The value in retaining non-pronuclear (0PN) zygotes for extended culture

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Objective: To determine the occurrence and ploidy status of usable embryos derived from oocytes not displaying two pronuclei (0PN) at the time of fertilization assessment.

Design: Retrospective study in a private in vitro fertilization laboratory.

Materials and Methods: A total of 3,370 blastocysts were identified as being morphologically suitable for fresh embryo transfer, vitrification or trophectoderm biopsy for genetic testing of ploidy status followed by vitrification (i.e. usable blastocysts). Usable blastocysts were obtained on Day 5, 6, or 7. Mature oocytes were fertilized using ICSI and pronuclear assessment was performed and recorded using an inverted microscope 16-18 h post insemination. Embryos with 2 pronuclei were separated from all other embryos arising from 0PN, 1PN, 3PN and ≥4PN zygotes. Embryos were group cultured in a continuous single culture medium (Irvine Scientific). Chromosomal copy number was assessed for suitable blastocysts with the Illumina MiSeq platform. Embryos reported as normal had no detectable copy number aberration, at a threshold of ≤30%. A threshold of ≥50% was defined for any whole chromosome gain/loss for chromosomes 1, 2, 3, 4, 5, 10, 12, 17, 19, and/or 22. A threshold of ≥30% was set for chromosomes 6, 7, 8, 9, 11, 13, 14, 15, 16, 18, 20, 21, X, Y and any segmental duplications and/or deletions for any of the chromosomes. A complex abnormality result was defined as combination of three or more copy number abnormalities consisting of trisomy and/or monosomy.

Results: Over the span of 18 months, 830 fresh oocyte retrievals resulted in 7,258 zygotes with two haploid pronuclei (2PN). These 2PN zygotes developed into 3,349 usable blastocysts (46%). 2.9% of the oocyte retrievals had usable blastocysts derived from oocytes not displaying pronuclei (0PN) at the time of fertilization assessment. 24 usable blastocysts (from 0PN zygotes) from 24 different patients were vitrified. 14 (58%) of these blastocysts were untested for chromosomal copy number confirmation. 10 blastocysts derived from 0PN zygotes were screened by PGS. Four embryos (44%) were reported as normal while two embryos were reported as abnormal XO and three embryos as complex abnormal XY. No transfer of a usable blasotocyst derived from a 0PN has yet occurred.

Table 1: Ploidy Status of Usable 0PN Blastocysts

Embryo	Trophectoderm Diagnosis
1	Complex +5, +8, +16; XY
2	Abnormal; XO
3	Complex -2, -3, +6, -8, -12; XY
4	Complex +3, -5, +19, -15, -16, -17, -18. -19, +20, +21; XO
5	Abnormal; XO
6	Normal XX
7	Normal XX
8	Normal XY
9	Normal XY
10-24	Unknown

Conclusion: Our results demonstrate that usable blastocysts derived from zygotes with abnormal pronuclear (0PN) formation can develop into a chromosomally normal embryo. However, these embryos may also have a high possibility of multiple chromosomal gains or losses. These results are similar to

previous reports of developmental potential, chromosomal analysis and ongoing pregnancies from zygotes containing abnormal pronuclei[1-3]. Patients should be counseled regarding the chromosomal abnormalities associated with untested usable blastocysts derived from atypical zygotes. Further investigation of ploidy status of usable blastocysts derived from abnormal zygotic embryos with a single pronucleus (1PN), three pronuclei (3PN) or higher (≥4PN) may provide further information regarding the retention value of abnormal pronuclear zygotes.

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