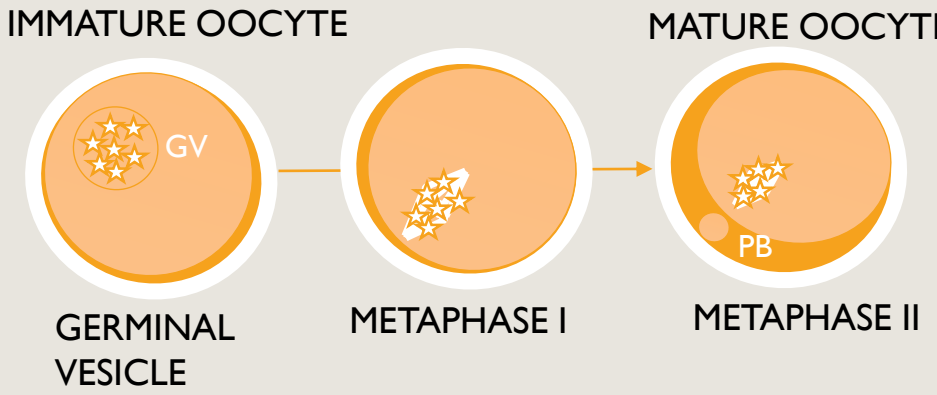
C.1 PAFAH1B3 Antibody





THE RELATIONSHIP BETWEEN PAFAH1B3 AND THE MEIOTIC SPINDLE

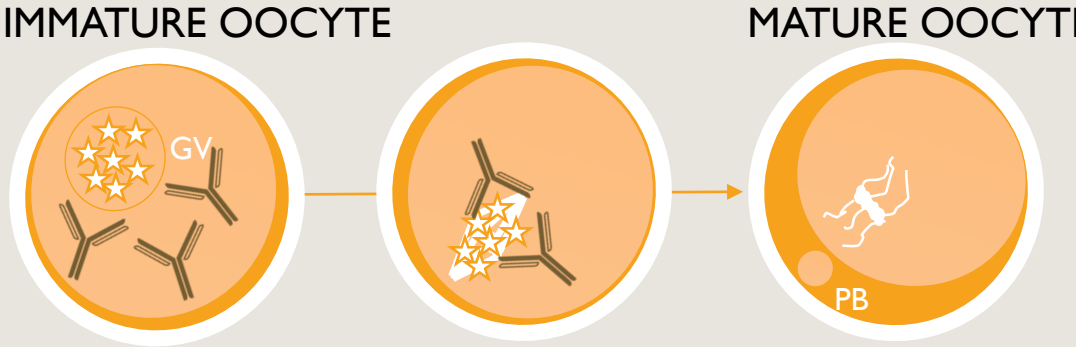
A. NORMAL

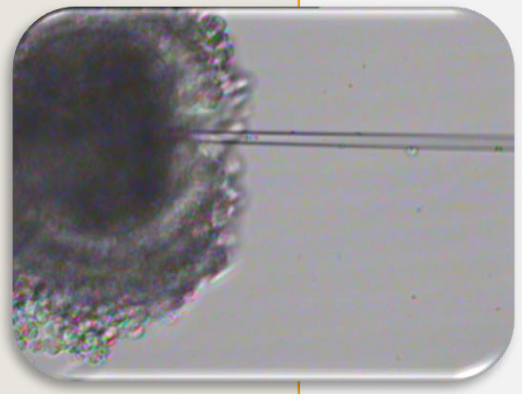


☆ PAFAH1B3

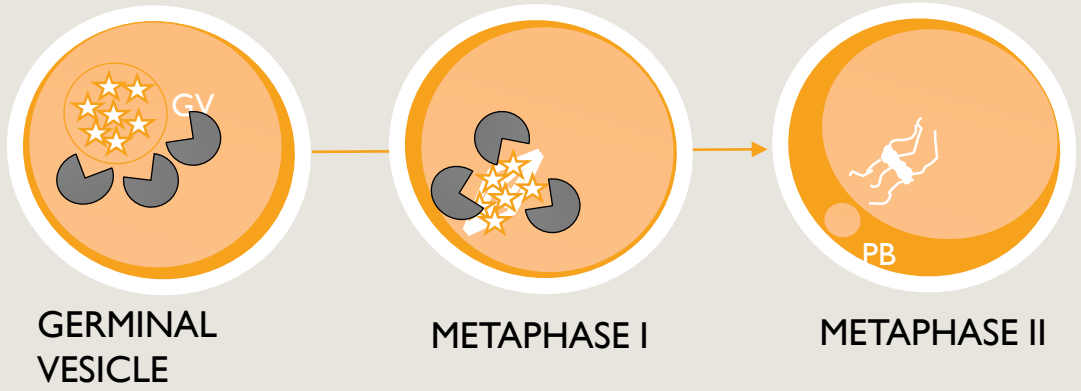
C. DISRUPTION OF PAFAH1B3

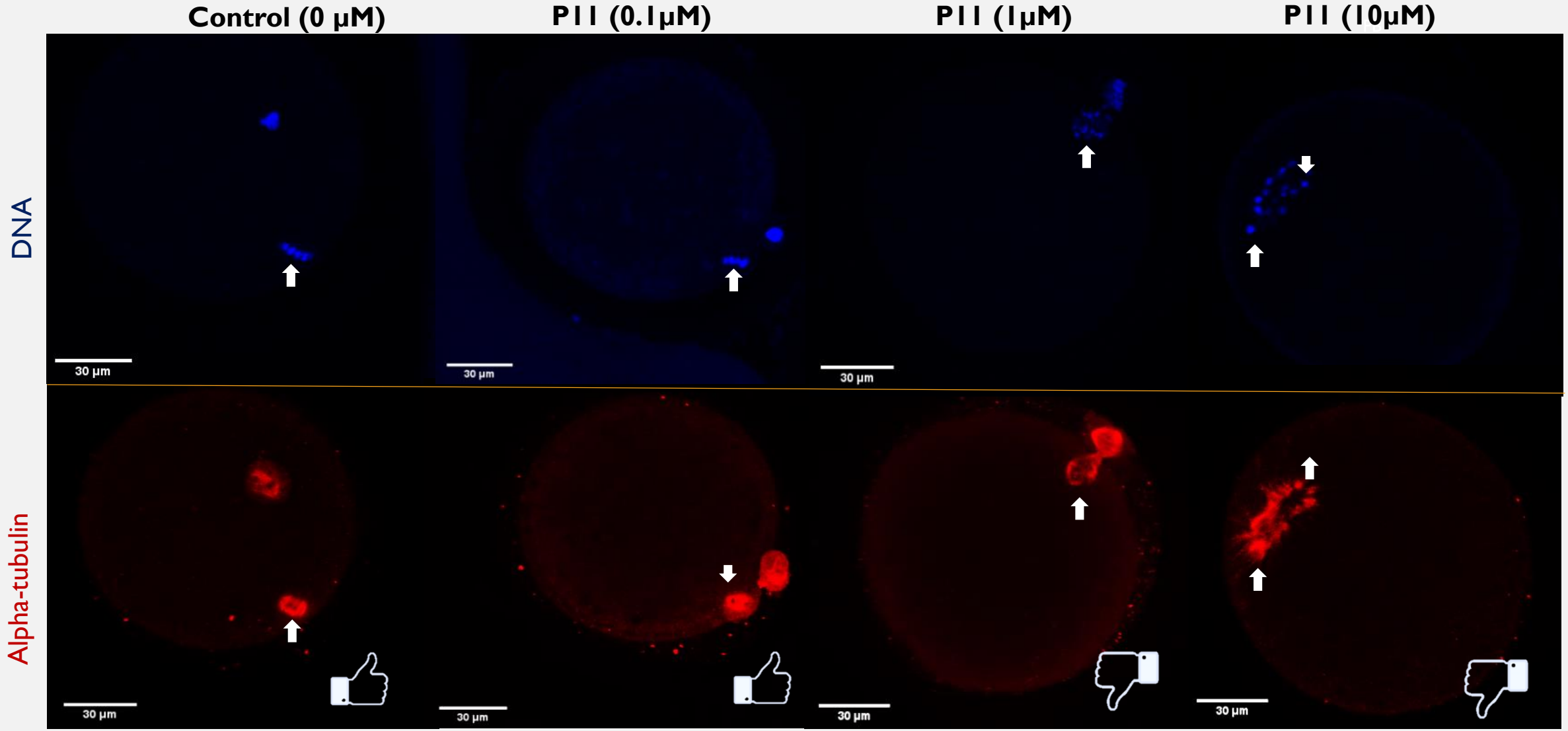
C.1 PAFAH1B3 Antibody





C.2 PI1

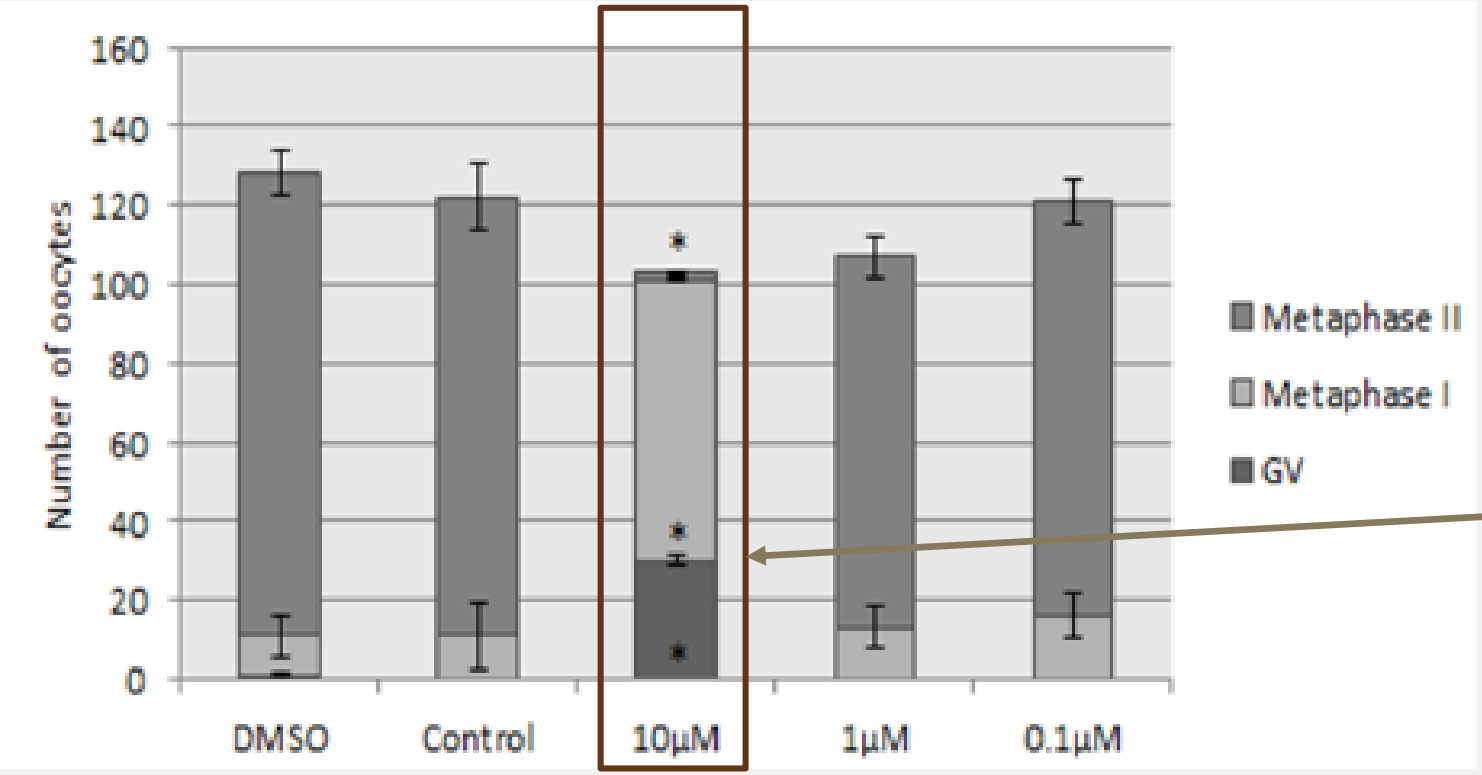



Control (0 μM)
P11 (0.1μM)
P11 (1μM)
P11 (10μM)
DNA
Alpha-tubulin
30 μm
30 μm
30 μm
30 μm
30 μm
30 μm
30 μm
30 μm
Concentration of P11
Disruption of the spindle

RESULTS

PAFAH1B3 in oocyte maturation

Aim: Evaluate the functional role of the catalytic subunit **PAFAH1B3** in **spindle formation** and **meiotic progression**.

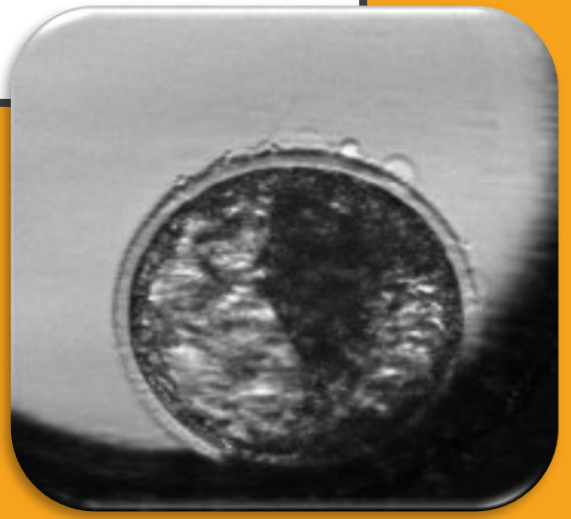


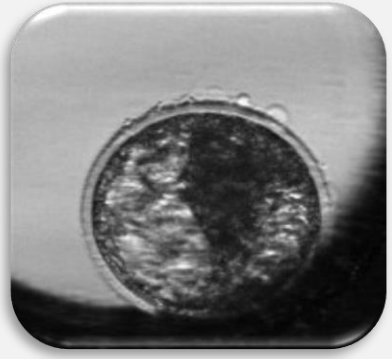
Conclusions

PAFAH1B3 is involved in

- spindle formation
- meiotic progression

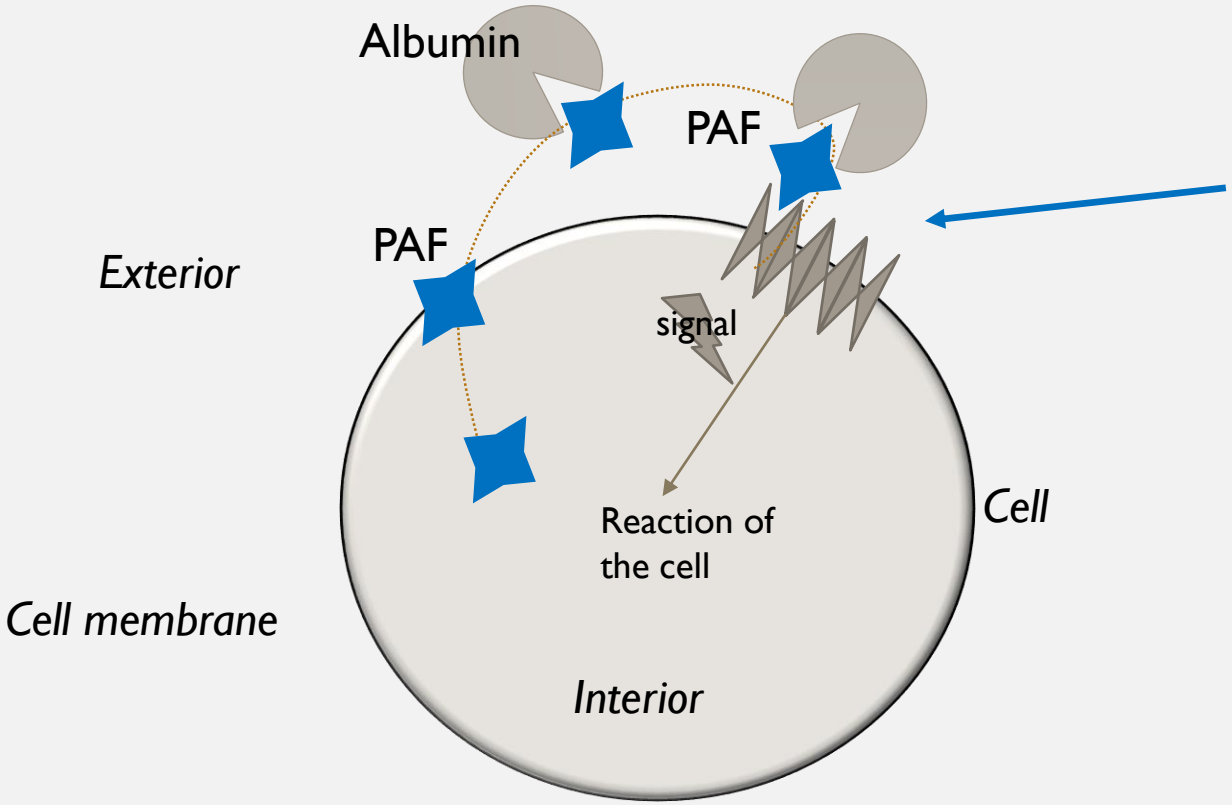
RESULTS – PART II: EMBRYO DEVELOPMENT





RESULTS

PAF in embryo development

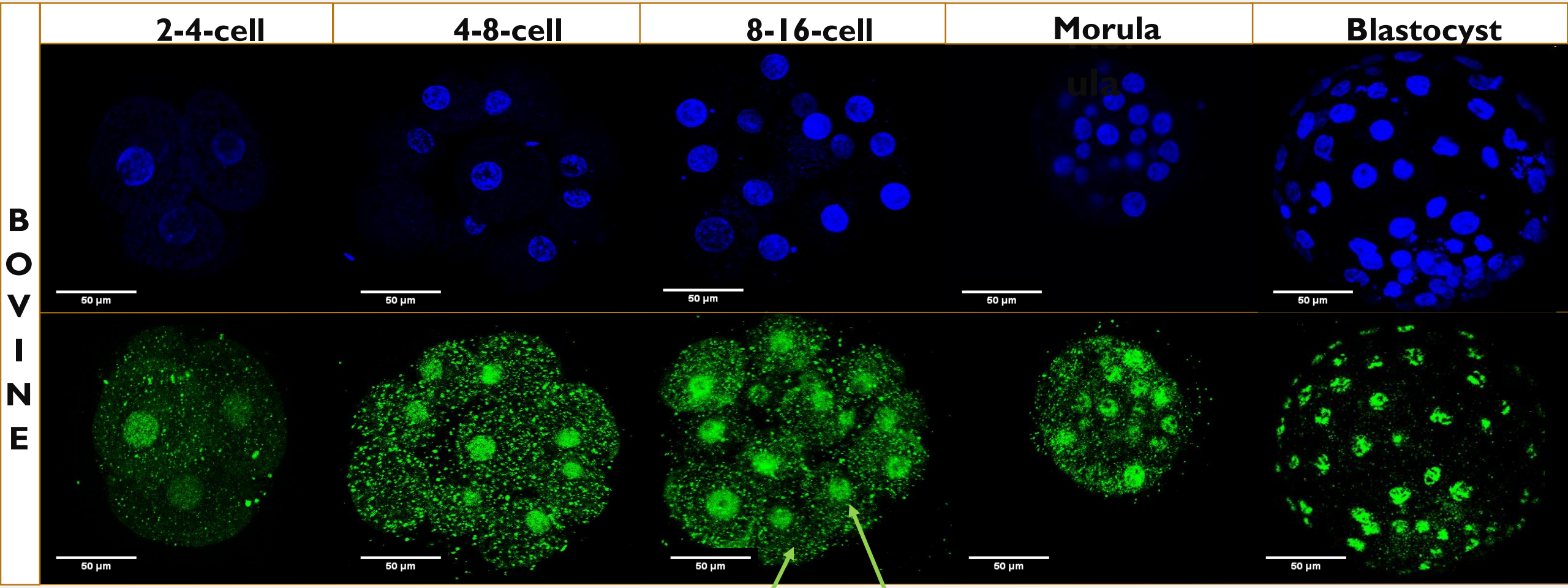


Aim: Evaluate the **presence** and **localization** of **PAF**

- Suggested to locate on the **cell membrane**
- PAF is necessary for embryo survival, quality, vitality and implantation potential

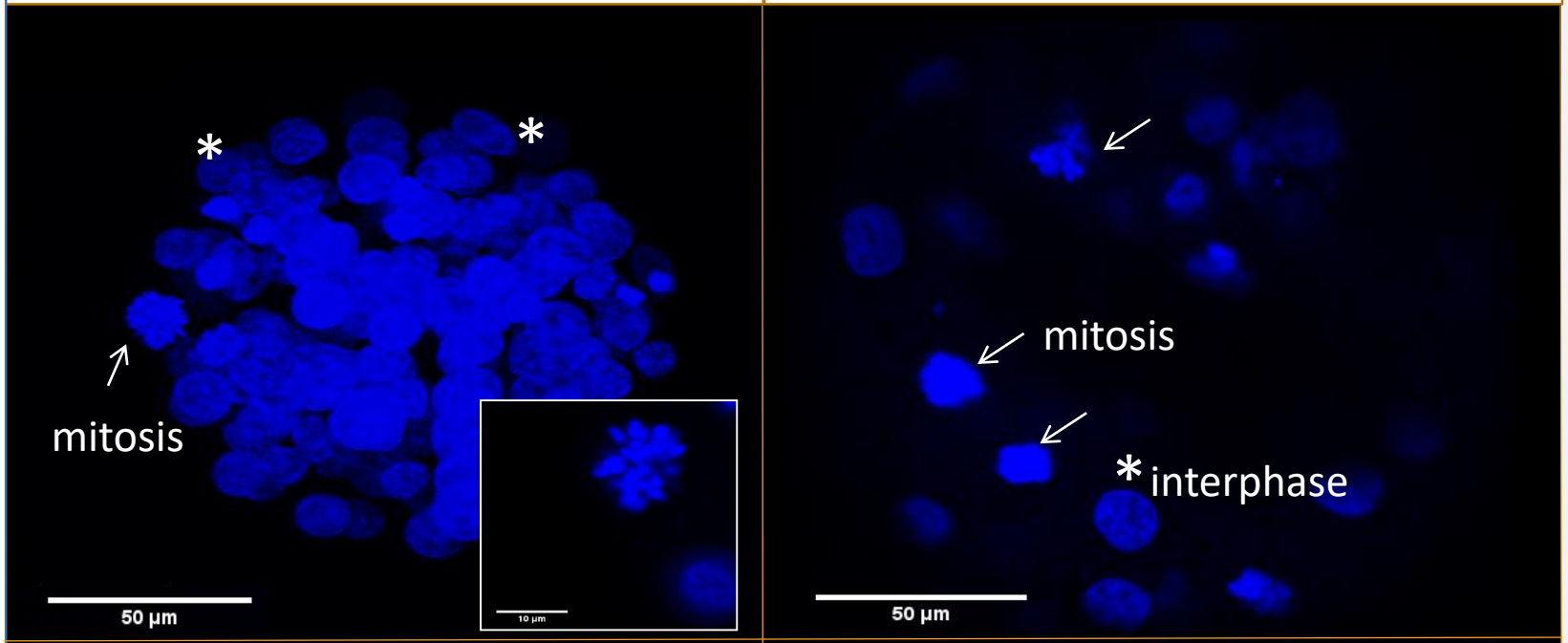
O'Neill, 2005

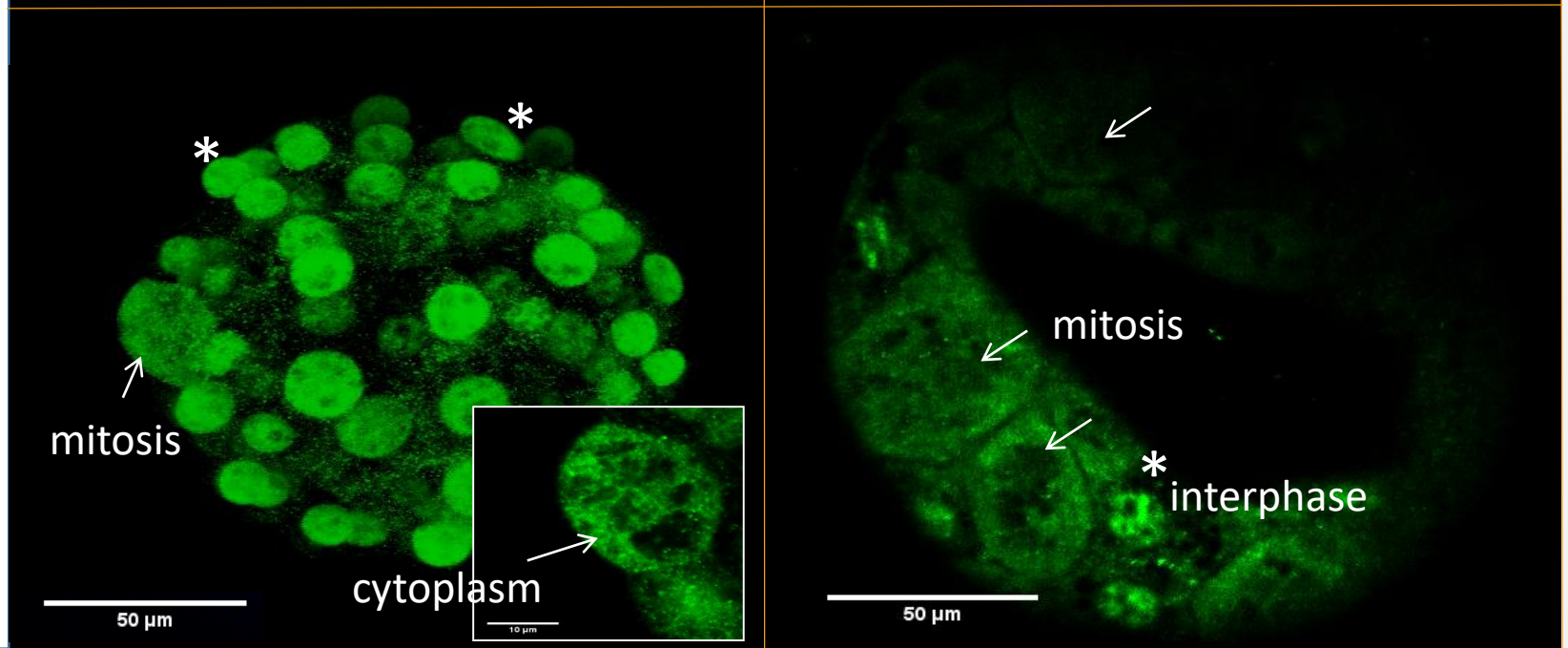
EMBRYO DEVELOPMENT



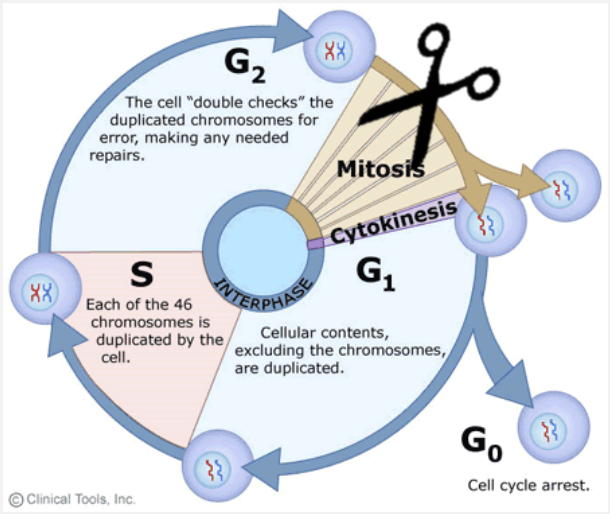
BOVINE Embryo

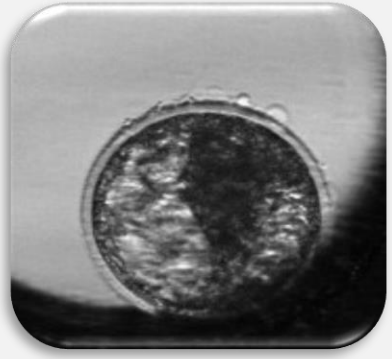
(A) NORMAL	(B) NOCODAZOLE





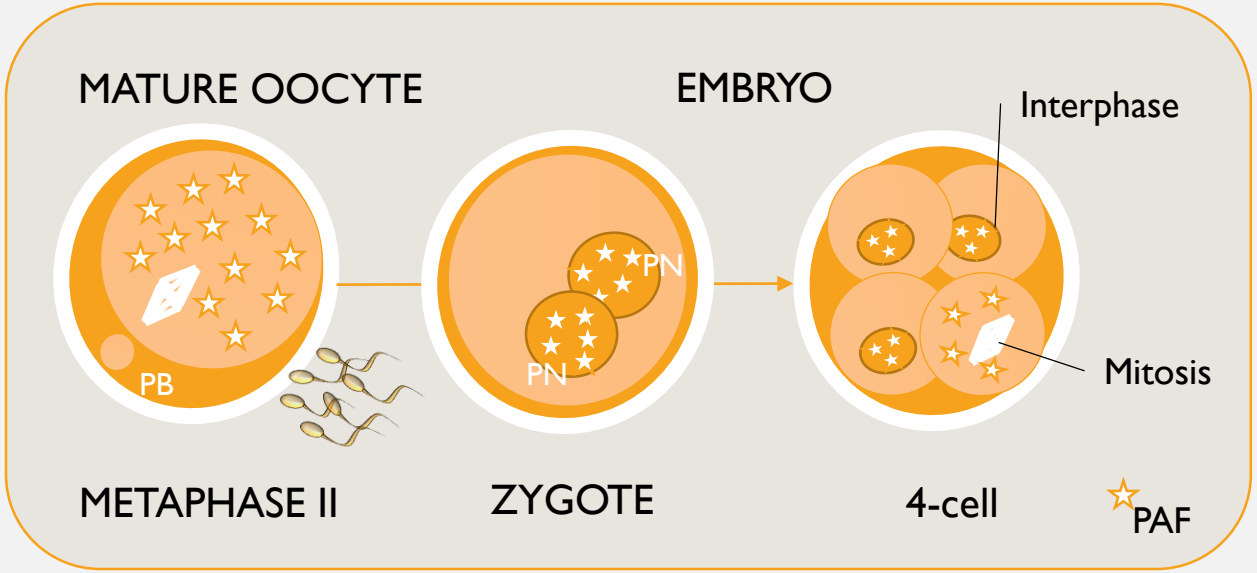
DNA
PAF





RESULTS

PAF in embryo development

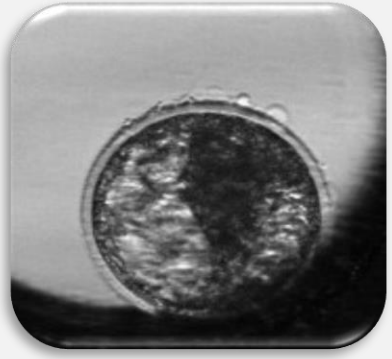


Aim: Evaluate the **presence** and **localization** of **PAF**

Conclusions:

- PAF is present in the **nucleus!**
- **PAF relocates to the cytoplasm when the cell enters mitosis**

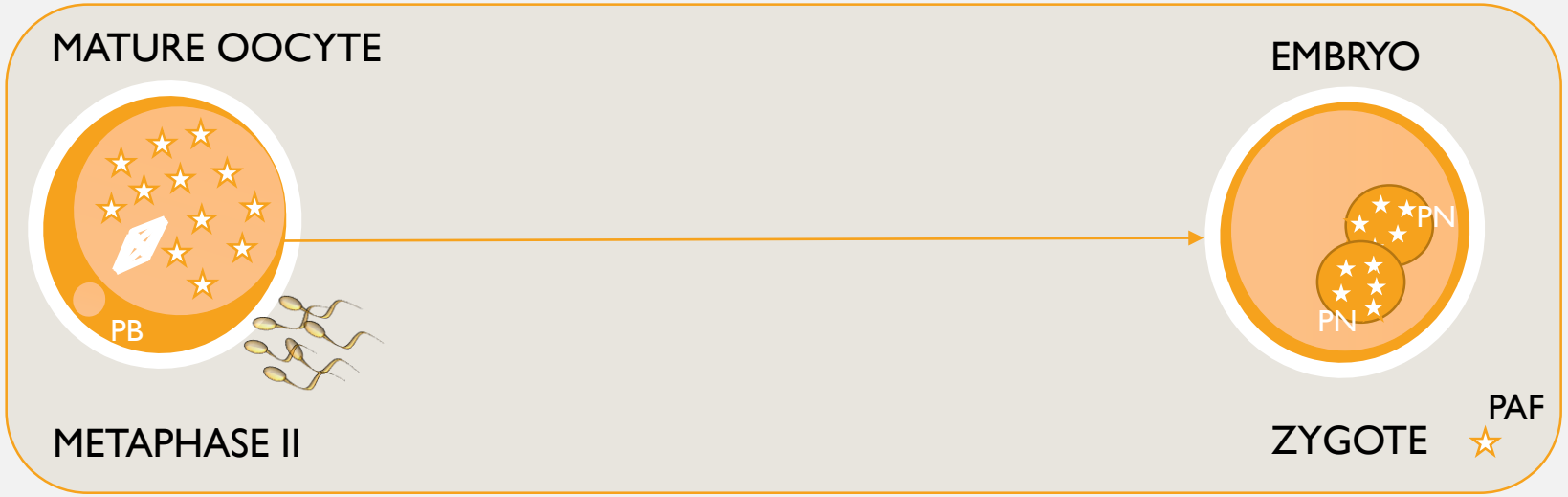
IS THIS IMPORTANT FOR EMBRYO DEVELOPMENT?

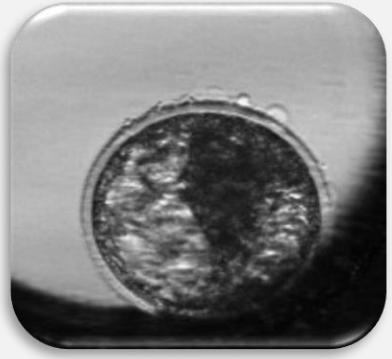


RESULTS

PAF in embryo development

Is the movement from nucleus to cytoplasm and back important for embryo development?

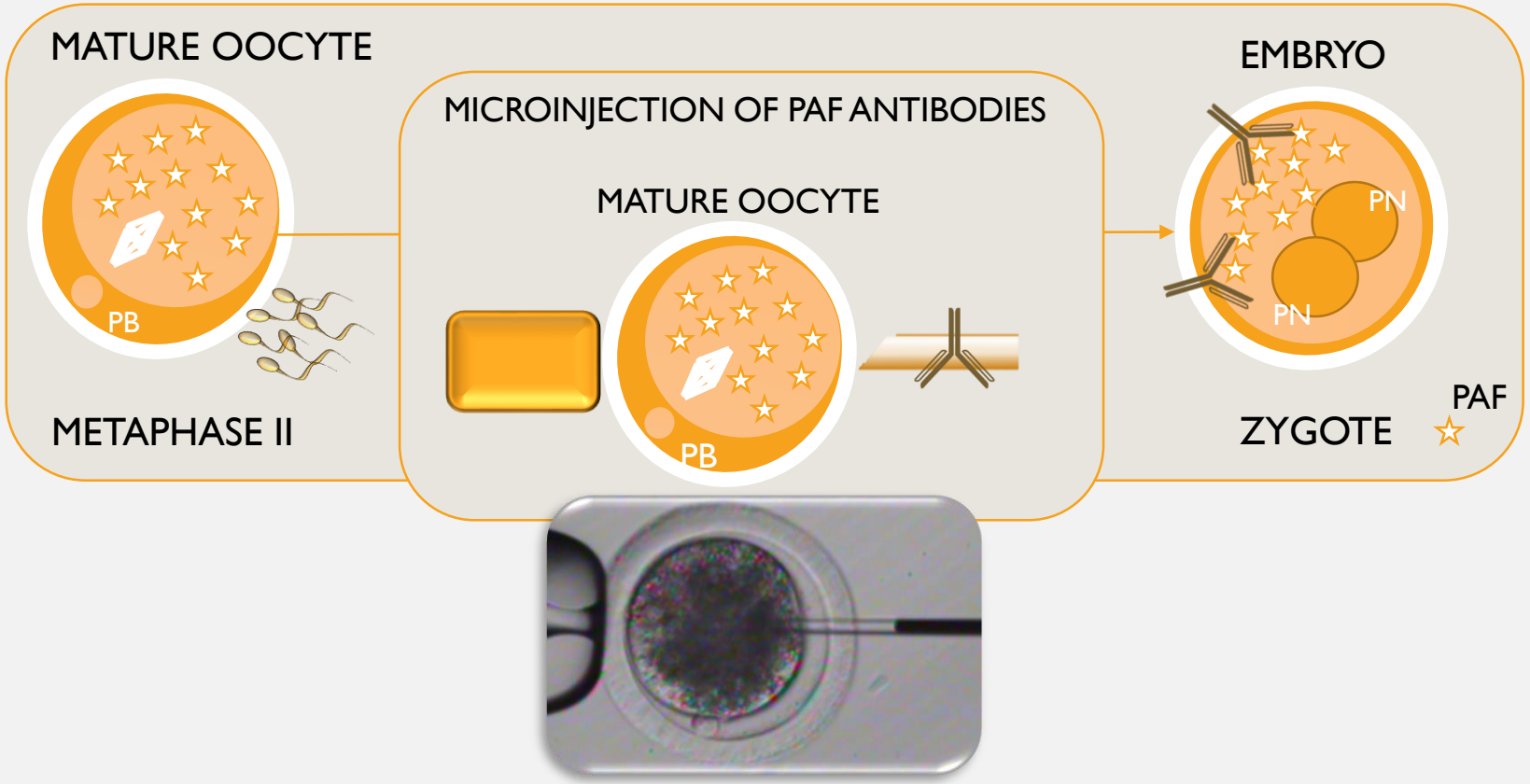


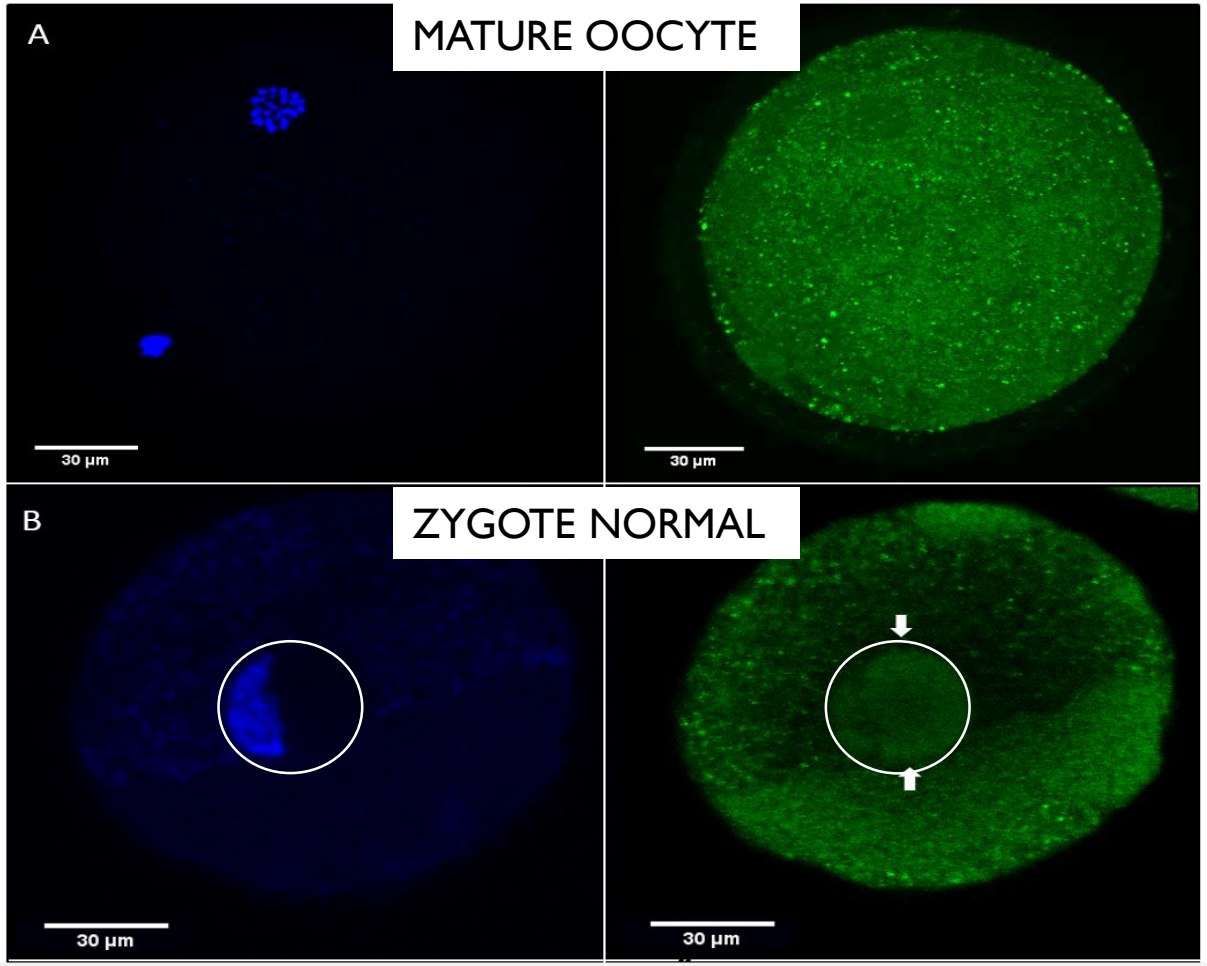


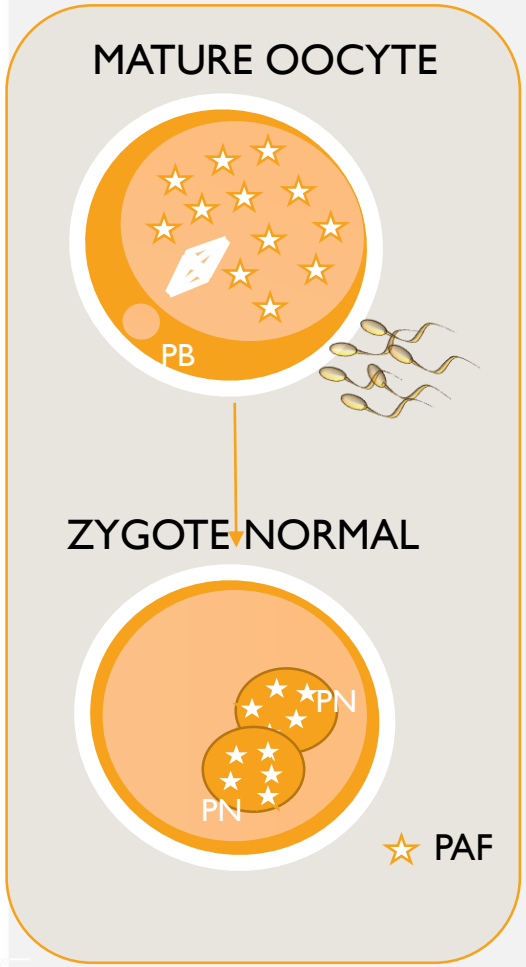
RESULTS

PAF in embryo development

Is the movement from nucleus to cytoplasm and back important for embryo development?

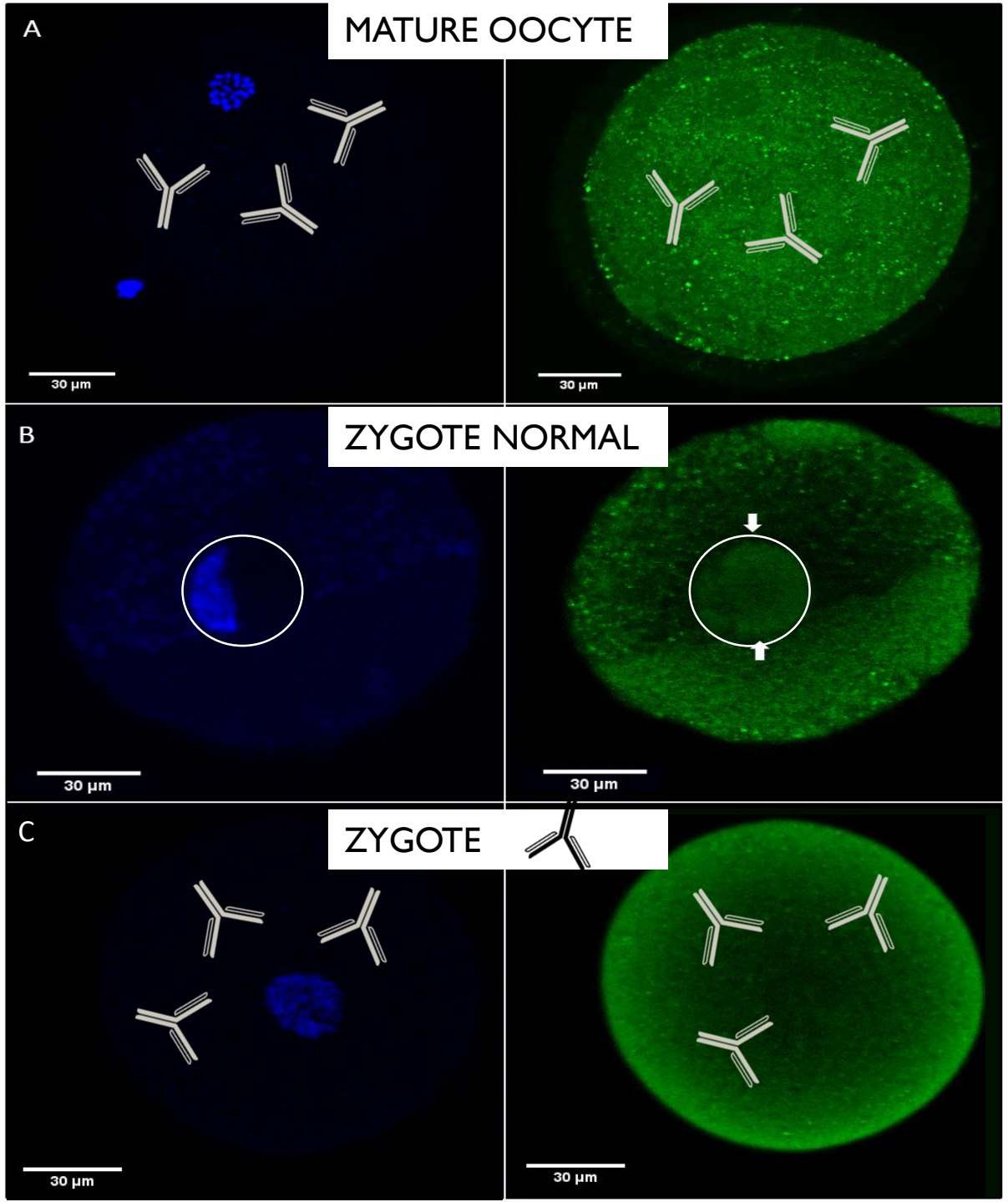


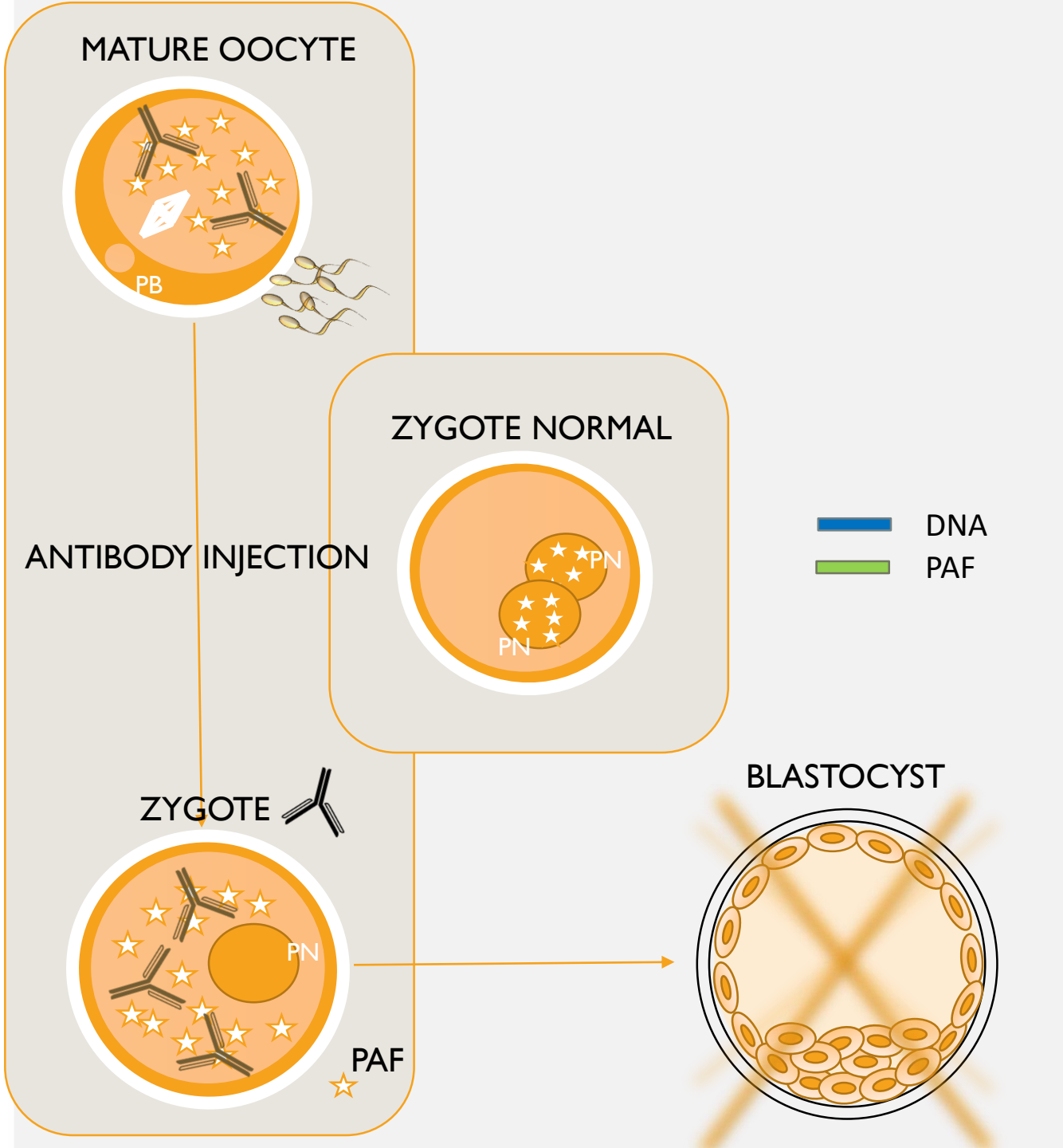

A
MATURE OOCYTE
30 µm
30 µm
B
ZYGOTE NORMAL
30 µm
30 µm


MATURE OOCYTE
PB
ZYGOTE NORMAL
PN
PN
PAF

DNA

PAF


A
MATURE OOCYTE
30 µm
30 µm
B
ZYGOTE NORMAL
30 µm
30 µm
C
ZYGOTE
30 µm
30 µm


MATURE OOCYTE
PB
ANTIBODY INJECTION
ZYGOTE NORMAL
PN
PN
DNA
PAF
ZYGOTE
PN
PAF
BLASTOCYST

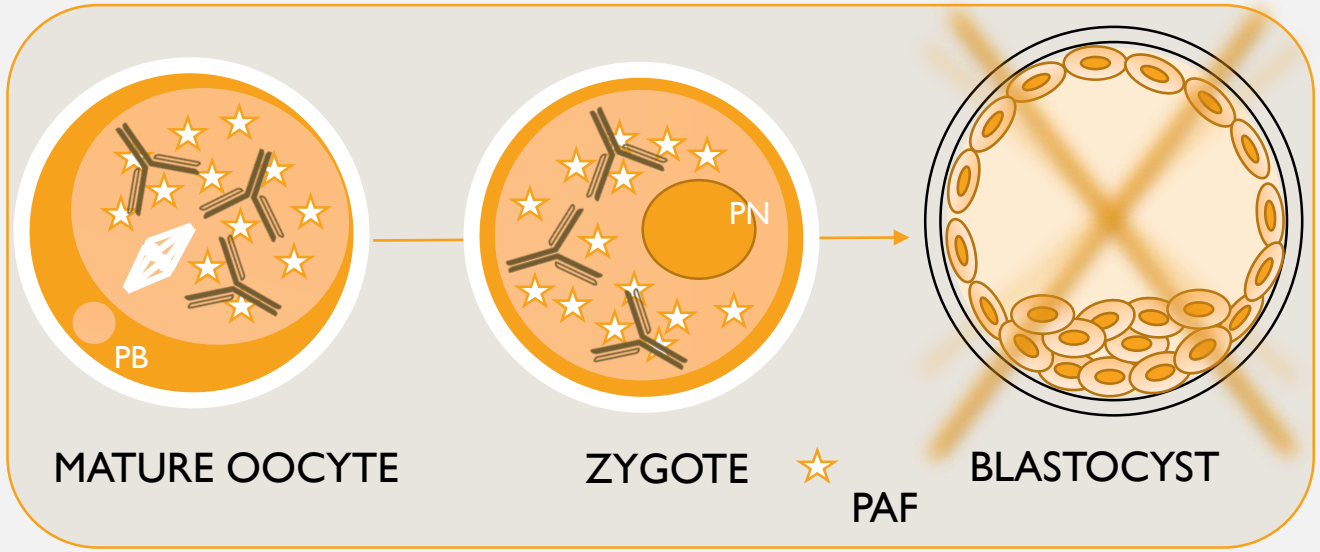
RESULTS

PAF in embryo development

Aim: Evaluate the **presence** and **localization** of **PAF**

Conclusions:

- **PAF relocates to the cytoplasm when the cell enters mitosis**

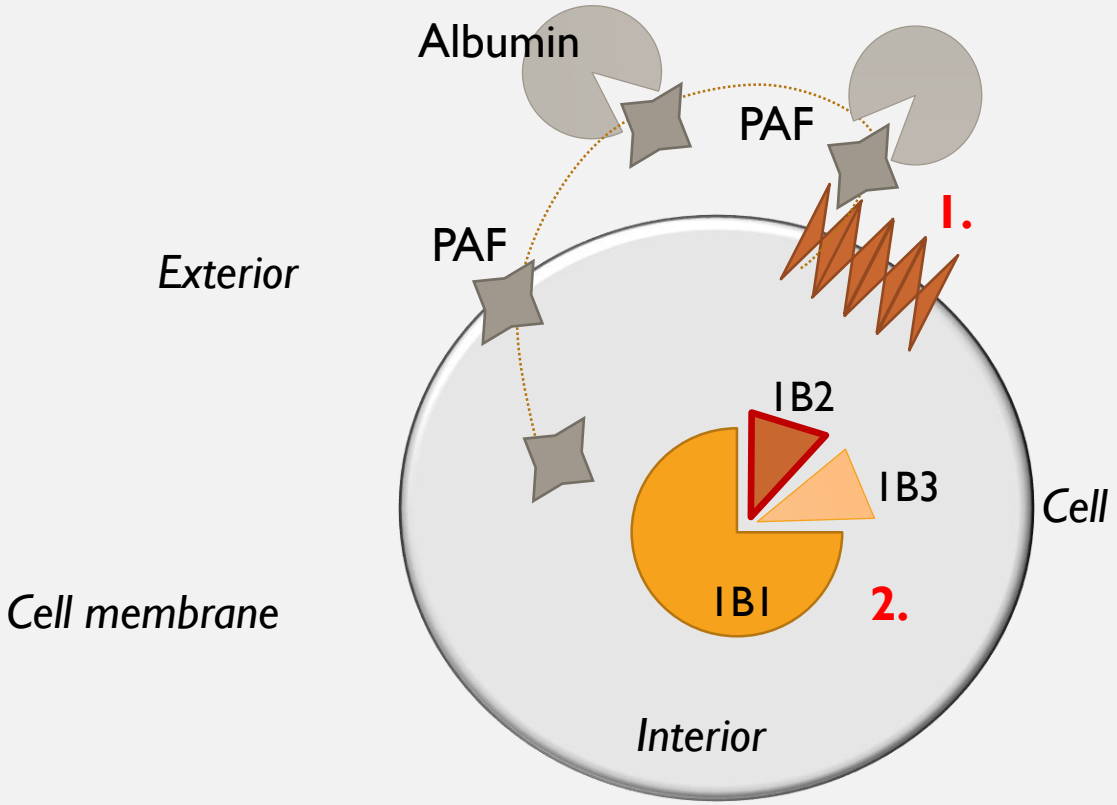


IS THIS IMPORTANT FOR EMBRYO DEVELOPMENT?

yes.

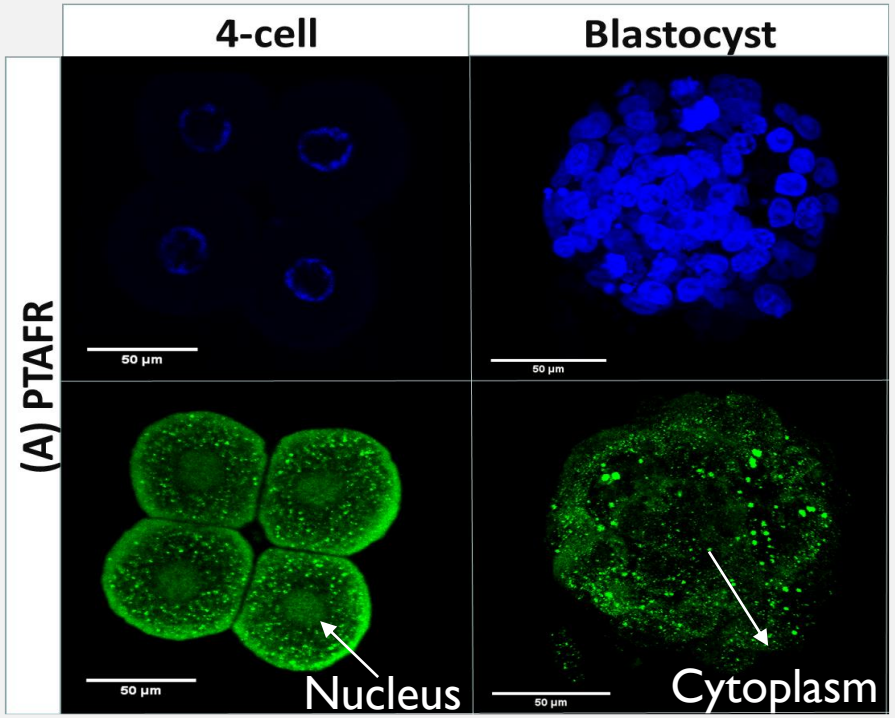
RESULTS

PTAFR & PAFAH1B in embryo development



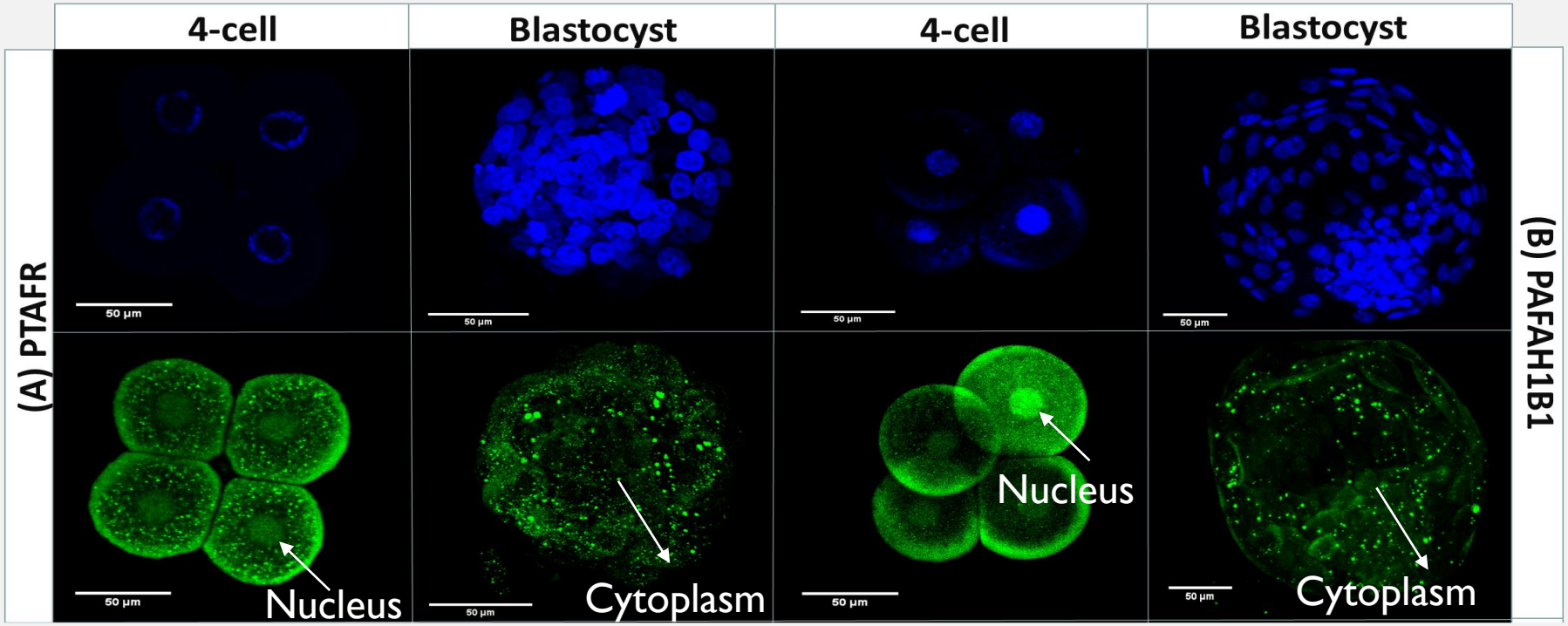
Aim: Evaluate the **presence** and **localization** of **PTAFR and PAFAH1B** in **embryo development** in cattle, mouse and human

1. Presumed to locate on the **cell membrane**
2. PAFAH1B: one study reported the localization of subunit PAFAH1B1 only.



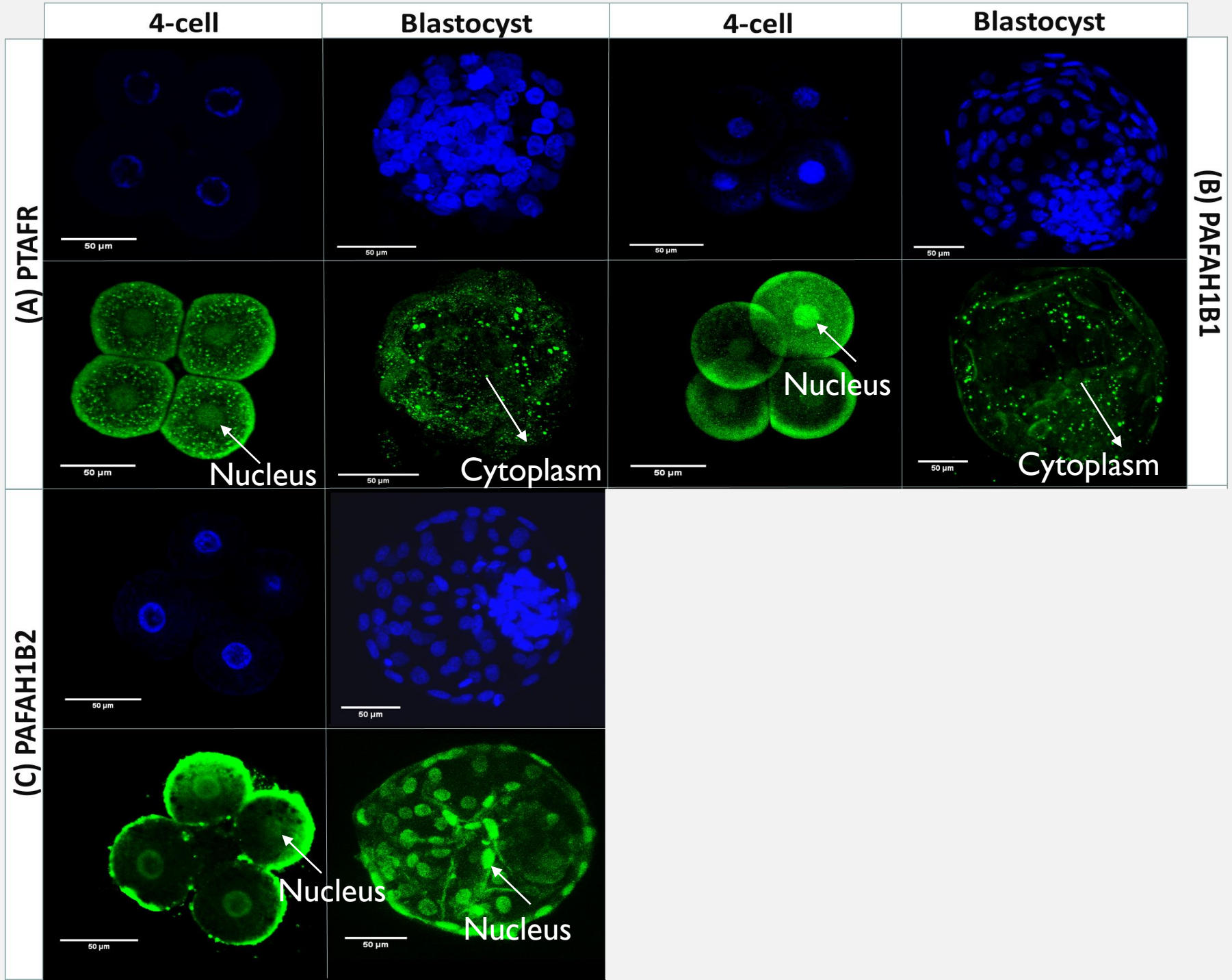
DNA

PTAFR/
PAFAH1B



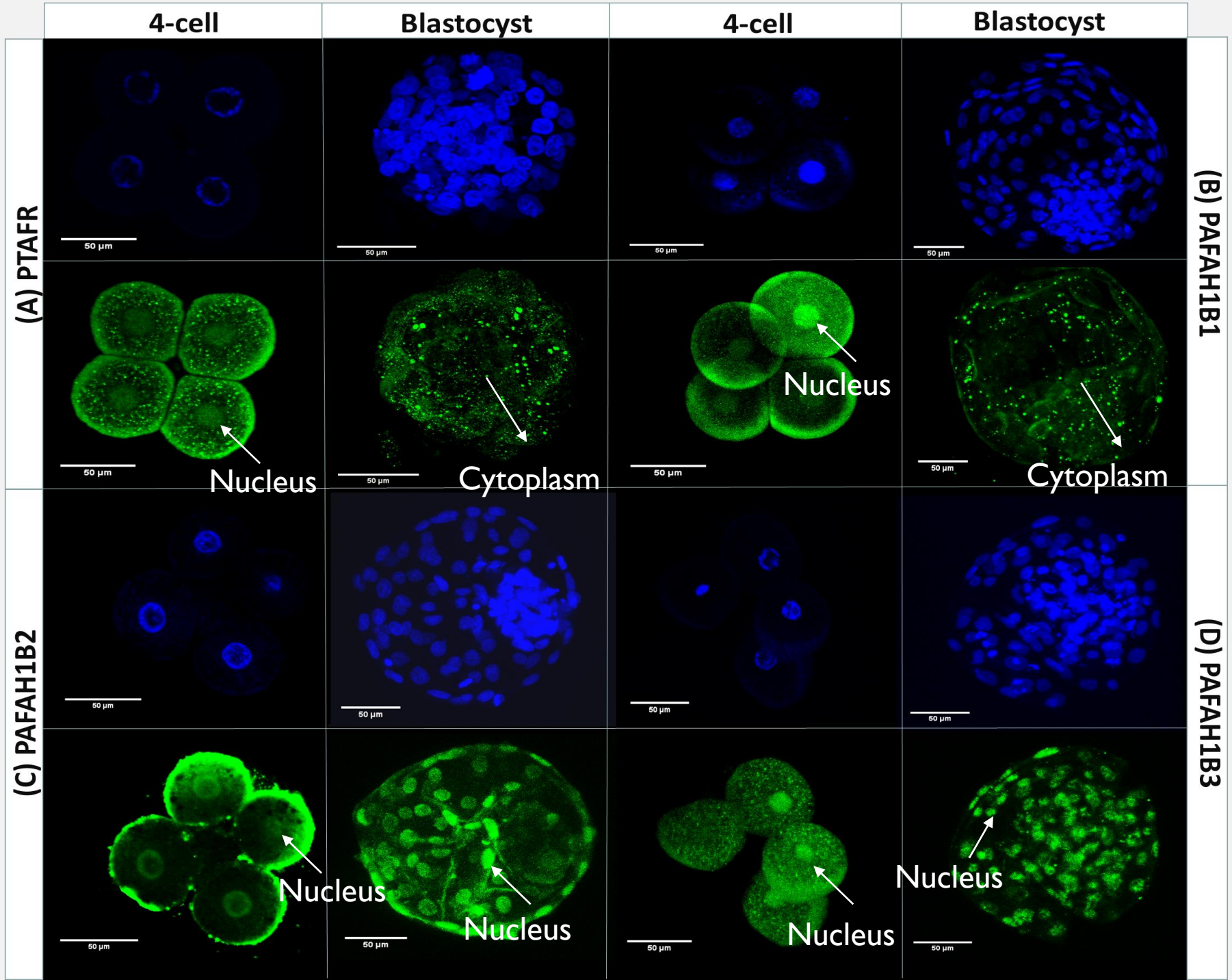
DNA

PTAFR/
PAFAH1B



DNA

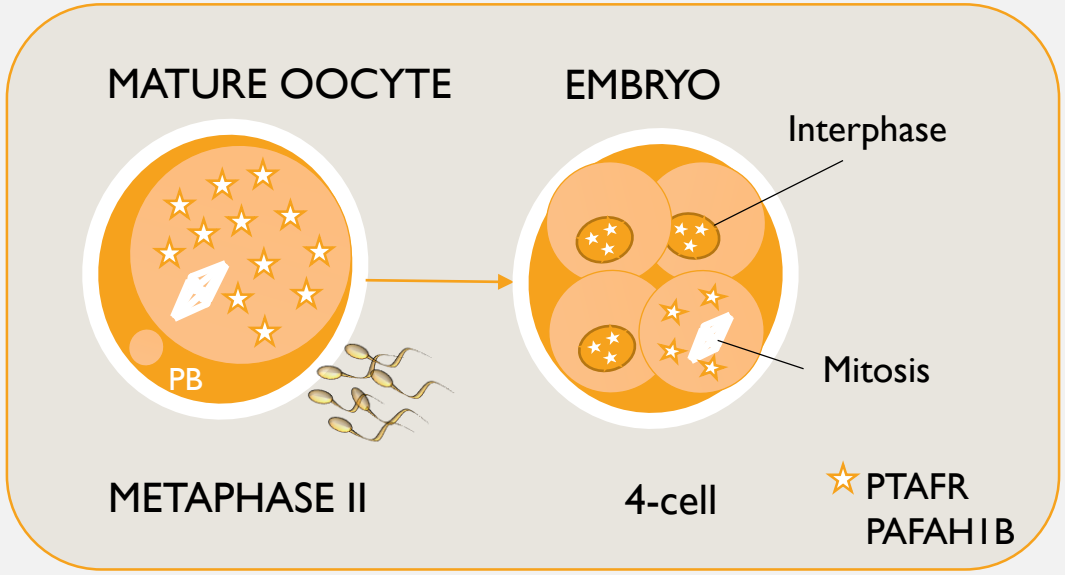
PTAFR/
PAFAH1B


4-cell
Blastocyst
4-cell
Blastocyst
(A) PTAFR
(B) PAFAH1B1
(C) PAFAH1B2
(D) PAFAH1B3
50 µm
Nucleus
Cytoplasm
Nucleus
Cytoplasm
Nucleus
Nucleus
Nucleus
DNA
PTAFR/
PAFAH1B

RESULTS

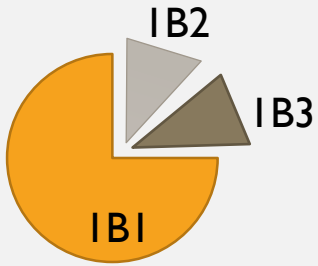
PAF in embryo development

Aim: Evaluate the **presence** and **localization** of **PTAFR and PAFAH1B**



Conclusions:

- PTAFR and PAFAH1B subunits are present in the **nucleus!**
- **Similar intracellular distribution as PAF**

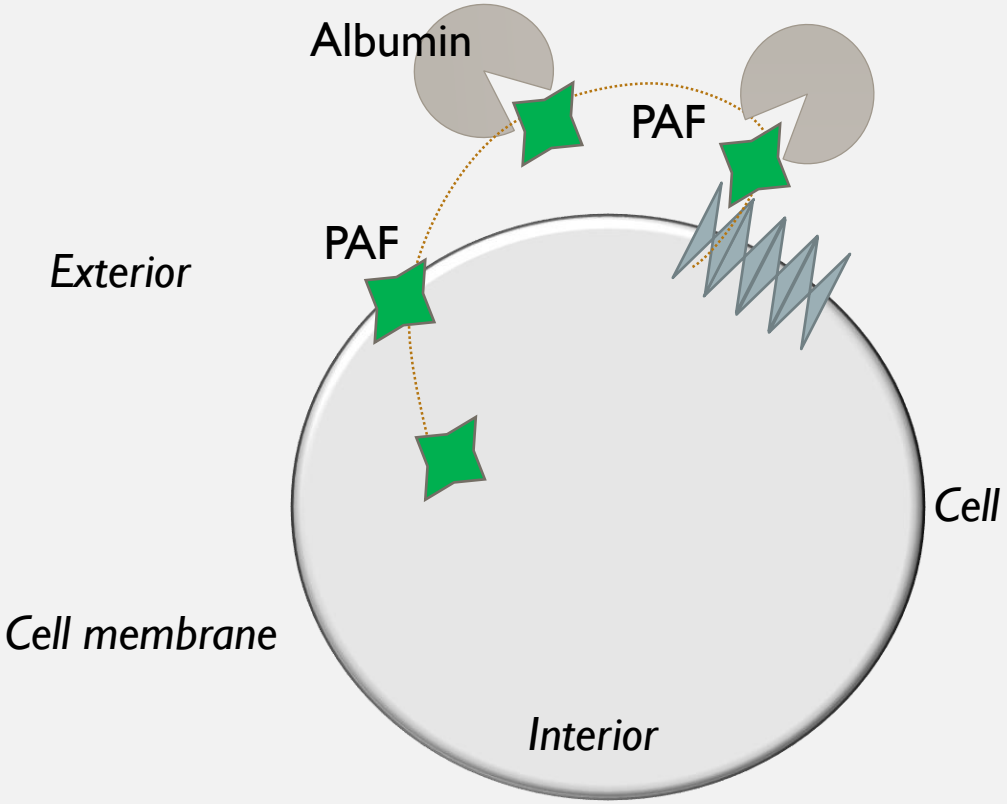


DISCUSSION

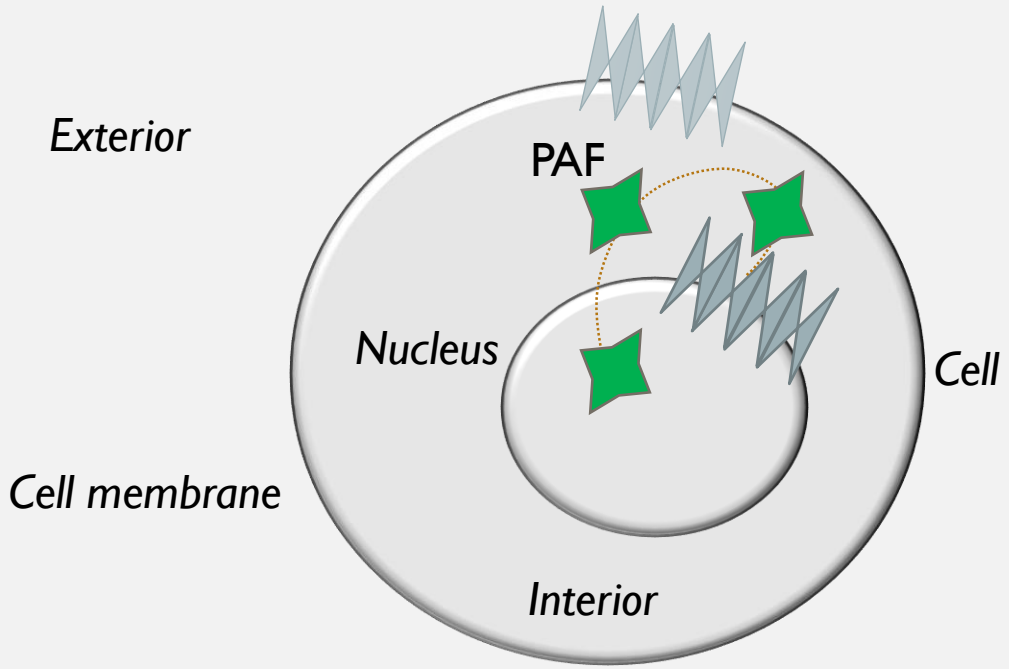
- PAF
- PTAFR – PAFAH1B
- PAFAH1B3 in spindle formation

DISCUSSION

PAF



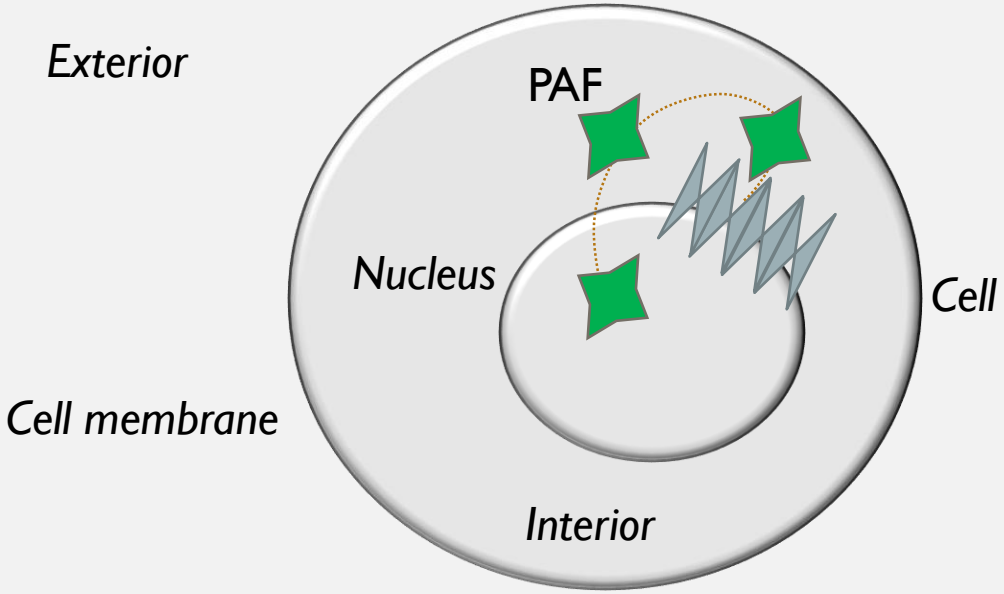
Old model: PAF only on the cell membrane



New model: PAF in the nucleus

DISCUSSION

PAF



PAF was believed to be present only on the **cell membrane**

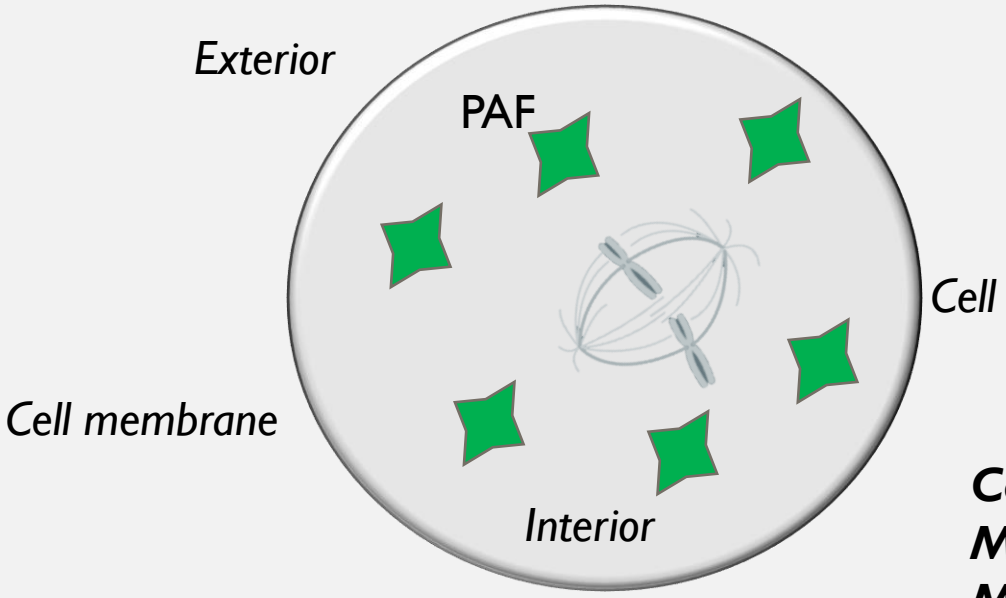
PAF is also present in the **nucleus and cytoplasm**

Implications?

Molecules showing similar behavior are involved in the process of oocyte maturation
➔ further research necessary

DISCUSSION

PAF



Cell in Mitosis/ Meiosis

PAF relocates to the **cytoplasm** upon **mitosis**

➔ Important for embryo development

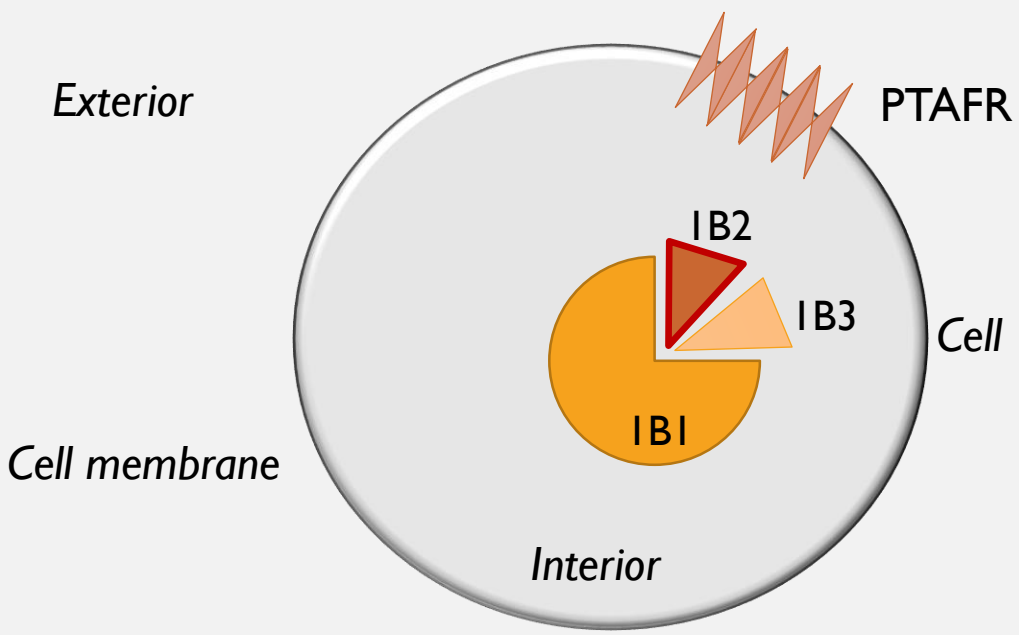
Implications?

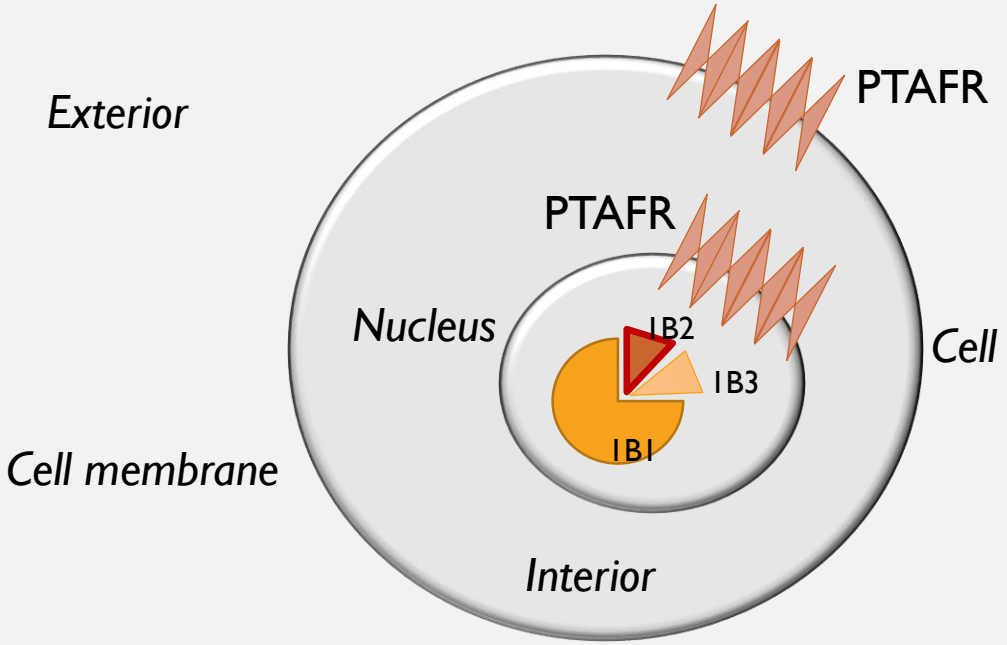
The oocyte stores important molecules in its cytoplasm that govern processes neccessary to form the embryo.

The dynamics of PAF (and PTAFR and PAFAH1B) suggest that PAF plays a role in the *transition from oocyte to embryo*.

DISCUSSION

PTAFR & PAFAH1B



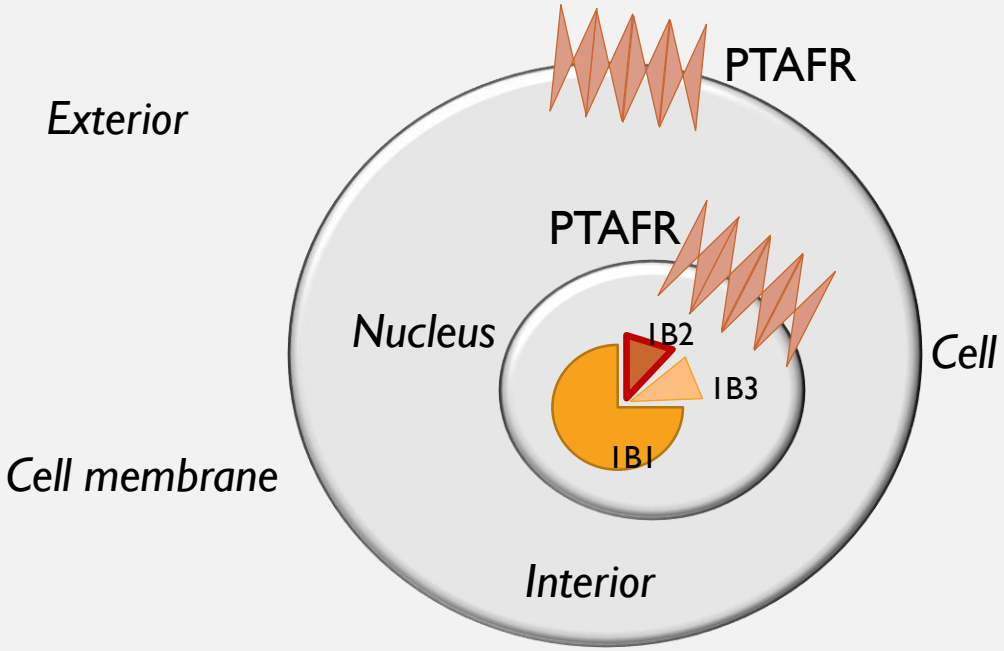


Old model: PTAFR on the cell membrane
PAFAH1B in the cytoplasm

New model: PTAFR & PAFAH1B in the nucleus

DISCUSSION

PTAFR & PAFAH1B



PTAFR was believed to be present only on the **cell membrane**

PAFAH1B was believed to reside in the **cytoplasm**

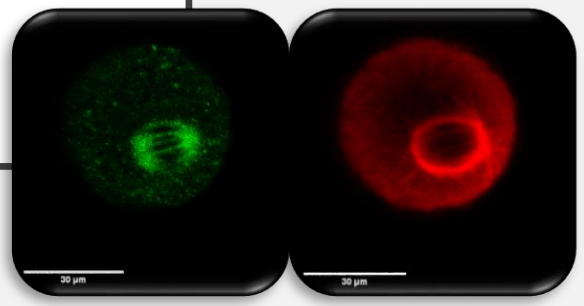
PTAFR and PAFAH1B are also present in the **nucleus**

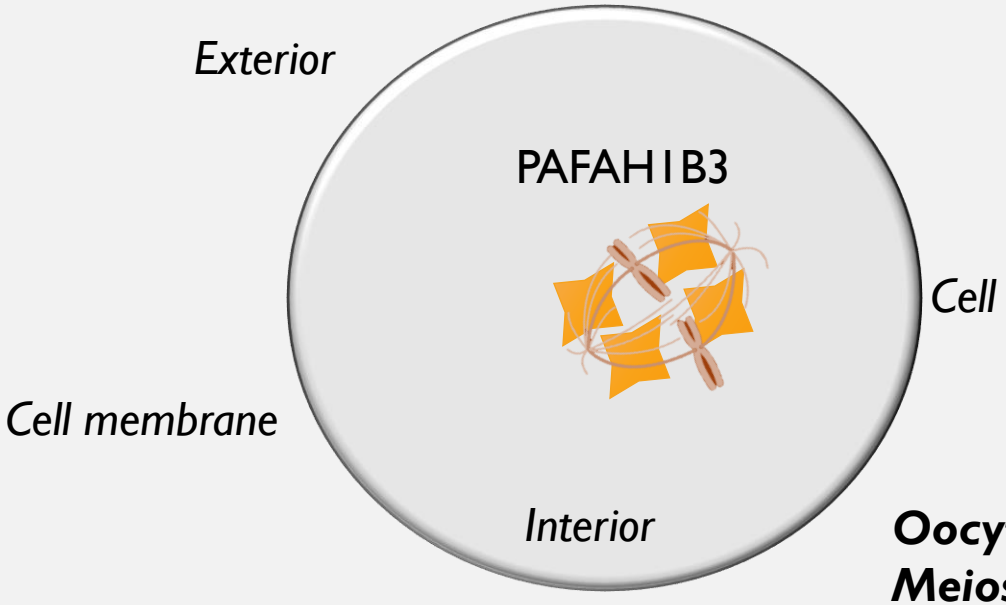
Implications?

Similar location of PAF, PTAFR and PAFAH1B strengthens our hypothesis and hints towards a specific pathway and function.

DISCUSSION

PAFAH1B3





Oocyte in Meiosis

Is PAFAH1B3 present in oocytes?

Role in formation of the **meiotic spindle** and **oocyte maturation**

Implications?

Highly similar to a syndrome in human IVF: the *maturation resistant oocyte*.

GENERAL CONCLUSIONS

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- **PAF, PTAFR** and all the subunits of the **PAFAH1B** enzyme are **present** in the bovine, murine and human **oocyte** and **embryo**.
- **PAF, PTAFR** and **PAFAH1B** subunits show a **dynamic distribution** pattern depending on the cell cycle. This was observed both during meiotic progression in the **oocyte** as during further **embryonic** development.
- The presence, localization and dynamics of PAF, PTAFR and PAFAH1B is a **fundamental, well conserved** system in bovine, mouse and human oogenesis and embryogenesis.
- The catalytic subunit **PAFAH1B3** is closely associated with the **spindle** in oocytes. Blockage of this unit by various techniques results in aberrant **spindle formation and meiotic arrest**.

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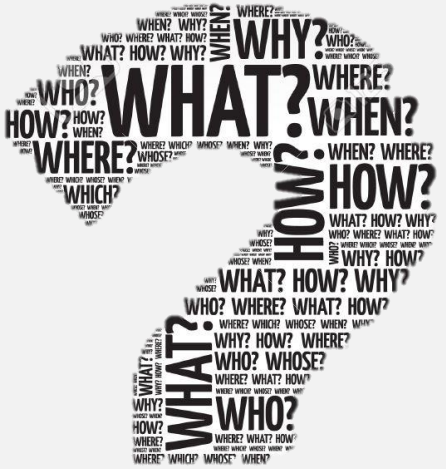
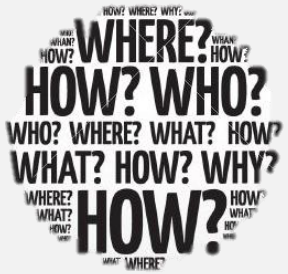
FUTURE RESEARCH

- Exact mechanism and function of this nuclear PAF is unkown
  - Is the PTAFR responsible for gene expression regulation in the embryo? If so, which genes are involved?
  - Is the receptor activated by PAF?
  - Does PAF act through PTAFR-independent pathways?
  - Besides PAF and PTAFR, what is the contribution of the PAFAH enzyme exactly?





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Promotor:

Prof. Dr. Catharina De Schauwer

Co-Promotor:

Prof. Dr. Ann Van Soom

Lab Technicians:

Petra Van Damme, Isabel Lemahieu

Sylvie Lierman, Vanessa Thys

Exam Committee:

Prof. Dr. E. Meyer, Prof. Dr. P. Bols, Prof. Dr. B. Gadella,

Prof. Dr. H. Van de Velde, Prof. Dr. B. Heindryckx,

Prof. Dr. W. De Spiegelaere, Dr. K. Smits, Dr. S. Heras

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That's all Folks!
Any Questions?