REPORT OF A VIABLE PREGNANCY ARISING FROM A ZYGOTE WITH A SINGLE PRONUCLEUS AT 16 HOURS POST INSEMINATATION

HEMA SARNA, ALPESH DOSHI, SUZANNE CAWOOD, URVASHI SARNA, ANASTASIA MANIA, RABI ODIA, PAUL SERHAL

Centre for Reproductive and Genetic Health, London, W1W 5QS, United Kingdom

Introduction:

Following intracytoplasmic sperm injection (ICSI), the oocyte cell cycle is resumed and a second polar body is extruded (McLay and Clarke, 2003). At 16-18 hours post insemination (hpi) the fertilised oocyte should display two pronuclei (PN); one pronucleus originates from the sperm and the other belongs to the oocyte (Nagy *et al.,*1994). An absence of fertilisation can be inferred when there are no visible PN's. Routinely, 1PN oocytes are discarded as they are thought to be abnormally fertilised (Lim *et al*., 2000).

Objective:

This report describes a case where a single embryo**,** showing one pronucleus at 16 hpi**,** was transferred following pre-implantation genetic diagnosis (PGD) and gave rise to a viable pregnancy.

Methods:

A female aged 37 years underwent assisted reproduction. The couple are beta-thalassaemia carriers and the primary aim of treatment was to have a healthy child**.** The secondary aim was to have a child who is a human leukocyte antigen (HLA) match for their affected child. The couple commenced treatment with ICSI followed by PGD at the blastocyst stage.

Results:

Post injection (16hpi), the patient had the following fertilisation results: 7x2PN, 4x1PN and 1xOPN. Following ICSI- PGD, the 1x1PN embryo was found to be normal (not a carrier/affected by beta thalassaemia) and was HLA identical. This embryo was graded 1BB using the Cornell blastocyst grading scheme (Veeck and Zaninovic, 2003) and resulted in a live birth when transferred to the patient.

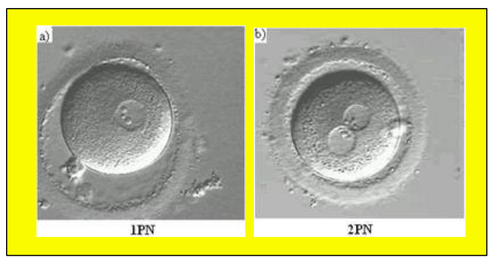

Image 1: The absence and presence of fertilisation. 1a) 1PN oocyte, 1b) 2PN oocyte. Image adapted from www.lindenberg.dk

Table 1: Table 1: PGD results for beta thalassaemia and HLA matched embryos

Embryo number	Diagnosis	Transferable
1	No diagnosis	Further analysis required
2	Carrier- HLA non identical	Not advised for this case
3	Carrier- HLA non identical	Not advised for this case
4	Affected- HLA identical	No
5	Normal- HLA non identical	Not advised for this case
6	Carrier- Monosomy 6	No
7	Carrier- HLA non identical	Not advised for this case
Unfertilised 1 (1PN with 2PB)	Normal HLA identical	Yes
Unfertilised 2 (1PN with 2PB)	Carrier- Monosomy 6	No
Unfertilised 3 (1PN with 2PB)	No diagnosis	Further analysis required

Following PGD analysis**:** two embryos were undiagnosed, one embryo was normal- HLA non identical, one embryo was normal HLA identical (transferred), three embryos were carriers- HLA non identical, two embryos were carrier's- monosomy 6 and one embryo was affected- HLA identical.

Discussion:

1PN embryos can arise due to:

- Parthenogenetic activation, however this is uncommon and karyotype analysis on such embryos suggests that around 80.5% of 1PN embryos are diploid (Staessen *et al.,*1993).
- Asynchronous pronuclear appearance at the time of fertilisation check or due to late pronuclear formation at the assessment stage. This is due to the possibility of early male or female pronuclear membrane disappearance or late formation of either the male or female PN (Nagy *et al*., 1998).

Conclusion:

As shown in this case report, 1PN embryos can potentially be genetically normal. Therefore if carrying out PGD it is advisable to analyse cells from unfertilised embryos which have clearly progressed and reached the blastocyst stage as these embryos may have fertilised correctly but outside of the fertilisation check period.

References

Lim A, Goh V, Su C and Yu S. Microscopic assessment of pronuclear embryos is not definitive. Hum Genet. 2000; 107: 62–68

McLay D and Clarke H. Remodelling the paternal chromatin at fertilisation in mammals. Reproduction. 2003; 125: 625-633

Nagy Z, Janssenswillen C, Janssens R, Vos A, Staessen C, Van de Velde H and

Van Steirteghem A. Timing of oocyte activation, pronucleus formation and cleavage in humans after Intracytoplasmic sperm injection (ICSI) with testicular spermatozoa and after ICSI or in-vitro fertilization on sibling oocytes with ejaculated spermatozoa. Human Reproduction. 1998; 13(6):1606–1612

Staessen C, Janssenwillen C, Devroey P and Steirteghem V. Cytogenetic and morphological observations of single pronucleated human oocytes after in-vitro fertilisation. Human Reproduction. 1993; 8: 221-223

Veeck L, Zaninovic N. An Atlas of Human Blastocysts. Human Health and Science. 2003