The utility of embryo banking in order to increase the number of embryos available for preimplantation genetic screening in advanced maternal age patients

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Abstract

Purpose To determine if embryo banking with PGS is more optimal than proceeding with PGS regardless of embryo number.

Methods Patients were divided into 2 groups, group 1 were those that banked embryos and proceeded through another round of IVF prior to PGS, and group 2 underwent PGS regardless of embryo number. Group 2 was divided into group 2A (patients with >10 embryos) and group 2B (patients who had <10 embryos).

Results There was no difference in embryos biopsied, normal embryos, number transferred, and pregnancy rate between group 1 and 2. A significant number of patients did not have a transfer in group 2B (6/11) compared to group 1 (3/19) ($P=0.0419$). There was no significance between pregnancy rates per transfer between group 1 (6/16) and group 2B (2/5).

Conclusion Our data suggests that banking will increase the odds of going to transfer but there was no increase in pregnancy rates.

Keywords Aneuploidy · Embryo banking · IVF · PGS · Preimplantation genetic screening

Introduction

Patients diagnosed with advanced maternal age (AMA) exhibit a high rate of chromosomal aneuploidy following preimplantation genetic screening (PGS) [1–3]. Previous reports demonstrate that roughly 60–80% of all embryos produced will be aneuploid for this age group [4–6]. Due to the low oocyte and embryo yield, this group must make a difficult choice in terms of PGS. If the patient proceeds with PGS with a low embryo number, it is possible that transfer will not occur. If the patient decides to proceed without PGS, a transfer will occur but the chromosome status is unknown [7].

Here we present a new option, freezing prior to PGS to effectively “bank” embryos for testing. For example, patients can go through one in vitro fertilization (IVF) cycle and freeze all their embryos at the zygote stage prior to PGS. Subsequently, the patient would go through another IVF cycle and the frozen embryos would be thawed and combined with the fresh embryos. This would increase the amount of embryos available for PGS and hopefully increase the likelihood that the patient will have normal embryos to transfer. The other option would be to go through multiple IVF cycles with PGS regardless of embryo number. Continuous attempts post PGS cycles where normal embryos were transferred have shown continually poor results in IVF outcome, not to mention the financial burden on the patients [8].

PGS is often the last course of action which allows patients and clinicians to use PGS results as a means of closure. Due to the relative constant rate of aneuploidy between cycles, advocates believe that patients should be consulted to proceed with other pregnancy options such as egg or embryo donation following failed PGS cycles (with the transfer of chromosomal normal embryos) or in cases of all abnormal embryos [9–11].

Capsule Banking embryos prior to PGS increases number of embryos to test and number normal in poor responders, but does not increase pregnancy rates.

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It may be possible to increase the pregnancy rate while decreasing the financial burden of IVF in conjunction with PGS by banking embryos prior to PGS. This would increase the embryo availability for PGS testing while decreasing the financial cost of such testing on so few embryos. The purpose of this study is to determine if embryo banking at the zygote stage yields a more positive result than proceeding with PGS regardless of embryo number.

Materials and methods

Study design

This was a comparative, retrospective study involving 38 patients diagnosed with AMA whom underwent IVF in conjunction with PGS (9 or 12 probe fluorescence in-situ hybridization) between December 2006 and April 2010. Group 2 was divided into two groups, patients who had >10 embryos (group 2A) and patients who had <10 embryos (group 2B).

Ovarian stimulation

Patients underwent controlled ovarian hyperstimulation using uFSH (Bravelle, Ferring Pharmaceuticals, Inc., Parsippany, New Jersey, USA) with GnRH agonist pituitary down-regulation (Lupron; TAP Pharmaceuticals, North Chicago, Illinois, USA) or GnRH antagonist (Ganirelix; Organon Pharmaceuticals, Inc., Roseland, New Jersey, USA) and hCG (Profasi; Serono Laboratories Inc, Norwell, Maine, USA). Cycles were monitored with follicular ultrasound measurements and serum estradiol levels (post day 6 of stimulation start). hCG was given when one or more follicles had a diameter of ≥ 18 mm. Egg retrieval was conducted by a transvaginal ultrasound 38 h after hCG administration.

Egg retrieval, sperm preparation, conventional insemination, and intracytoplasmic sperm injection (ICSI) are discussed elsewhere [12].

Fertilization and embryo assessment

Sixteen to 20 h after insemination, the oocytes were evaluated for fertilization. Fertilization was considered normal when two pronuclei were seen. The fertilized oocytes were transferred to cleavage media (Cooper/Sage, Bedminster, New Jersey, USA) with 10% SPS, under oil, and returned to the incubator.

Embryo assessment took place 42 to 46 h (day 2) and 66 to 70 h (day 3) post insemination. Day 3 embryos were given a letter score of “A” through “D”, where “A” was the highest and “D” the lowest quality embryo.

Freezing and thawing of zygotes and banking

All patients regardless of embryo number were given the option to bank their zygotes and proceed with another IVF cycle. Zygote freezing occurred 1–2 h post fertilization check on those patients that wished to bank their embryos. Zygote freezing and thawing was conducted utilizing the one step cryopreservation method [13]. Frozen and thawed zygotes were cultured separately from fresh zygotes.

Embryo biopsy, randomization and blastocyst culture

Embryos were biopsied on day 3 regardless of cell number. Each embryo was placed in individual 20 μ L drops of Calcium-free and Magnesium-free PBS supplemented with 5% SPS. Embryos were properly positioned with a holding pipette (Humagen, Charlottesville, VA). A laser (SaturnActive Laser, Research Instruments, Cornwall, United Kingdom) was used to make a hole in the zona pellucida. Using a biopsy pipette (Humagen, Charlottesville, VA), a cell containing a visible nucleus was removed and isolated from the embryo. Embryos were rinsed and placed into blastocyst media (Cooper/Sage, Bedminster, NJ) supplemented with 10% SPS and placed into the incubator.

Isolated cells were fixed to a slide (Fisher Scientific, Pittsburgh, PA) using 3:1 methanol/glacial acetic acid stored at -30°C prior to use. Six cells were fixed per slide and sent to a reference lab for either 9 probe or 12 probe fluorescence in-situ hybridization.

Blastocyst assessment took place 120 to 124 h (day 5) and 144 to 148 h (day 6) post insemination. The evaluation consisted of the presence and score of inner cell mass and trophoctoderm. Day 5/6 embryos were given a letter score of “A” through “D”, where “A” was the highest and “D” the lowest quality embryo.

Statistical analysis

Unpaired *t*-tests, fisher’s exact test, and chi-square test for independence were applied where appropriate and statistical significance was set at $P < 0.05$.

Results

A total of 38 patients were included in this study. 19 patients banked (group 1) their embryos and 19 patients did not (group 2). Group 2 was divided into two groups, patients who had >10 embryos (group 2A) and patients who had <10 embryos (group 2B). Group 2A was not used in the calculations with the control group because they had >10 embryos and stimulated well enough to do PGD without banking.

There was no significant difference in number of embryos biopsied, number normal embryos, average number transferred, and pregnancy rates between the group 1 and 2 (Table 1). A greater number of patients did not have a transfer in group 2 compared to group 1, 3/19 (15.8%) and 8/19 (42.1%) respectively ($P=0.1510$; fisher's exact test; Table 1). When the pregnancy rate (+hCG) is calculated based on transfers, there is a strong insignificant trend between group 1 (6/16 +hCG; 37.5%) and group 2 (7/11 +hCG; 63.6%) ($P=0.2519$; fisher's exact test; Table 1). Of the 6 that were pregnant from group 1, 1 (16.7%) did not continue to a heartbeat. Of the 7 patients that were pregnant from group 2, 3 (42.8%) did not continue to a heartbeat ($P=1.0000$; fisher's exact test; Table 1).

As a control group we compared group 2B (<10 embryos; poor responders) to group 1. There was no difference between ages of group 1 (41.6 ± 1.7 years) and group 2B (42.3 ± 2.3) ($P=0.3480$; unpaired *t*-test; Table 2). The average number biopsied was higher in group 1 compared to group 2B, 10.6 ± 4.1 and 6.1 ± 2.3 embryos, respectively ($P=0.0024$; unpaired *t*-test; Table 2). The average number of embryos diagnosed as normal was significant between group 1 and group 2B, 2.0 ± 1.8 and 0.6 ± 0.8 embryos, respectively ($P=0.0217$, unpaired *t*-test, Table 2). The total number of normal embryos was not significant between group 1 (38/201, 18.9%) and group 2B (7/67, 16.4%) ($P=0.1320$, fisher's exact test, Table 2). Group 1 had an increased average number to transfer compared to group 2B, 1.5 ± 1.2 and 0.6 ± 0.8 embryos, respectively ($P=0.0354$, unpaired *t*-test, Table 2). In group 2B 6/11 (54.5%) of the patients did not have a transferred compared to 3/19 (18.9%) in group 1 ($P=0.0419$, fisher's exact test, Table 2). There were no differences in pregnancy rates (either hCG or clinical) between group 1 and group 2B calculated per egg retrieval or per embryo transfer (Table 2).

Table 1 Cycle characteristics of banked vs. not banked patients

	Banked cycles (group 1)	Not banked cycles (group 2)	<i>P</i> value
# Patients	19	19	
Avg. Age	41.6 ± 1.7	41.1 ± 2.5	0.4756 ^a
Avg. # Biopsied	10.6 ± 4.1	9.3 ± 5.4	0.4088 ^a
Avg. # Normal	2.0 ± 1.8	1.8 ± 2.3	0.7670 ^a
# Normal (%)	38 (17.1%)	33 (18.9%)	0.6932 ^b
Avg. # Transferred	1.5 ± 1.2	1.1 ± 1.2	0.3111 ^a
# No ET (%)	3 (15.8%)	8 (42.1%)	0.1510 ^b
+ hCG (%) per ET	6 (37.5%)	7 (63.6%)	0.2519 ^b
+ FCA (%) per ET	5 (26.3%)	4 (21.1%)	1.0000 ^b

^a = unpaired *t*-test

^b = fisher's exact test

Table 2 Cycle characteristics of banked cycles vs. control patients

	Banked cycles (group 1)	Control* (group 2B)	<i>P</i> value
# Patients	19	11	
# Egg Retrievals	38	11	
Avg. Age (years)	41.6 ± 1.7	42.3 ± 2.3	0.3480 ^a
Avg. # Biopsied	10.6 ± 4.1	6.1 ± 2.3	0.0024 ^a
Avg. # Normal	2.0 ± 1.8	0.6 ± 0.8	0.0217 ^a
# Normal (%)	38 (18.9%)	7 (16.4%)	0.1320 ^b
Avg. # Transferred (ET)	1.5 ± 1.2	0.6 ± 0.8	0.0354 ^a
# No ET (%)	3 (15.8%)	6 (54.5%)	0.0419 ^b
+ hCG (%) per ER	6 (15.8%)	3 (27.3%)	0.4003 ^b
+ FCA (%) per ER	5 (13.1%)	2 (18.2%)	0.6465 ^b
+ hCG (%) per ET	6 (37.5%)	2 (40.0%)	1.0000 ^b
+ FCA (%) per ET	5 (26.3%)	2 (40.0%)	1.0000 ^b

*AMA, poor responders (<10 embryos) who did not bank

^a = unpaired *t*-test

^b = fisher's exact test

Discussion

The purpose of banking embryos is to increase the number of embryos that undergo PGS. In theory, increasing the number of embryos to test would increase the number of normal embryos, increase the number to transfer, and therefore increase the pregnancy rate. In order for banking to even be considered, a high survivability of frozen embryos is crucial.

In our study, 91/124 (73.4%) zygotes survived being thawed and were biopsied. Interestingly, even though there was no difference in aneuploidy between the two groups, embryo quality and cell number was compromised between groups (Table 3). Previous research suggests that cryopreservation influences embryo quality but does not increase aneuploidy [14]. The low survival could be attributed to the quality and age of the patient population. Different cryopreservation techniques may yield different results in terms of embryo quality and survival. Research indicates that pre-freeze morphology is a predictor of cryopreservation survival [15]. Due to this groups age, we would expect the morphology of zygotes and embryos to be compromised which could result in poor thaw survival. Another possibility is that the frozen and fresh embryos were from different IVF cycles and each cycle may yield different embryo qualities.

Iwarsson and colleagues [16] found a high degree of chromosomal abnormalities in frozen-thawed embryos (25% normal). Our data shows that 16.5% of the frozen embryos were normal. This difference could be attributed to the number of chromosomes observed, Iwarsson and

Table 3 Embryo morphology of frozen and fresh embryos in a banked cycle

	Frozen embryos	Fresh embryos	<i>P</i> value
Number Frozen	124	—	
# Embryos	91	131	
Avg. Cell #	5.1±1.7	6.3±1.8	0.0132 ^a
Day 3 Embryo Quality			
# A (%)	17 (18.9%)	51 (38.9%)	0.0025 ^c
# B (%)	34 (37.4%)	39 (29.8%)	
# C (%)	31 (34.1%)	38 (29.0%)	
# D (%)	9 (9.9%)	3 (2.3%)	
Avg. # Biopsied	4.6±2.4	6.6±2.8	0.0236 ^a
Day 5 Embryo Quality			
# A (%)	5 (5.5%)	17 (13.0%)	0.0020 ^c
# B (%)	15 (16.5%)	18 (13.7%)	
# C (%)	20 (22.0%)	52 (39.7%)	
# D (%)	51 (56.0%)	44 (33.6%)	
# Normal (%)	15 (16.5%)	27 (20.6%)	0.4890 ^b
# ET (%)	12 (13.2%)	18 (13.7%)	1.0000 ^b

^a = unpaired *t*-test^b = fisher's exact test^c = Chi-squared test for independence

colleagues [16] utilized 5 probe FISH while we performed 9 or 12 probe FISH. We did not standardize based on either 9 probe or 12 probe FISH because it would decrease our numbers. Previous research has found that 12 probe only accounts for 3.5% more chromosomal abnormalities than 9 probe FISH [17]. Of the 38 patients in the study only 7 patients underwent 9 probe FISH (18.4%). The small number of patients utilizing 9 probe FISH in this study should not influence our results.

In our study there was no significant difference in the number of embryos biopsied from each group (Table 1). There are two possible reasons for this outcome, patients whom opted not to bank yielded a higher number of embryos compared to those that did bank (as the more embryos the less likely the patient would bank) or banking successfully increased the total number of embryos available for biopsy. If the patients whom opted not to bank yielded a higher number of embryos then the patient populations would be different as they would not be considered poor responders. To account for this we compared fresh cycle parameters between our control group (group 2B) and group 1 (Table 4). Our data shows that the average number of follicles and fertilization rate were significantly lower in group 2B compared to group 1 (Table 4). The lower number of follicles indicates that patients in group 2B stimulated more poorly than those patients that banked (Group 1).

Table 4 Fresh cycle parameters of patients who banked and those that did not bank

	Banked cycles	Not banked cycles (Group 2B)	<i>P</i> value
# Patients	19	11	
Avg. Age	41.6±1.7	42.3±2.3	0.3480 ^a
Avg. # Oocytes	10.1±3.5	9.4±5.5	0.6723 ^a
# Fertilized	134 (70.0%)	59 (57.3%)	0.0397 ^b
Avg. # Fertilized	7.1±2.6	5.4±2.8	0.1044 ^a
Day of hCG	9.5±1.6	9.3±1.4	0.7329 ^a
Avg. # Follicles	13.4±4.0	10.0±4.1	0.0344 ^a
E2 day of hCG	2052.8±1068.6	1684.0±931.4	0.3489 ^a

^a = unpaired *t*-test^b = fisher's exact test

To act as a control we isolated patients from group 2 that can be considered poor responders (<10 embryos; group 2B). We compared group 2B (11 patients) to group 1. We found that banking did increase the number to biopsy, average number normal and number for subsequent transfer. Our data shows that banking increases the odds of obtaining a transfer in poor responders. The pregnancy rates per embryo transfer did not differ in poor responders that opted to bank or not bank. Interestingly, the pregnancy rate per egg retrieval between banked cycles and the poor responders that did not bank was not significant (Table 2). The pregnancy rate per egg retrieval in banked cycles is identical to the pregnancy rate in patients from the same age group from our clinic.

Research suggests that there may be a threshold at which performing PGS may not be beneficial in patients with AMA. Munne and colleagues [18] suggest eight or more zygotes. However our data shows that even if patients increase the number available for biopsy through banking the pregnancy rate is not influenced. Research by Ferraretti and colleagues [19] indicates that previous PGS cycles are predictive of subsequent IVF outcomes. Our data supports this claim indicating that increasing the number available for biopsy does not increase the overall pregnancy rate. We attribute this to the difference in patient populations between group 1 and group 2B.

In summary, banking increased the odds of having a transfer in poor responding patients diagnosed with AMA however the pregnancy rate per egg retrieval or transfer was not influenced. Our data suggests banking may be beneficial in a patient obtaining a transfer however it seems that pregnancy rates are not influenced. This could be due to our patient population who opted not to bank as they had a lower follicle number and fertilization rate compared to patients that banked embryos (Tables 2 and 4).

References

- Verlinsky Y, Cieslak J, Freidine M, Ivakhnenko V, Wolf G, Kovalinskaya L, et al. Pregnancies following pre-conception diagnosis of common aneuploidies by fluorescence in situ hybridization. *Hum Reprod*. 1995;10:1923–7.
- Magli MC, Gianaroli L, Munne S, Ferraretti AP. Incidence of chromosomal abnormalities from a morphologically normal cohort of embryos in poor prognosis patients. *J Assist Reprod Genet*. 1998;15:297–301.
- Munne S, Sandalinas M, Escudero T, Marquez C, Cohen J. Chromosome mosaicism in cleavage-stage human embryos: evidence of a maternal age effect. *Reprod Biomed Online*. 2002;4:19–28.
- Munne S, Chen S, Colls P, Garrisi J, Zheng X, Cekleniak N, et al. Maternal age, morphology, development and chromosome abnormalities in over 6000 cleavage-stage embryos. *Reprod Biomed Online*. 2007;14:628–34.
- Mastenbroek S, Twisk M, Van Echten-Arends J, Sikkema-Raddatz B, Korevaar JC, Verhoeve HR, et al. In vitro fertilization with preimplantation genetic screening. *New Engl J Med*. 2007;357:9–17.
- Staessen C, Platteau P, Van Assche E, Michiels A, Tournaye H, Camus M, et al. Comparison of blastocyst transfer with or without preimplantation genetic diagnosis for aneuploidy screening in couples with advanced maternal age: a prospective randomized control trial. *Hum Reprod*. 2004;19:2849–58.
- Practice Committee of Society for Assisted Reproductive Technology, Practice Committee of American Society for Reproductive Medicine. Preimplantation genetic testing: a practice committee opinion. *Fertil Steril*. 2008;90:S136–43.
- Gianaroli L, Magli C, Ferraretti AP, Munne S. Preimplantation diagnosis for aneuploidies in patients undergoing in vitro fertilization with a poor prognosis: identification of the categories for which it should be proposed. *Fertil Steril*. 1999;72:837–44.
- Gianaroli L, Magli MC, Ferraretti AP, Tabanelli C, Trombetta C, Boudjema E. The role of preimplantation diagnosis for aneuploidies. *Reprod Biomed Online*. 2002;4:31–6.
- Munne S, Escudero T, Colls P, Xuezhong Z, Oter M, Garrisi M, et al. Predictability of preimplantation genetic diagnosis of aneuploidy and translocations on prospective attempts. *Reprod Biomed Online*. 2004;9:645–51.
- Platteau P, Staessen C, Michiels A, Steirteghem AV, Liebaers I, Devroey P. Preimplantation genetic diagnosis for aneuploidy screening in women older than 37 years. *Fertil Steril*. 2005;84:319–24.
- Taylor TH, Wright G, Jones-Colon S, Mitchell-Leef D, Kort HI, Nagy ZP. Comparison of ICSI and conventional IVF in patients with increased oocyte immaturity. *Reprod Biomed Online*. 2008;17:46–52.
- Leibo SP. A one-step method for direct nonsurgical transfer of frozen-thawed bovine embryos. *Theriogenology*. 1984;21:767–90.
- Edirisinghe R, Jemmott R, Allan J. Comparison of growth rates of fresh and frozen-thawed embryos according to chromosomal status. *J Assist Reprod Genet*. 2005;22:295–300.
- Karlstrom PO, Bergh T, Forsberg AS, Sandkvist U, Wikland M. Prognostic factors for the success rate of embryo freezing. *Hum Reprod*. 1997;12:1263–6.
- Iwarsson E, Lundqvist M, Inzuna J, Ahrlund-Richter L, Sjoblom P, Lundkvist O, et al. A high degree of aneuploidy in frozen-thawed human preimplantation embryos. *Hum Genet*. 1999;104:376–82.
- Taylor TH, Hallowell SV, Phillips M, Orris JJ, Glassner MJ, Wininger JD. Comparison of 9 probe and 12 probe fluorescence in-situ hybridization. *Fertil Steril*. 2008;90:S490.
- Munne S, Sandalinas M, Escudero T, Velilla E, Walmsley R, Sadowy S, et al. Improved implantation after preimplantation genetic diagnosis of aneuploidy. *Reprod Biomed Online*. 2003;7:91–7.
- Ferraretti AP, Magli MC, Kopcow L, Gianaroli L. Prognostic role of preimplantation genetic diagnosis for aneuploidy in assisted reproductive technology outcome. *Hum Reprod*. 2004;19:694–9.