Genetic Outcomes and Transferability of triplonucleated Zygotes: Insights from 2.1 and Same Size Triplonucleated Embryo

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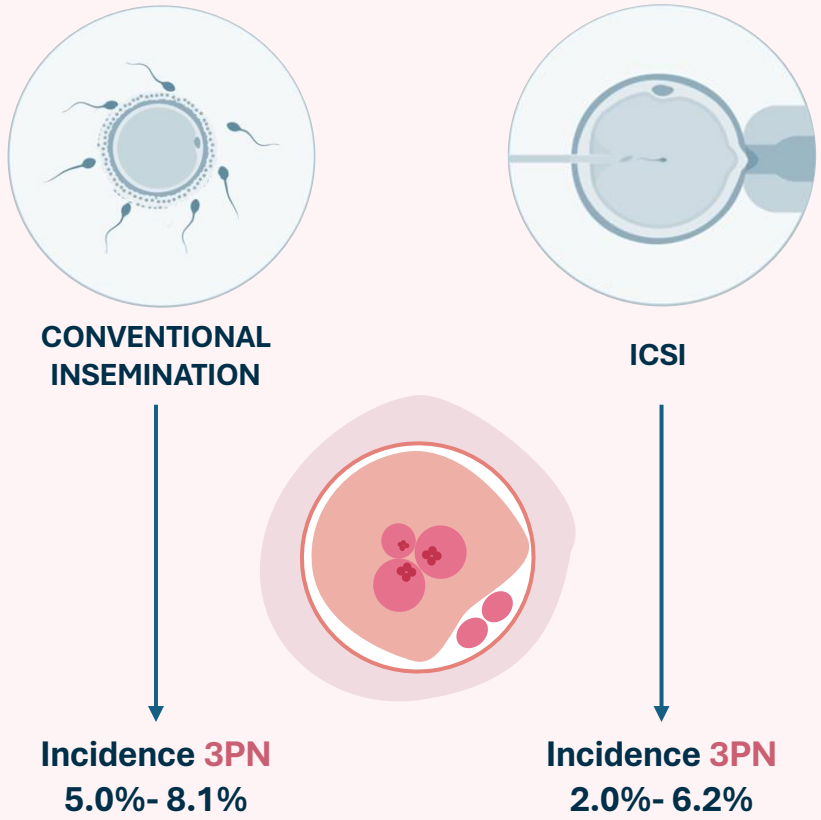
EQUIPO JUANA CRESPO



- In the in vitro fertilization (IVF) setting, normal oocyte fertilization is commonly confirmed 16 – 18 hours after conventional insemination or (ICSI) with the appearance of two pronuclei (2PN).

- The presence three pronuclei (3PN) shows an atypical oocyte fertilization. 3PN fertilized embryos are commonly discarded due to the suspected increased risk of a triploid chromosomal complement.

Alpha Scientists in Reproductive Medicine European Society for Human Reproduction and Embryology Special Interest Group of Embryology. The Istanbul consensus workshop on embryo assessment: proceedings of an expert meeting. Hum Reprod 2011; 26:1270–83.

Do all zygotes with three observed pronuclei have an actual triploid chromosomal complement?

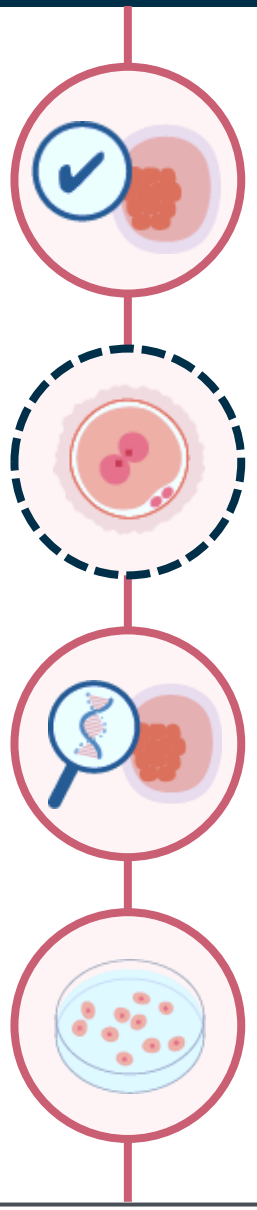
BACKGROUND: Rethinking 3PN Embryos

Abnormally fertilized oocytes can result in healthy live births: improved genetic technologies for preimplantation genetic testing can be used to rescue viable embryos in in vitro fertilization cycles

-In 2017, a study by Capalbo et al. proposed a new way to look at 3PN embryos. They divided them into two types depending on the size of the third pronucleus. They only analysed embryos with two pronuclei of similar size and a third smaller, believing these might have a better prognosis. They called to this type 2.1 embryos

-The study even included transferring these 2.1 embryos, resulting in the birth of two healthy babies. **This finding proved that viable embryos could potentially be rescued from the pool of 3PN embryos.**

*Capalbo A, Treff N, Cimadomo D, Tao X, Ferrero S, Vaiarelli A, Colamaria S, Maggiulli R, Orlando G, Scarica C, Scott R, Ubaldi FM, Rienzi L. Fertil Steril. 2017 Dec;**108(6)**:1007-1015.e3.*



SMART PGT-A

PLOIDY DETECTION AND GENETIC PN CHECK

CONTAMINATION

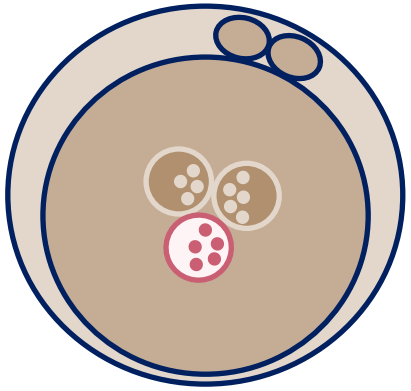
COHORT CHECK

- In 2023 a new genetic technique emerged that allows the detection of the ploidy and euploidy of an embryo in the same biopsy and can be used on a routine basis in clinic, with a massive next-generation sequencing (NGS) strategy using single nucleotide polymorphisms (SNPs), without the need for parental samples.

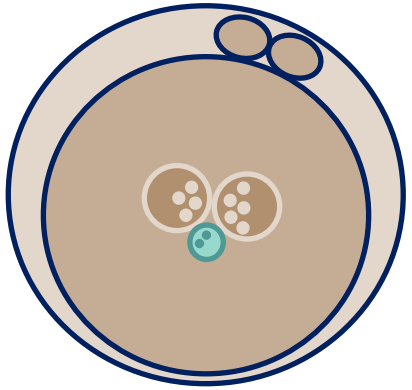
Therefore, 3PN embryos could be routinely analysed in an easy way to rescue embryos that would have been discarded under current practices.

Comparing types of 3PN Embryos with Smart PGT-A Plus

- Having this background and the availability of this new technique, the need arises to analyze the ploidy rate of two distinct types of 3PN embryos separately.
- These types are: 3PN embryos with all three pronuclei being similar in size, and 3PN embryos with two pronuclei of similar size and a third one that is smaller.
- This comparison will reveal whether these two types of embryos behave similarly or differently.



Same-size 3PN (SS)



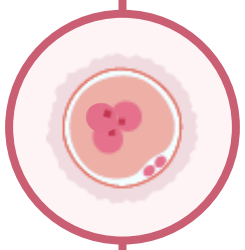
Micro 3PN (2.1)

- An Ethics Committee for Investigation accredited us to perform this prospective observational study in June 2023.

STUDY DESIGN

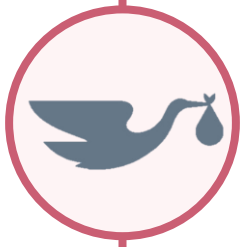
Our study...

Updated
December 2024



349 3PN embryos

317 cycles
297 patients



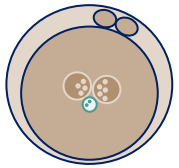
Equipo Juana Crespo

June 2023 until
December 2024

During that period, 2953 cycles were performed in the clinic resulting in 24506 oocytes injected. (490 3PN in total)

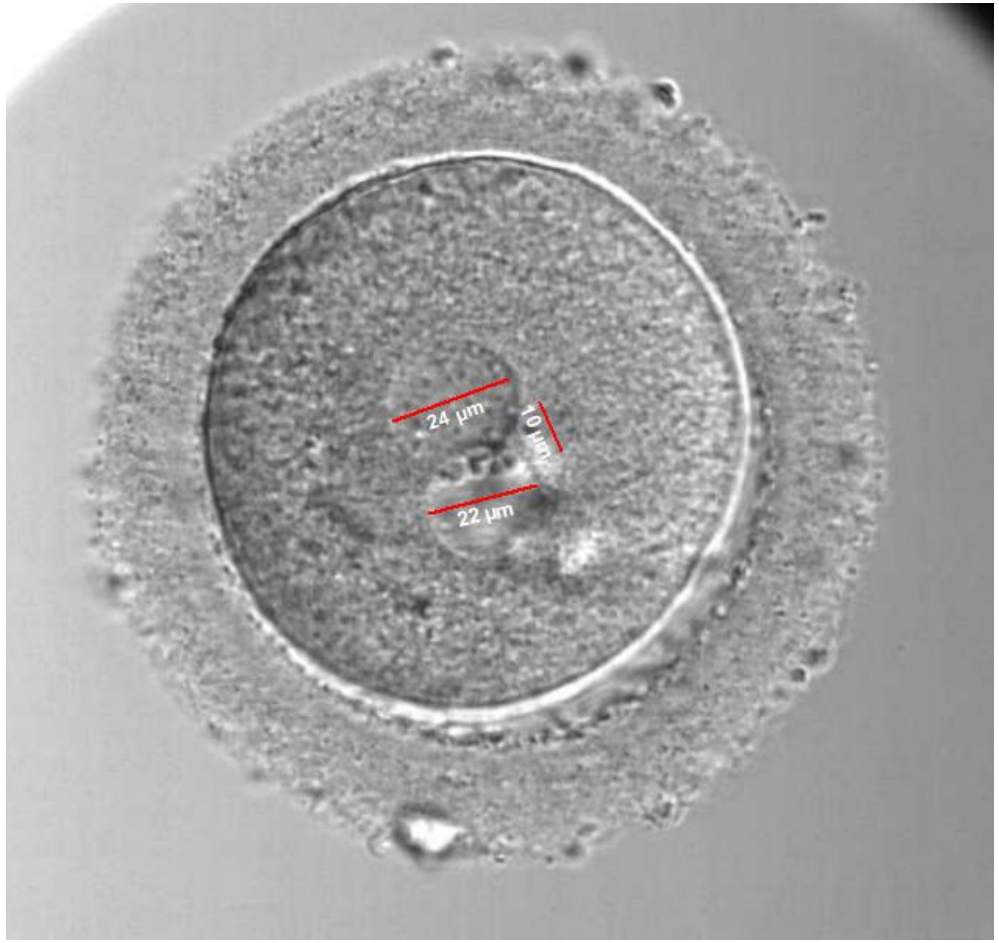
2.0% 3 PN INCIDENCE

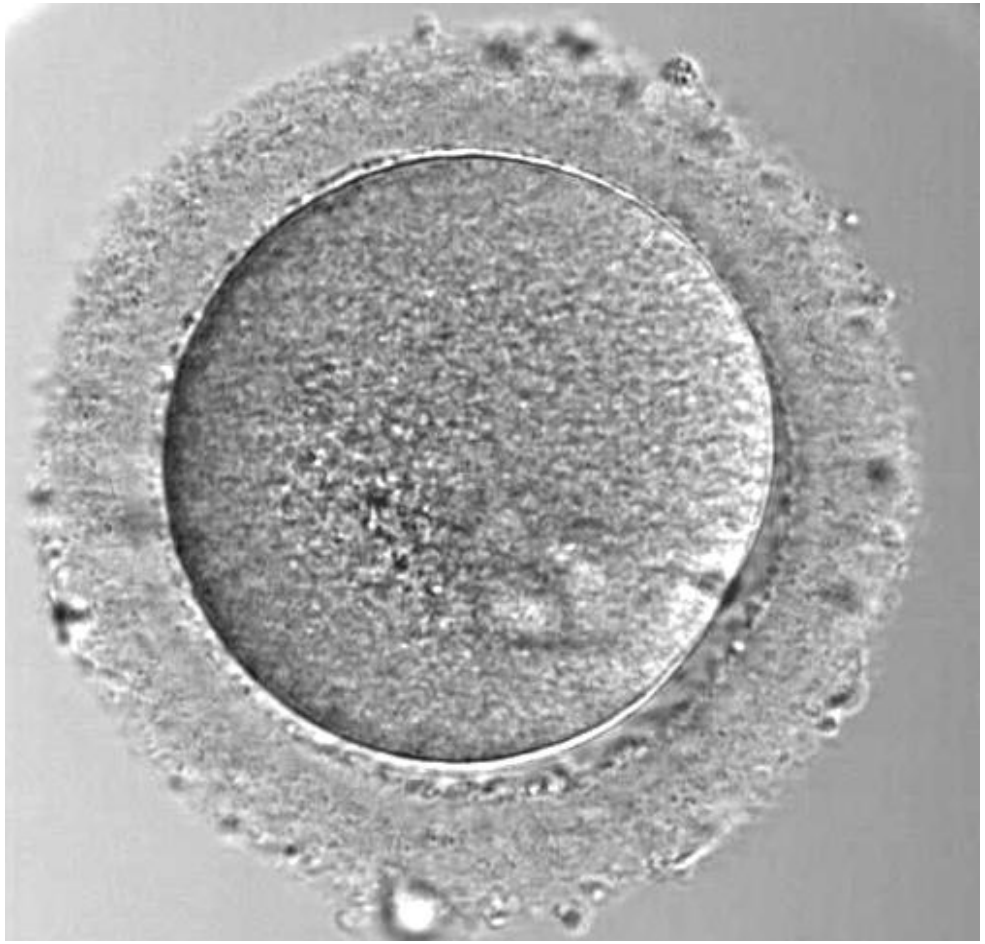
STUDY DESIGN



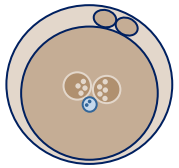
(2.1)

Diameter of the smallest pronucleus <70% of the diameter of the largest pronucleus



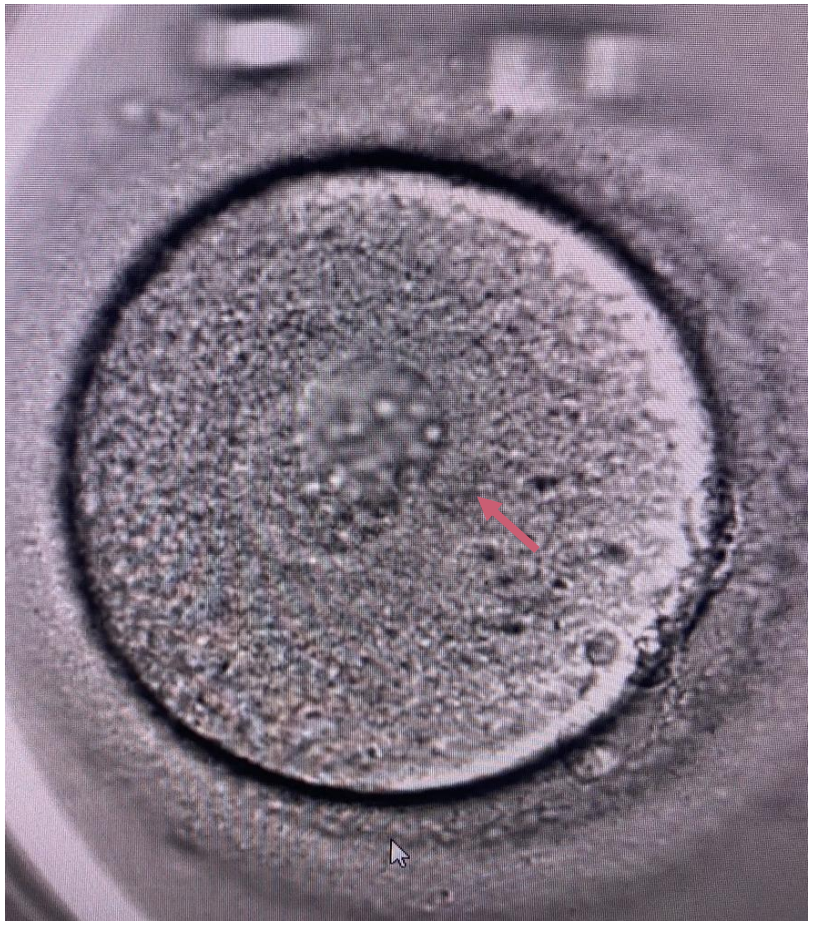


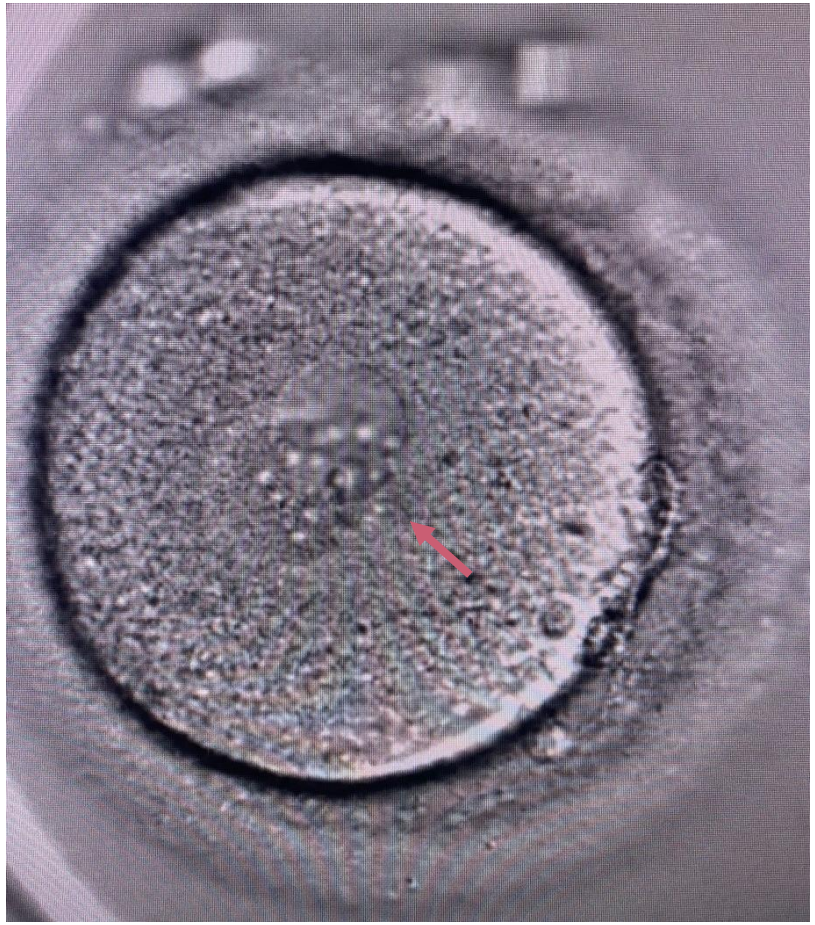
STUDY DESIGN

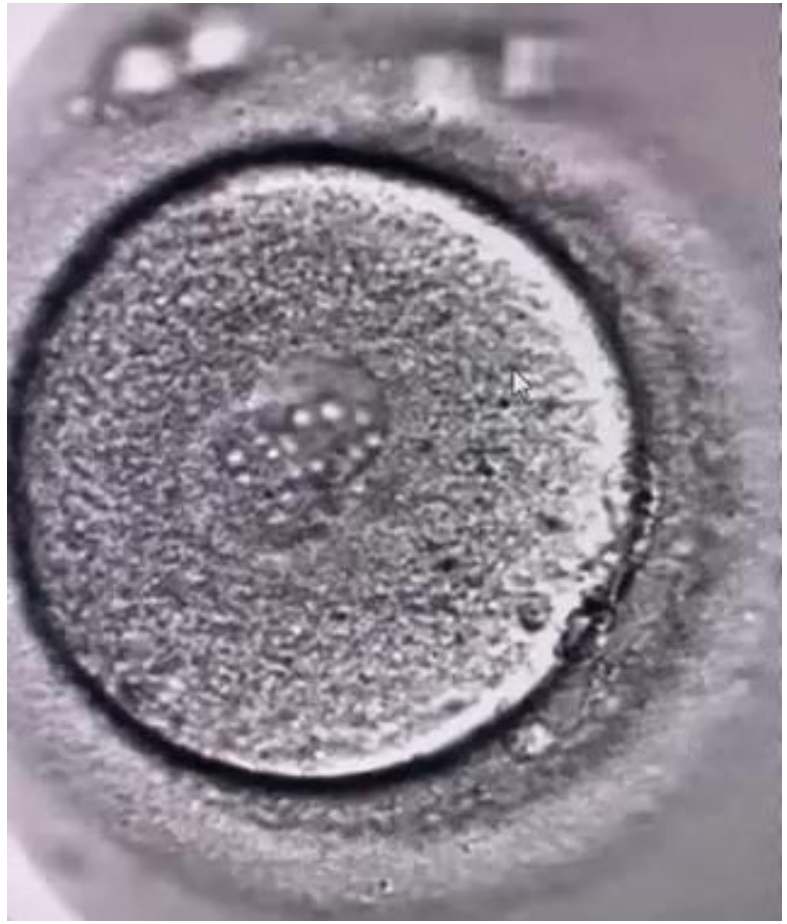


(2.1)

The synchronised movement of the small and uncertain pronucleus alongside the two larger ones, when rewound and played forward, is very helpful in determining whether it's a true 2.1 or not.







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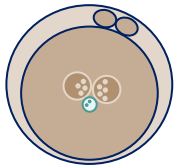
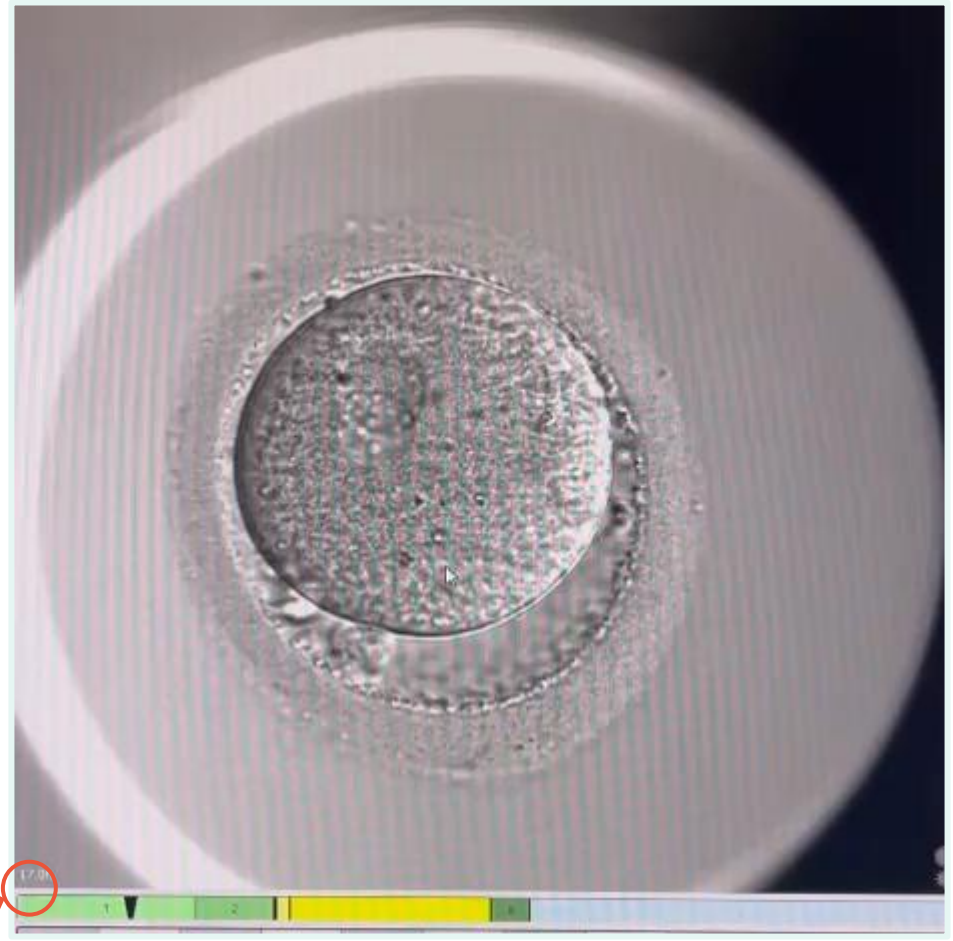
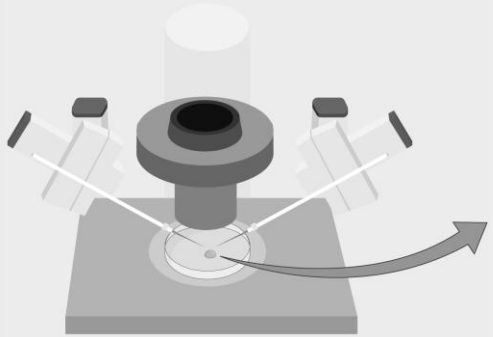


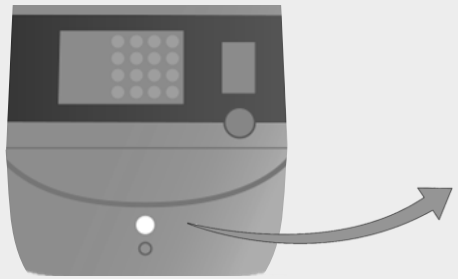
(2.1)

Diameter of the smallest pronucleus <70% of the diameter of the largest pronucleus



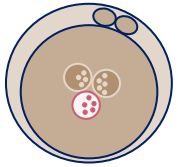


2.1 embryos might be difficult to identify.



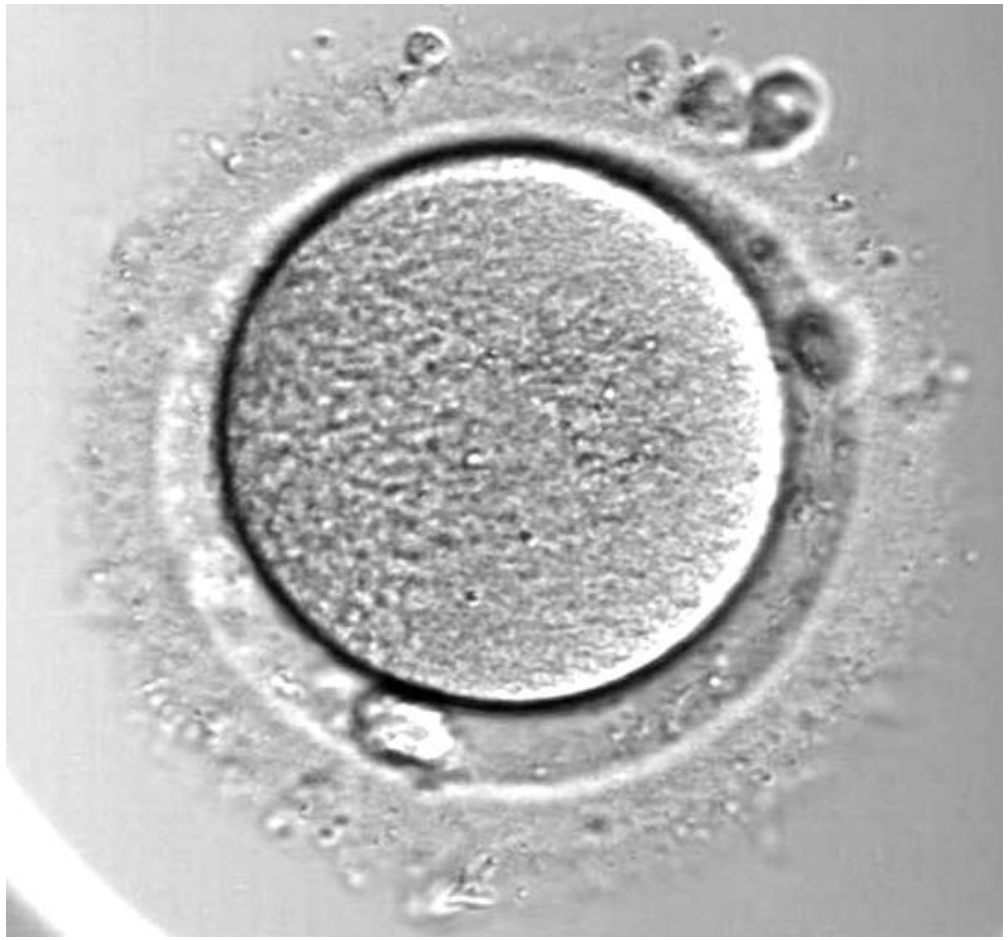
Timelapse incubators are helpful in identifying these small pronuclei, especially in challenging cases.

STUDY DESIGN



Same-size 3PN (SS)

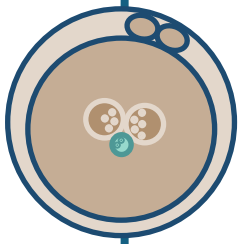
Diameter of the smallest pronucleus >70% of the diameter of the largest pronucleus



RESULTS

2.1 GROUP
(mean age 34,2 years)

189 embryos

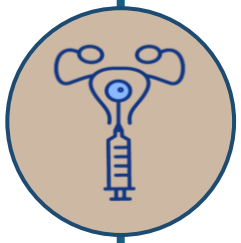


57 diploid and 7 triploid

69 reached GQ blastocyst stage

12 euploid, 3 mosaic and 49 aneuploid

64 informative por ploidy and aneuploidy analysis

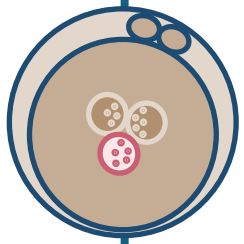


15 transferable embryos (12 diploid euploid, 3 diploid mosaic)

RESULTS

SS GROUP
(mean age 36,9 years)

160 embryos



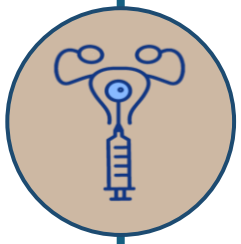
2 diploid and 46 triploid

60 reached GQ blastocyst stage

19 euploid and 29 aneuploid

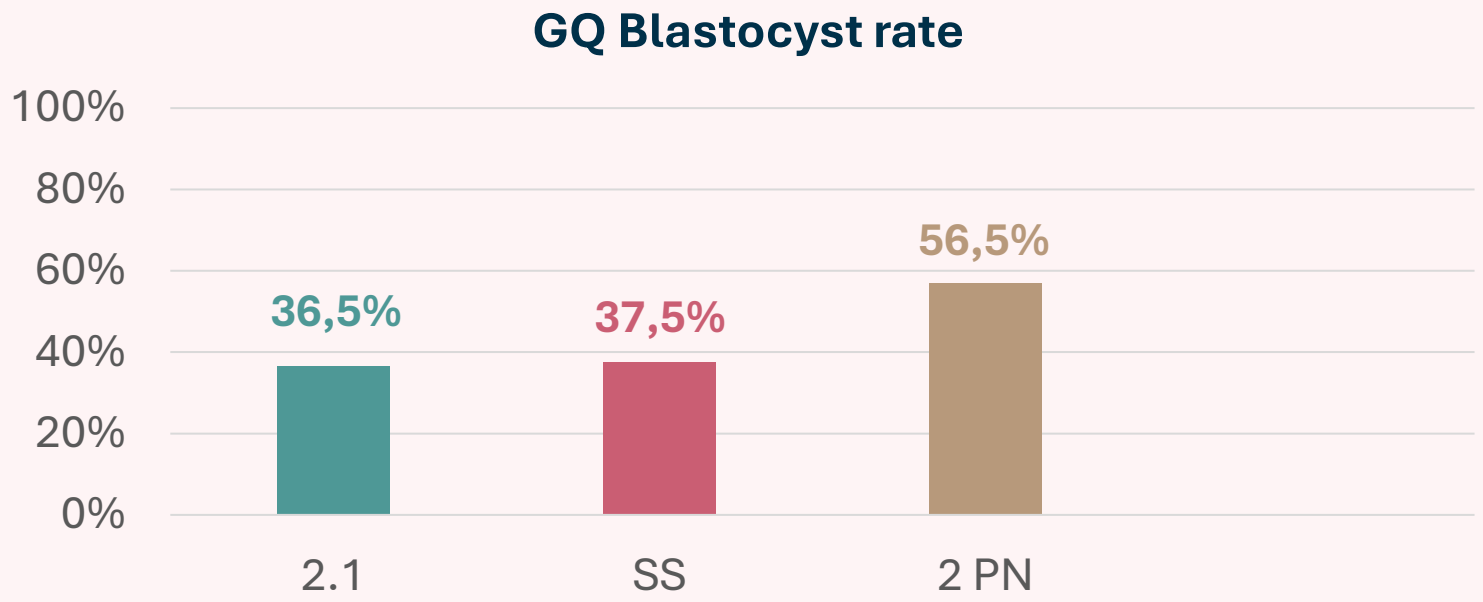
48 informative por ploidy and aneuploidy analysis

1 transferable embryo (1 diploid euploid)



RESULTS

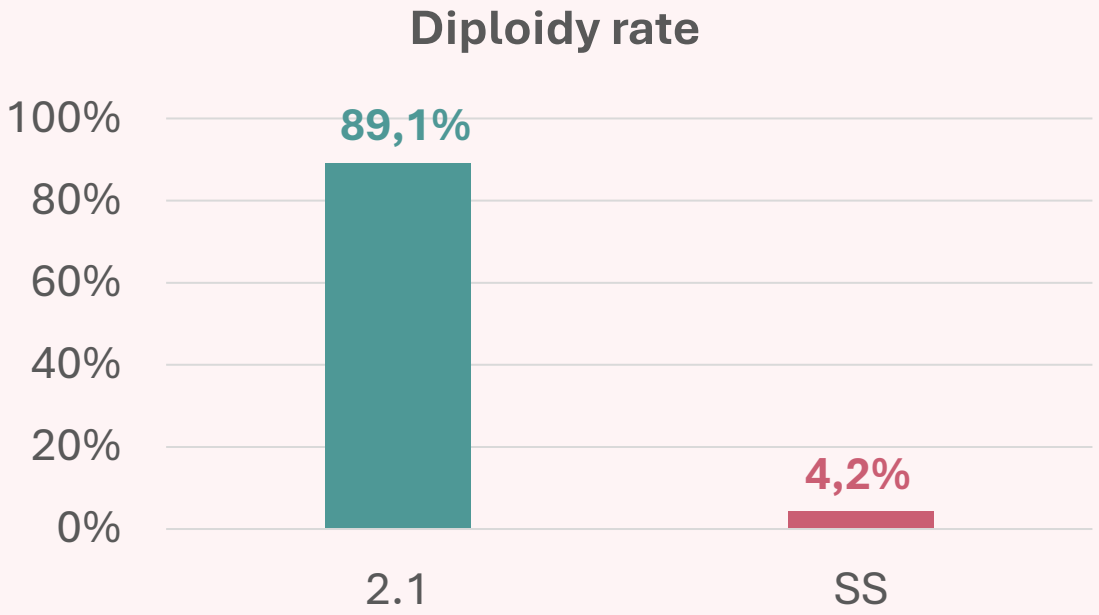
Blastocyst formation



No significant differences were found between the Good Quality (GQ) blastocyst rates of the 2.1 group (36,5%) and the SS group (37,5%) *(pvalue 2.1-SS>0.897),* but these rates are significantly lower than the GQ blastocyst rate of the 2PNs embryos on those 317 cycles, their sibling embryos (56,5%). *(pvalue 2.1-2PN<0.001). (pvalue SS-2PN<0.001).*

RESULTS

Ploidy status



64 2.1
informative biopsied blastocysts

57 DIPLOID
7 TRIPLOID

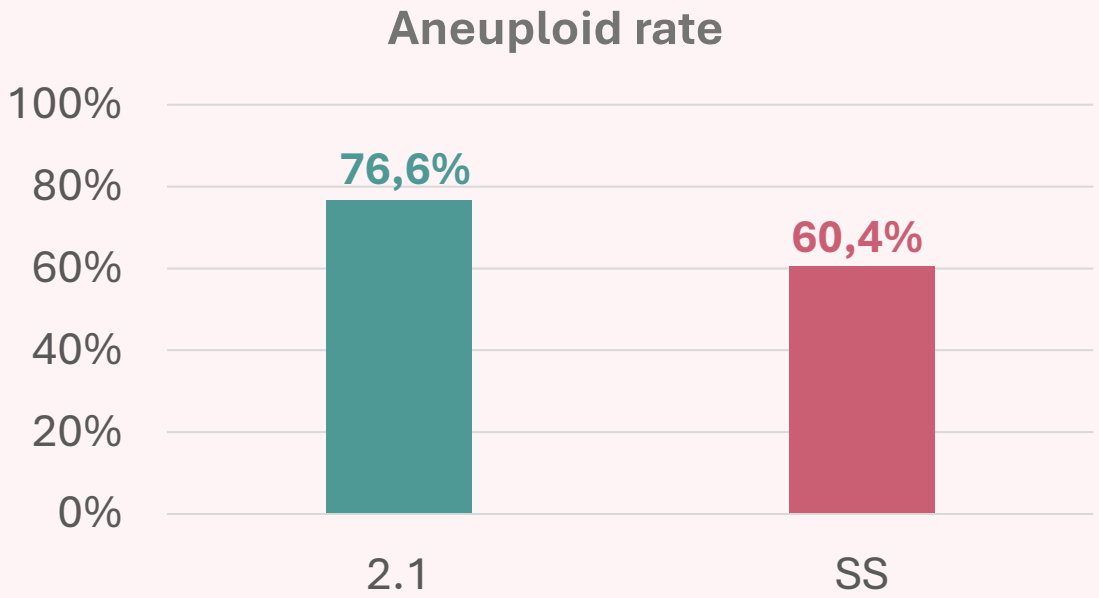
48 SS
informative biopsied blastocysts

2 DIPLOID
46 TRIPLOID

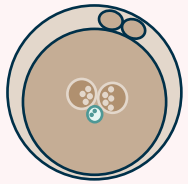
- The 2.1 group had 89,1% of diploidy rate, while the SS group had 4,2% of diploidy rate, showing significant differences between them ($p<0.001$).

RESULTS

Euploidy status



Mean age 34,2



12 Euploid
3 Mosaic
49 Aneuploid

Age 33,2
Age 32,3
Age 34,6

Mean age 36,9

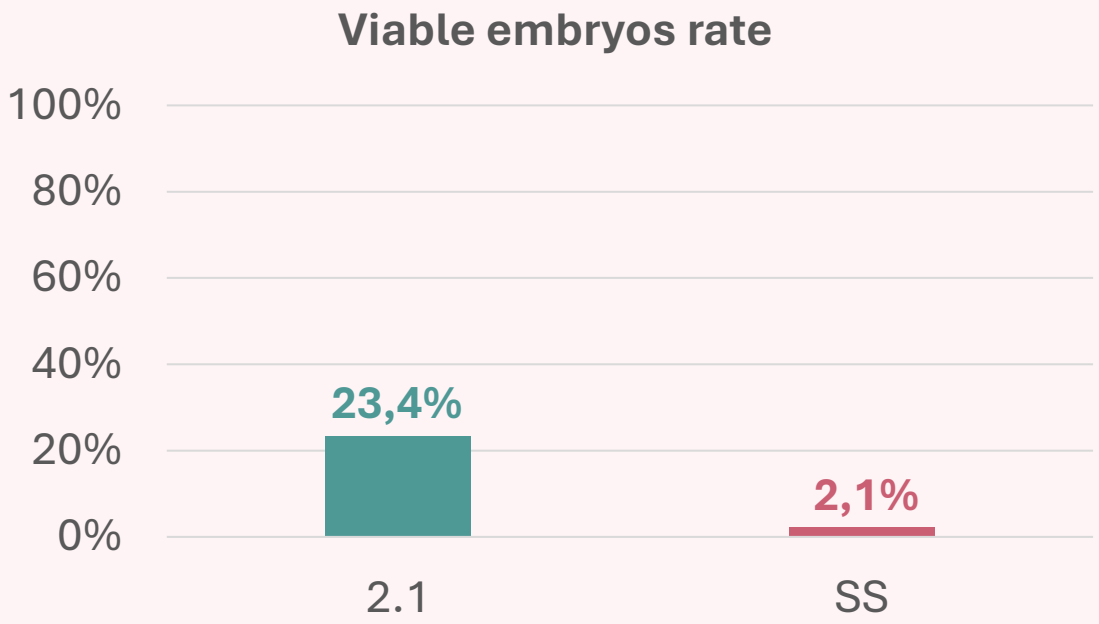
19 Euploid
29 Aneuploid

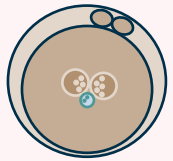
Age 34,5
Age 38.5

- Despite being higher in 2.1 group, no significant difference was observed in aneuploidy rate (p 0,443).
- A larger sample size is required to draw definitive conclusions in a PGT-A program. At present, it appears that the SS group has a similar euploidy rate to the 2PN embryo population, which varies with age. However, the euploidy rate in the 2.1 group is lower, despite being younger, potentially due to the characteristics of this type of embryo. We will be able to make more conclusive findings as the number of biopsied 3PN embryos increases.

RESULTS

Viable embryos: Available for transfer





57 DIPLOID
(64 2.1)

12 EUPLOID
3 MOSAIC
42 ANEUPLOID

2 DIPLOID
(48 SS)

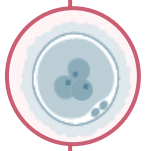
1 EUPLOID
1 ANEUPLOID

16 Viable embryos Available for transfer

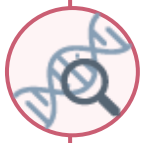
- **23,4%** of 2.1 biopsied embryos result in a transferable embryo (15/64)
- **2,1%** of SS biopsied embryos result in a transferable embryo (1/48)

CONCLUSION

Take home messages

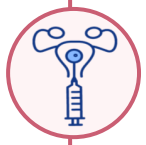


It is important to differentiate the two types of 3PNs, this would help us to know the potential of the embryo and advising the patient in the best way.

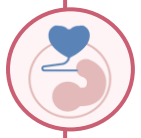


Most of the 2.1 3PNs are diploid (89,1%) so it is worth to biopsy and analyse them using SNPs technology, as it informs us about the ploidy and euploidy status of the embryo.

Despite most of them being diploid, the remaining 10,9% of 2.1 embryos are truly triploids so we should not transfer them without a previous analysis , as they could result in a triploid pregnancy and a molar pregnancy.



Regardless being triploid or diploid, embryos could still be euploid, mosaic or aneuploid so with PGT-A is still needed to check if embryos are suitable for transfer.



23,4% of the 2.1 biopsied blastocysts are viable embryos, increasing the number of viable embryos available for transfer for the benefit of the patient.

Thank you!

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