

This project has received funding from the European Union's Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement No 713714



OVARIAN CLUB XI
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November 1-3, 2018 | Paris, France

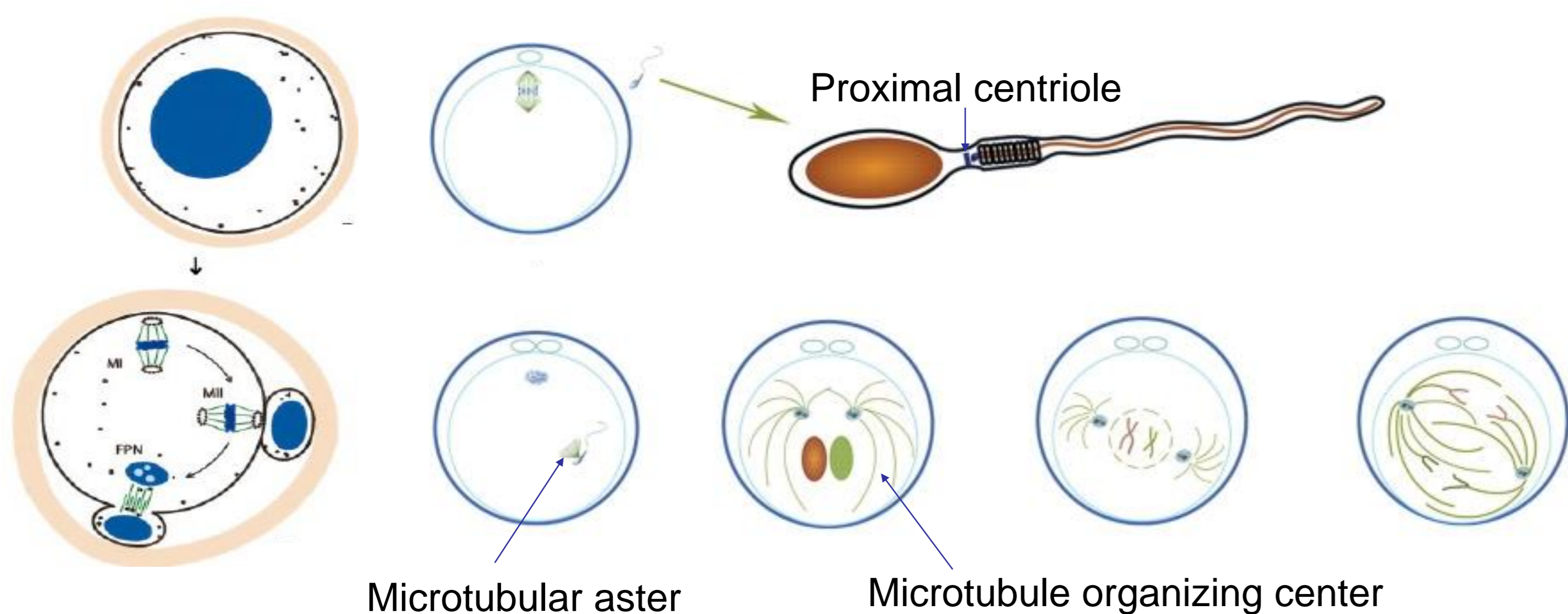
Monitoring the microtubule nucleation dynamics of sperm centriole after IFV and ICSI in sheep zygotes

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Introduction



- Matured oocytes lack centrioles and functional centrosome is contributed by the sperm

*Reported in sheep by Crozet et al., 2000**

Using Assisted Reproductive Technologies (ART) the aim of our study was

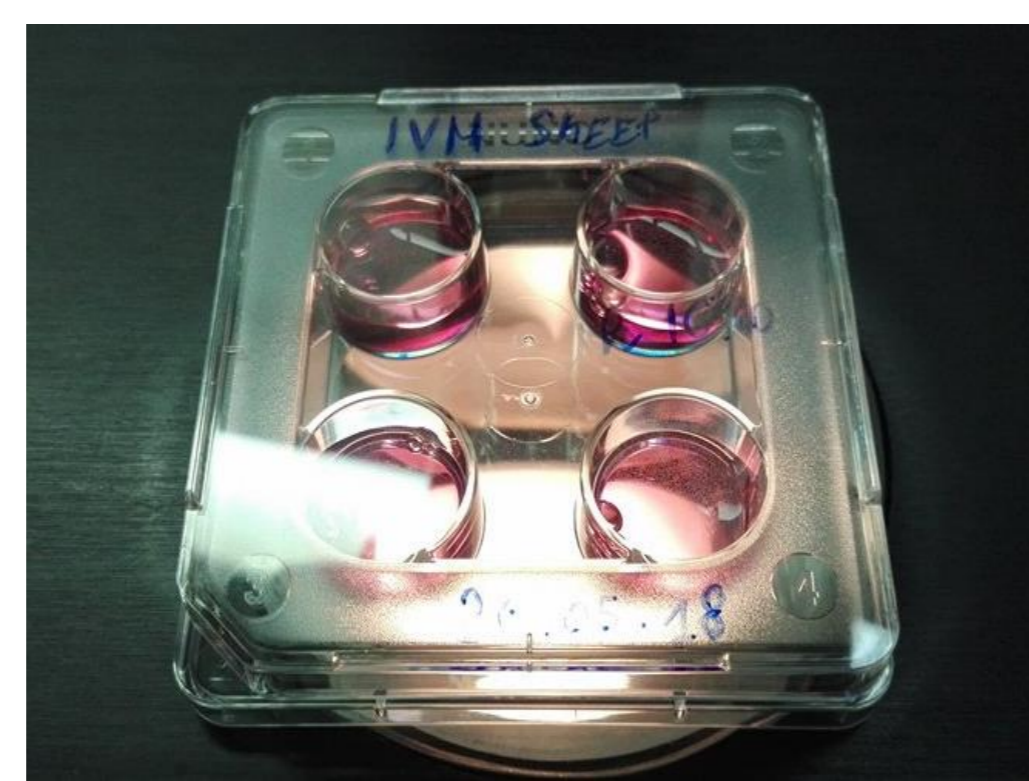
- Tracing the fate of sperm centriole after fertilization
- Evaluating the timing of sperm microtubular aster nucleation in early sheep zygotes

* Crozet, N., Dahirel, M., Chesne, P. (2000) Centrosome Inheritance in Sheep Zygotes: Centrioles Are Contributed by the Sperm. Microscopy Research and Technique 49: 445-450.

Material and Methods



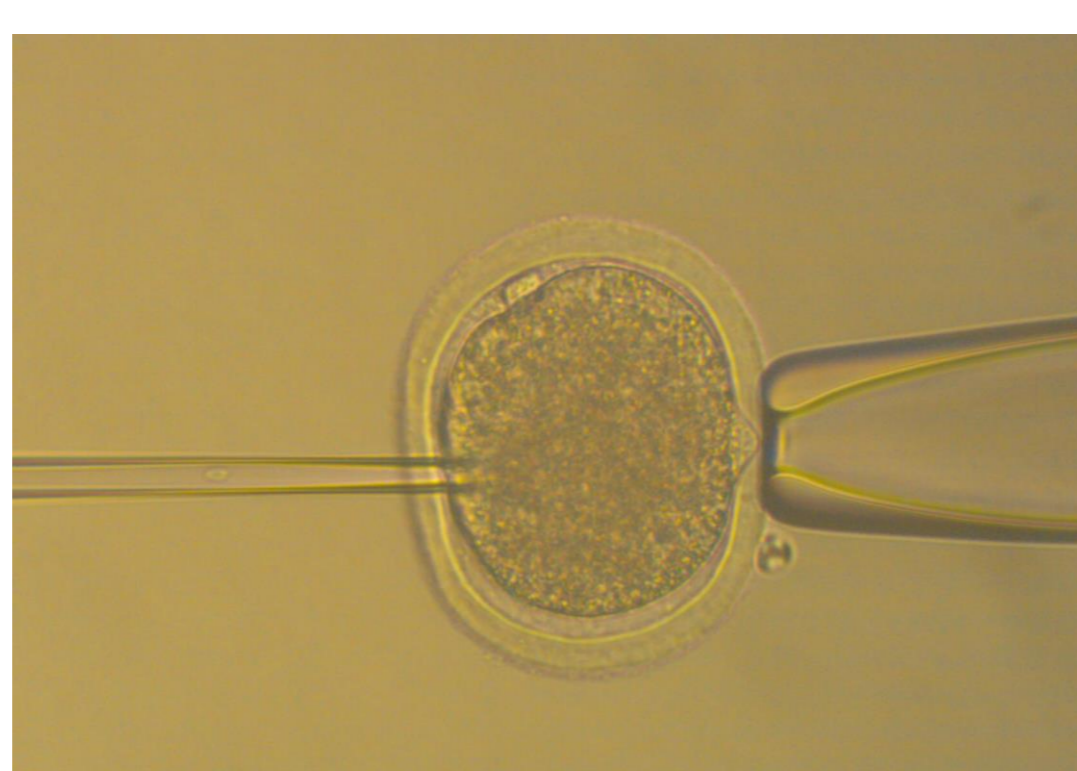
Oocytes collection (1)



In vitro maturation (24h) (2)



In Vitro Fertilization (IVF) (3)

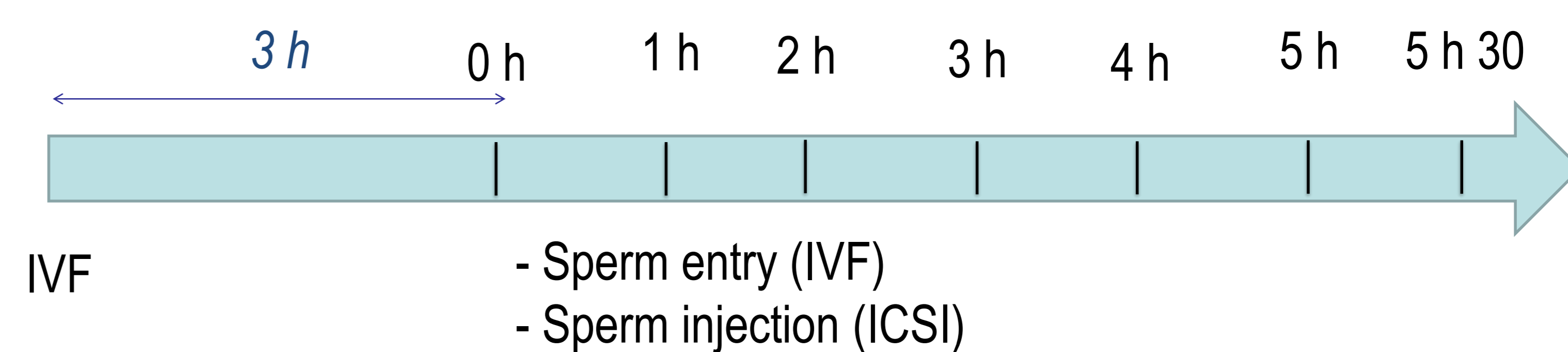


IntraCyttoplasmic Sperm Injection (ICSI) (4)

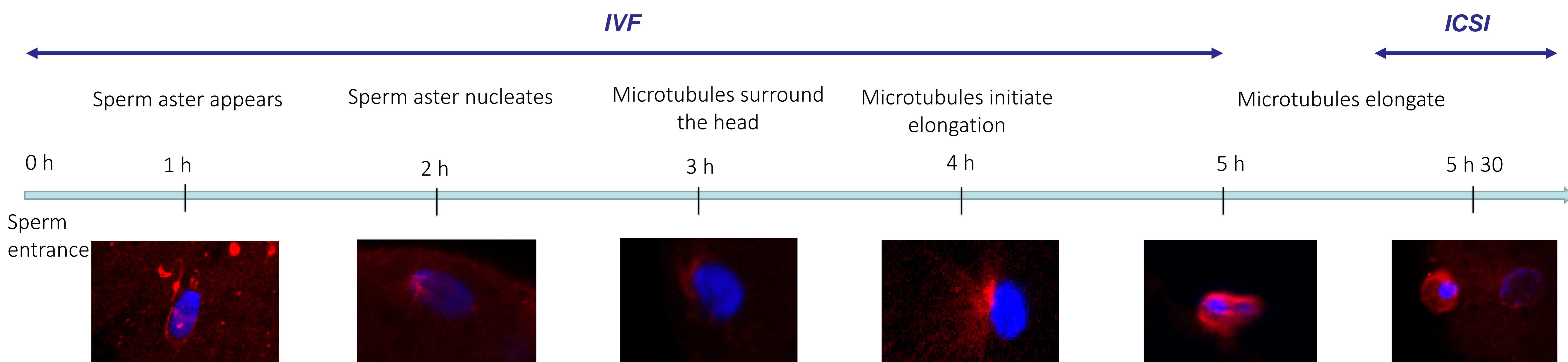
Piezo-pulsed spermatozoa

Immunological detection of tubulin (5)

- Free Zona Pellucida (ZP) presumptive Zygotes (Tyrode + Pronase)
- Fixed with 4% paraformaldehyde
- Permeabilized by 0.5% Triton X-100
- Immunological detection of tubulin with Goat anti- α -tubulin primary antibody (1:50)
- Observation under confocal microscope



Results



Conclusions

No differences were noticed in the dynamics and timing of sperm microtubular aster nucleation, that started around 5h post ICSI (5h30). Therefore, we conclude that abnormal microtubular nucleation by the centriole is not responsible for the low development of ICSI fertilized sheep oocytes.

Acknowledgement

We thank the European Union and the Abruzzo region to cofound the Rep-Eat project which supported this work with the Marie Skłodowska-Curie grant agreement No 713714. We also thank Luca Valbonetti to help us with confocal microscope.