

TWO-PRONUCLEI (2PN) BANKING TO REDUCE COSTS FOR PATIENTS UNDERGOING MULTIPLE PGT-A CYCLES: ONE CENTER'S EXPERIENCE

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Background: For many patients with infertility, in vitro fertilization (IVF) and embryo banking is one strategy to create an option for future fertility. However, this can be very expensive, especially when multiple cycles are required to create a usable embryo. This is often the case for those with diminished ovarian reserve or older patients, as only 20% of embryos from patients over 41 years old lack chromosomal abnormalities (1). In addition to the increased expense, the need to complete multiple rounds of IVF to obtain a usable embryo has a physical and emotional burden(2). Typically, embryos are created after retrievals, cultured, and biopsied after each retrieval cycle. However, this can be time consuming and demoralizing if cycles do not yield usable embryos. A new approach of vitrifying embryos at the 2PN stage with group culture and blastocyst biopsy was initiated to consolidate procedures and provide a more streamlined and cost-effective treatment strategy for poor prognosis patients. However, is a paucity of published data as to whether vitrified-thawed 2PN embryos have similar outcomes, as measured by blastocyst rate, compared to continually cultured embryos after each individual cycle (3).

Objective: Investigate the impact of vitrification at the 2PN stage with subsequent culture, biopsy, and revitrification of embryos from multiple cycles report on outcomes of multi-cycle vitrification at the 2PN stage strategy with resultant combined culture to the blastocyst stage for biopsy.

Materials and Methods: Data was prospectively collected on patients who opted to undergo multiple cycles with embryos vitrified at the 2PN stage, then thawed, cultured to blastocyst, biopsied, and re-vitrified as a group. This was compared to standard blastocyst culture cycles, matched for age and number of 2PN embryos created. Primary outcomes were blastocyst and euploid embryo rates. Analysis and comparison between the 2PN and the control group was performed using the Chi-Square tests and Student's t-test as appropriate.

Results: The 2PN group of 63 patients (mean age 39.5 years) completed 158 oocyte retrievals, matched with 158 control cycles. From the 681 2PN embryos in each group, there were 255 (2PN) vs. 306 (controls) blastocysts biopsied ($p=0.07$). The average blastocyst (35.7% vs. 41.9%; $p=0.19$) and euploid embryo/2PN rate per patient (12.7% vs. 16.5%; $p=0.24$) were similar, but there were overall significantly fewer euploid blasts/2PN in the 2PN group (78/681 vs. 114/681; $p=0.02$). When stratified by age, the 2PN group <40-years-old had higher blastocyst development (44.7% vs. 25.2%; $p<0.01$) and more euploid embryos available for transfer than the ≥ 40 -year-old 2PN group, but similar blastocyst development to the <40-year-

old controls. The older 2PN group had a lower percentage of euploid embryos than the older control group (3.0% vs. 6.9%; p=0.03).

Conclusions: For patients <40 years old planning multiple IVF cycles, banking embryos at the 2PN stage provides an option for consolidation of procedures reducing overall cost and simplifying the process. However, multiple vitrification sessions in older patients may result in less embryos available for transfer.

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References:

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Table 1:			P-Value
Per Patient	2PN (n = 63)	Control (n = 158)	
Patient Age (years)	39.5 +/- 3.4	39.1 +/- 3.0	0.26
AMH (ng/ml)	1.1 +/- 1.0	1.8 +/- 1.9	0.01
Number of cycles (n)	2.5 +/- 0.8	1.0	-
Blastocyst Rate/2PN	35.7 +/- 24.1	41.9 +/- 34.7	0.19
(%)	12.7 +/- 18.7	16.5 +/- 22.3	0.24
Euploid Rate/2PN (%)	681	681	1.0
2PN total (n)	255	306	0.07
Blastocysts (n)	78	114	0.02
Euploid blastocysts (n)	23	26	0.67
Mosaic blastocysts (n)			
Per Cycle	2PN (n = 158)	Control (n = 158)	
Oocytes (n)	6.9 +/- 5.9	8.1 +/- 5.7	0.07
MII (ICSI) (n)	5.7 +/- 4.9	6.0 +/- 4.4	0.65
2PNs (n)	4.3 +/- 3.9	4.3 +/- 3.9	1.0
Blastocysts (n)	1.6 +/- 1.7	1.9 +/- 2.2	0.15
Euploid blastocysts (n)	0.5 +/- 0.7	0.7 +/- 1.0	0.02
Usable (LLM + Euploid)	0.5 +/- 0.8	0.8 +/- 1.1	0.02

Table 1: Data for 2PN vs. control patients and data for 2PN individual cycles vs. control cycles. 2PN = Two Pronuclei; AMH = Anti-Mullerian hormone; ICSI = intracytoplasmic sperm injection; LLM = low level mosaic. Data is reported as mean +/- SD.

2PN Patients by Age	Age < 40.0 (n = 34)	Age > 40 (n = 29)	P-Value
Age (years)	37.0 +/- 2.8	42. +/- 1.4	<0.01
AMH (ng/ml)	0.9 +/- 0.7	1.3 +/- 1.2	0.10
Number of cycles	2.4 +/- 0.8	2.7 +/- 0.8	0.19
Oocytes (n)	16.8 +/- 12.4	18.0 +/- 17.2	0.78
2PN/patient	10.3 +/- 7.3	11.0 +/- 10.9	0.30
Blastocysts/patient	4.6 +/- 5.1	3.4 +/- 4.0	0.29
Euploid blastocyst/patient	2.0 +/- 2.2	0.3 +/- 0.6	<0.01
Blastocyst rate/2PN (%)	44.7 +/- 25.2	25.2 +/-17.5	<0.01
Euploid rate/2PN (%)	21.1 +/- 21.3	3.0 +/- 6.7	<0.01
Number of Embryos (n)			
2PNs	350	331	-
Blastocysts	157	98	0.05
Euploid blastocysts	68	10	<0.01
Mosaic blastocysts	19	4	0.03
Usable blastocysts	87	14	<0.01

Table 2: Data for 2PN patients comparing above and below age 40.0 years. 2PN = Two Pronuclei; AMH = Anti-Mullerian hormone; Data is reported as mean +/- SD

Model effect	Values	OR (95% CI)	P-Value
Odds of having a usable blastocyst			
Patient Age	Per year	0.69 (0.59-0.78)	
AMH	Per 1 pg/ml	1.48 (1.20-1.88)	
2PN Banking	2.5 cycles vs 1 cycle	2.27 (1.15-4.55)	

Table 3: Logistic regression analysis per patient for total and usable number of blastocysts created

Figure 1. Graphs comparing (a) blastocyst and euploids rates (b) total blastocyst and euploid numbers and (c) embryos per cycle between the 2PN and control groups.

