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Title:
IS REPRODUCTIVE POTENTIAL COMPROMISED WHEN EMBRYOS ARE RE-BIOPSIED?

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Background:

Genomic analysis of embryos has revolutionized reproductive medicine. Periodically, technical limitations (allele drop-out, DNA amplification failure, etc.) may generate inconclusive or non-diagnostic results. In these instances, embryos can be thawed to undergo a second biopsy to ascertain ploidy status.

Objective:
We investigated whether >1 trophectoderm biopsy impacts embryonic implantation potential.

Materials and Methods:
This case control study included patients who underwent euploid frozen embryo transfer (FET) from November 2012 to April 2016. Quantitative polymerase chain reaction (PCR) and array comparative genomic hybridization (aCGH) were used to perform comprehensive chromosomal screening (CCS). Patient cohorts were segregated by the count of trophectoderm biopsies (TB) performed on a blastocyst prior to being selected for FET. The Single TB group included patients whose previously frozen, unscreened embryos were thawed, biopsied and re-vitrified. Once confirmed to be euploid, these blastocysts were rewarmed and transferred. The Double TB group included patients whose CCS results from an initial trophectoderm biopsy were interpreted as inconclusive. Their blastocysts were thawed and underwent a second trophectoderm biopsy (TB) to confirm their euploid status prior to undergoing repeat freeze-thaw and subsequent FET. Patient demographics including age, BMI, ovarian reserve and endometrial thickness at FET were compared. Primary outcomes included rates of implantation, clinical pregnancy and early

pregnancy loss. Student's t-test, chi-square, linear and binary logistic regression analysis were performed.

Result(s):

Seventeen patients who required two TBs to obtain a conclusive CCS result prior to a FET were compared to the Single TB group (n = 42 patients). Both cohorts underwent vitrification and thawing twice. Baseline demographics, FET cycle characteristics and outcomes are shown in Table 1. After controlling for BMI (which was significantly lower in the Double TB cohort) and number of embryos transferred per cycle, the odds of implantation (OR 0.4 [95% CI 0.1-1.4], p=0.15) and clinical pregnancy (OR 0.5 [95% CI 0.2-1.6], p=0.24) were not reduced in patients who pursued two TBs. Furthermore, the odds of early pregnancy loss (OR 0.5 [95% CI 0.1-2.2], p=0.4) were not increased in the double TB cohort.

Conclusion(s):

While current laboratory techniques safely allow for repeated embryo vitrification and thawing, this study suggests that the performance of a second TB does not appear to compromise blastocyst implantation potential. In the near future, biopsy and analysis of cryopreserved/thawed embryos will likely be more frequently performed as the scope of genomic information accessible from amplified embryonic cells continues to expand.

Table 1:

	Single Biopsy (n=42)	Double Biopsy (n=17)	P value
Patient age at ET	37.8 ± 4.1	36.6 ± 4.8	NS
Oocyte age	35.5 ± 3.7	35.0 ± 4.3	NS
Parity	0.2 ± 0.5	0.4 ± 0.6	NS
BMI at ET	23.8 ± 3.9	21.6 ± 3.1	<0.05
Day 3 FSH	7.0 ± 2.9	6.3 ± 1.8	NS
Endometrial Thickness at ET (mm)	9.1 ± 1.8	8.5 ± 0.9	NS
Average number of blastocysts transferred	1.1 ± 0.3 (n=46)	1.2 ± 0.4 (n=20)	NS
Implantation rate	50.0% (21/42)	70.6% (12/17)	NS
Clinical pregnancy rate	47.6% (20/42)	64.7% (11/17)	NS
Early pregnancy loss rate	14.3% (6/42)	23.5% (4/17)	NS