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ARTICLE

Blastocyst culture selects for euploid embryos: comparison of blastomere and trophectoderm biopsies

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Abstract Preimplantation genetic diagnosis and screening improves the chances of achieving a viable pregnancy, not only free of undesired single-gene defects but also aneuploidy. In addition, improvements in vitrification provide an efficient means of preserving embryos (blastocysts). By combining trophectoderm biopsy with recent improvements in vitrification methods, only those embryos that have proved themselves viable and potentially more competent are tested. Using array comparative genomic hybridization (aCGH) to assess all 24 chromosomes, aneuploidy rates were compared between day-3 blastomere biopsy and day-5 trophectoderm biopsy. Of those 1603 embryos, 31% were euploid, 62% were aneuploid and 7% not analysable. A significantly larger proportion of embryos were euploid on day-5 biopsy (42%) compared with day-3 biopsy (24%, $P < 0.0001$). The number of euploid embryos per patient was not significantly different. Combining extended culture, trophectoderm biopsy and aneuploidy assessment by aCGH and subsequent vitrification can provide a more efficient means of achieving euploid pregnancies in IVF.

KEYWORDS: aneuploidy, array comparative genomic hybridization, blastomere biopsy, preimplantation genetic screening, trophectoderm biopsy, whole-genome amplification

Introduction

A limiting issue in IVF is the age-dependent high incidence of aneuploidy with an abnormal number of chromosomes in the resulting embryos/fetuses. There are aneuploidies that are paternal in origin or are post meiotic, occurring after fertilization but the majority has been shown originating from meiotic nondisjunction in the oocyte (Handyside, 2012).

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This has been shown to increase with advancing maternal age (Munne, 2003; Munne et al., 1994). These untested, abnormal embryos, whether transferred or conceived via intercourse, do not implant, lead to miscarriage (Hodes-Wertz et al., 2012) or result in a fetus with an abnormal chromosome compliment often leading to termination.

In order to screen embryos for euploid status, traditionally day-3 cleavage-stage blastomere biopsy is performed with chromosome analysis of the most common aneuploidies diagnosed by fluorescence in-situ hybridization (FISH). The cells' DNA can also be amplified using PCR to test for known single-gene disorders and can provide results in time for a day-5 embryo transfer (Gutierrez-Mateo et al., 2009). Higher pregnancy rates are obtained in some patient groups using day-3 blastomere biopsy with fresh transfer on day 4 or 5 (Munne et al., 2003).

With the advancement in the detection of the full chromosome complement available with array comparative genomic hybridization (aCGH), embryos can be more fully screened to avoid transferring embryos that do not contain a normal karyotype (Hellani et al., 2008). In addition, with improvement in media as well as culture conditions, blastocyst formation can reach an average of 40–60% (Gardner and Lane, 2003). Blastocyst culture has been shown to improve pregnancy and implantation rates by allowing selection to occur in the Petri dish. The extended culture gives an embryo time to better express its potential and has become an invaluable tool for embryo selection. Observing development in a cohort of embryos and basing selection of embryos for transfer on blastocyst morphology alone gives better implantation and birth rates (Grifo et al., 2007). However, there are some embryos that arrest at the cleavage stage: they simply stop growing. These embryos prove that they have no further potential, and most have degraded DNA and/or are aneuploid (Munne, 2006).

This work hoped to improve success by combining two selection tools: selecting euploid embryos (genetic selection) that had achieved the blastocyst stage (developmental competence selection). This was accomplished by performing trophectoderm biopsy and 24-chromosome preimplantation genetic screening using aCGH onlyon embryos reaching the blastocyst stage (Forman et al., 2012; Schoolcraft et al., 2010). We compared the euploidy rates of day-3 blastomere biopsy to trophectoderm biopsy at the blastocyst stage to determine if extended embryo culture also selects for euploid status and then compared pregnancy and implantation rates between the two different days of biopsy.

Materials and methods

Patients

Patients presented to New York University Fertility Center with interest in preimplantation genetic screening since it had been performed here for years prior to this study. The use of aCGH as the method for genetic screening began in 2010 as the centre was convinced that screening all chromosomes was an improvement over screening a limited number of chromosomes using FISH probes. Recruiting patients for a new unproven technique (trophectoderm biopsy) was difficult. Laboratory personnel and physicians counselled patients regarding the possible benefits of performing biopsies only on blastocysts. Recruiting patients to participate in this study with cryopreservation and no fresh transfer as described below was a challenge. Each case was evaluated and options discussed with patients. Some patients were interested in the variation in treatment options, while others were more reticent. Others became aware of the new procedure and requested trophectoderm biopsy. Patients were enlisted for the study between 1 January 2010 and 1 September 2012. All patients desiring to undergo blastomere biopsy with preimplantation genetic screening for aneuploidy determination with or without gender selection were considered for the study. Patients with preimplantation genetic diagnosis for single-gene disorders or using donor oocytes were excluded from this study. Patients who presented prior to onset of trophectoderm biopsy were provided only the day-3 biopsy option.

Several patients undergoing trophectoderm biopsy had suffered repeated miscarriages and were diagnosed with Asherman's syndrome or other uterine issues and subsequently successfully used gestational carriers to carry their euploid embryos to term. These patients were excluded from the study.

Ovarian stimulation

All underwent ovarian stimulation involving: (i) luteal-phase down-regulation with a gonadtrophin-releasing hormone (GnRH) analogue; (ii) follicular-phase flare using GnRH analogue; and (iii) antagonist-controlled stimulations in which GnRH antagonist was administered when lead follicles achieved diameters of roughly 12 mm. All cycles included daily injections of gonadotrophins, either recombinant FSH (Gonal F, EMD Serono, Rockland, MA, USA; or Follistim, Merck, Whitehouse Station, NJ, USA) or a combination of recombinant FSH and human menopausal gonadotrophin (Menopur, Merck). Injection of human chorionic gonadotrophin (HCG; 10,000 IU urinary HCG or two vials of recombinant HCG (Ovidrel; EMD Serono) or leuprolide acetate (Lupron, Tap Pharmaceuticals, Lake Forrest, IL, USA) plus 1000 IU urinary HCG was used to trigger the final maturation, when at least two follicles had reached 18 mm, although individualized criteria are often used when a patient has had prior cycles. The oocyte retrieval was scheduled 36 h after the trigger.

Day-3 biopsy and genetic screening

Cleavage-stage blastomere biopsy was performed on day-3 embryos that had at least 4 cells. A hole was created in the zona pellucida using acidified Tyrode's solution (Sigma, St Louis, MO, USA). Calcium/magnesium-free medium (Global, Guilford, CT, USA) was used to loosen the cell–cell adhesion allowing for less traumatic suction removal of the cell from the embryo. The biopsied cell was then loaded into a PCR tube (Eppendorf) labelled with the corresponding embryo number and sent to the reference laboratory (Reprogenetics, Livingston, NJ, USA) on dry ice for analysis using whole-genome amplification. Embryos were then placed back in culture until aCGH results were obtained in time for a day-5 embryo transfer. The best-quality euploid

embryo(s) were then transferred using a Sureview Wallace catheter (Smith's Medical, UK) with ultrasound guidance. Progesterone supplementation was provided to patients with fresh transfers of biopsied embryos by injection of progesterone in oil or by vaginal administration (Crinone, Endometrin or suppositiories) beginning on the day following oocyte retrieval.

Trophectoderm biopsy and genetic screening

For blastocyst biopsy, a narrow channel was created in the zona pellucida using a laser (Cronus, Research Instruments, UK) on day 3. On days 5 and 6 and on rare occasions day 7, embryos were assessed and any fully differentiated good-quality blastocyst was biopsied. Using suction, the trophectoderm cells extruding from the expanded blastocyst were gently pulled and then a few pulses of the laser were used at cell junctions in order to safely remove a few cells without disrupting the inner cell mass. The cells were washed and loaded into PCR tubes labelled with corresponding embryo number and sent to the reference laboratory on dry ice for 24-chromosome analysis. Following trophectoderm biopsy, blastocysts were vitrified using Sage (Trumbull, CT, USA) or Irvine (Santa Ana, CA, USA) media and vitrification kits and CryoLocks (BioTech, Cummin, GA, USA) according to the manufacturer's instructions.

All biopsies were sent to a reference genetics laboratory for preimplantation genetic screening using whole-genome amplification and aCGH as described previously (Harton et al., 2013).

After the aCGH results were obtained, the patient's preparation for transfer of vitrified–warmed embryos began with oral oestradiol tablets (Estrace; Warner Chilcott, Rockaway, NJ, USA) beginning on day 2 of the next menstrual cycle. Once the uterine lining was at least 7 mm with a ring pattern, injections of progesterone in oil (Watson Pharma, Parsippany, NJ, USA) or suppositories (prepared by local pharmacies) were begun. On day 6 of progesterone, the desired number (generally one and sometimes two) euploid embryos were warmed using Sage or Irvine warming kits and then transferred to the patient's uterus under ultrasound guidance. Pregnancy results were obtained on day 28 of the cycle and confirmed on day 35 with ultrasound on day 42–49 for presence of intrauterine sac and fetal heart activity.

Specific outcome measures included rates of euploidy, implantation and clinical pregnancy. Euploidy rate was calculated using the number of euploid embryos divided by the total number of embryos that were biopsied. Implantation rate was calculated as the number of intrauterine gestational sacs visualized on ultrasound divided by the total number of embryos transferred. A clinical pregnancy was defined as the presence of an intrauterine gestational sac(s) with fetal cardiac activity as documented by ultrasound. Variables in the study groups were compared by chi-squared analyses and Fisher's exact test or Student's t-test, as appropriate. Statistical analysis was performed using Microsoft Excel, SPSS software and/or GraphPad online. Patients' acceptance of informed consent was indicated by their signatures in accordance with Institutional Review Board protocol (NYU IRB no. 512-03373, approved 10 October 2012).

Results

A total of 305 patients underwent ovarian stimulation: 131 in anticipation of blastomere biopsy and 174 in anticipation of trophectoderm biopsy. There were no significant differences in patient age, maximum day-2 or -3 FSH or incidences of most of the diagnoses or stimulation protocols between the day-3 and trophectoderm biopsy groups (Table 1). Patients in the trophectoderm biopsy group had a higher incidence of uterine factor infertility. Oocyte retrieval was cancelled because of poor ovarian response in 9.9% (13/131) of patients in the day-3 biopsy group and 4.6% (8/174) of patients in the trophectoderm biopsy group.

A total of 284 patients underwent oocyte retrievals: 118 for day-3 biopsy and 166 for trophectoderm biopsy. Patient treatment parameters were not significantly different, including day-2 FSH and oestradiol concentrations, total gonadotrophin, FSH and/or human menopausal gonadotrophin administered and days of gonadotrophin administration (Table 2).

In total, 1603 embryos were analysed (936 biopsied on day 3, 667 biopsied at the blastocyst stage) over the 2.5-year period. Maternal age at biopsy was (mean ± standard deviation) 38.1 ± 4.3 years (range 22–47 years) and was not significantly different between the two groups. Trophectoderm biopsies were performed on day 5 (59.6%), day 6 (38.7%) and day 7 (1.7%) just prior to vitrification.

There was no difference between patient parameters in the two biopsy groups when considering the numbers of oocytes retrieved, patients with intracytoplasmic sperm injection, mature oocytes, 2PN zygotes, embryos cultured beyond day 3 (extended culture) or cells in day-3 embryos (Table 3). There were significant differences between the number of embryos biopsied per patient (8.8 day-3 versus 4.7 trophectoderm biopsy; $P < 0.0001$). There were also significant differences in the number of blastocysts forming on days 5 or 6 (4.1 day-3 versus 5.3 trophectoderm biopsy; $P = 0.031$) and the number of blastocysts that achieved stage 2 or greater (3.4 day-3 versus 4.7 trophectoderm biopsy; $P = 0.012$; Table 2).

Ploidy results

Of those 1603 embryos that were biopsied, 31.3% ($n = 501$) were euploid, 62.1% were aneuploid ($n = 995$) and 6.7% ($n = 107$) had no diagnosis. There were 6.5 ± 4.6 embryos (range 1–35) biopsied and 2.0 ± 3.0 (range 1–17) were found to be normal. The proportion of biopsied embryos that were euploid was significantly larger following trophectoderm biopsy compared with day-3 biopsy (42% versus 24% expressed per biopsied embryo, $P < 0.0001$; 32% versus 23% per biopsied patient, $P = 0.011$; Table 3). The number of euploid embryos per patient was not significantly different between the day-3 and trophectoderm biopsy groups (2.1 versus 2.0).

Thirty-one patients (29%) who had embryos biopsied on day 3 had no euploid embryos. Twelve additional patients with at least one euploid embryo had no euploid blastocysts at the time of transfer (day 5) or the following day (day 6). (These patients underwent transfer of euploid morulae or euploid cleavage-stage embryos without blastocysts on

Table 1 Characteristics of patients who underwent ovarian stimulation.

	Blastomere biopsy (n = 131)	*Trophectoderm biopsy (n = 174)*
Age (years)	38.3 ± 3.9	39.2 ± 3.6
Maximum FSH (IU/l)	8.2 ± 4.5	7.9 ± 2.9
Infertility diagnosis		
Diminished ovarian reserve	20 (15.3)	23 (13.2)
Ovulatory dysfunction (PCO)	11 (8.4)	8 (4.6)
Male factor	17 (13.0)	23 (13.2)
Endometriosis	9 (6.9)	9 (5.2)
Tubal factor	1 (0.8)	8 (4.6)
Uterine factor[a]	5 (3.8)	23 (13.2)
Unexplained	12 (9.2)	8 (4.6)
Other	95 (72.5)	136 (78.2)
Protocol		
Luteal down-regulation	21 (16.0)	16 (9.2)
Flare	19 (14.5)	21 (12.1)
Antagonist	91 (69.5)	137 (78.7)
Cycle cancellation	13 (9.9)	8 (4.6)

Values are mean ± SD or % (*n*).
PCO = polycystic ovaries.
[a]*P* = 0.003.

Table 2 Cycle and embryological data of patients who underwent oocyte retrieval.

	Blastomere biopsy (n = 118)	*Trophectoderm biopsy (n = 166)*
Age (years)	38.5 ± 4.0	39.1 ± 3.9
Day-2 FSH (mIU/ml)	5.9 ± 3.1	6.4 ± 2.9
Day-2 oestradiol (pg/ml)	51 ± 43	49 ± 26
Gonadotrophin (total IU)	3950 ± 1520	4020 ± 1510
FSH (total IU)	2490 ± 1070	2480 ± 1000
HMG (total IU)	1460 ± 970	1540 ± 830
Days of gonadotrophin	9.7 ± 2.1	9.5 ± 1.6
Oestradiol (pg/ml) on trigger day	2300 ± 1130	2550 ± 1140
Oocytes	13.4 ± 7.8	14.2 ± 8.4
Patients with ICSI	39/118 (33)	45/166 (27)
Mature oocytes	11.0 ± 7.0	11.7 ± 7.5
2PN zygotes	8.6 ± 5.5	8.7 ± 5.5
Embryos in extended culture	8.2 ± 5.3	9.3 ± 5.7
Cells on day 3	6.1 ± 1.6	6.4 ± 1.8
Blastocysts[a]	4.1 ± 4.0	5.3 ± 4.7
Stage-2 blastocysts[b]	3.4 ± 3.7	4.7 ± 4.4
Patients with biopsy	106 (90)	141 (85)

Values are mean ± SD or % (*n*).
HMG = human menopausal gonadotrophin; ICSI = intracytoplasmic sperm injection.
[a]*P* = 0.031.
[b]*P* = 0.012.

day 5.) Therefore, there were 43 patients (41%) in the blastomere biopsy group with no euploid blastocysts on day 5 or 6. Fifty-four patients who had trophectoderm biopsies (38%) had no euploid blastocysts. The incidence of patients with no euploid blastocysts was not significantly different when expressed per biopsied patient (41%, 43/106 versus 38%,

Table 3 Results of patients who underwent embryo biopsy.

	Blastomere biopsy (n = 106)	*Trophectoderm biopsy (n = 141)*	*P-value*
Embryos biopsied	936	667	
Euploid embryos	224 (24)	277 (42)	<0.0001
Aneuploid embryos	643 (69)	352 (53)	<0.0001
Embryos with no determination	69 (7)	38 (6)	NS
Embryos biopsied/biopsy patient	8.8 ± 5.3	4.7 ± 4.0	<0.0001
Euploid embryos/biopsy patient	2.1 ± 2.4	2.0 ± 2.9	NS
Euploid embryos/biopsied embryo (%)	23 ± 21	32 ± 31	0.011
Euploid embryos/retrieval	1.9 ± 2.3 (118)	1.7 ± 2.8 (166)	NS
Euploid embryos/cycle	1.8 ± 2.3 (131)	1.6 ± 2.7 (174)	NS
Euploid blastocysts/retrieval	1.2 ± 1.6 (118)	1.7 ± 2.8 (166)	NS
Euploid blastocysts/cycle	1.1 ± 1.5 (131)	1.6 ± 2.7 (174)	NS
Cycles with no euploid embryos	55/131 (42)	87/174 (50)	NS
Cycles with no euploid blastocysts	68/131 (52)	87/174 (50)	NS

Values are mean ± SD (total numbers in parentheses) or % (*n*).
NS = not statistically significant.

54/141), per retrieval (47%, 55/118 versus 48%, 79/166) or per cycle initiated (52% 68/131 versus 50%, 87/174).

Transfer and pregnancy results

There were 75 embryo transfers in the blastomere biopsy group and 68 in the trophectoderm biopsy group, for which 125 embryos (1.7 ± 0.6 embryos per transfer) and 78 (1.2 ± 0.4 embryos per transfer) were transferred. There was a significantly higher implantation rate in the trophectoderm biopsy group compared with the embryo biopsy group (60% versus 41%, respectively, $P = 0.004$), but the clinical pregnancy rate was not significantly different (47% versus 57%, respectively) (Figure 1). However, since the numbers of embryos transferred in the two groups were significantly different (t-test, $P = 0.0001$), comparisons of clinical pregnancy rates may not be appropriate.

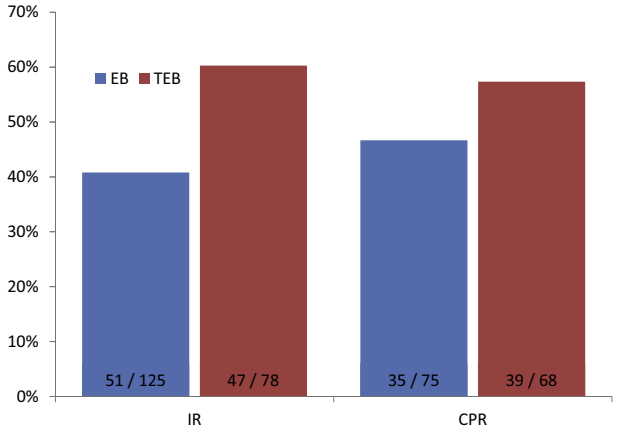

Figure 1 Implantation rates and clinical pregnancy rates per transfer for embryo and trophectoderm biopsies. Implantation rates were significantly different between embryo and trophectoderm biopsy ($P = 0.004$) when considering all patients with embryo transfers. Clinical pregnancy rates were not significantly different between the two groups. CPR = clinical pregnancy rate; EB = embryo biopsy; IR = implantation rate; TEB = trophectoderm biopsy.

Of the euploid embryos that were transferred, 31 babies were born in the blastomere biopsy group (25% of those transferred) and 45 babies were born in the trophectoderm biopsy group (58% of those transferred). There were three miscarriages and one stillbirth in the blastomere biopsy group (11.4%) versus six miscarriages in the trophectoderm biopsy group (15.4%). There were no misdiagnoses found in any of the babies born or in tissue recovered from the terminations that occurred due to nonviable pregnancies.

In the blastomere biopsy group, there were 12 fresh transfers that included only euploid nonblastocysts (morulae and cleavage-stage embryos). Only one pregnancy resulted from transfer of two morulae (implantation rate 14%, 2/14). In contrast, when only euploid embryos were transferred and the transferred embryos included at least one blastocyst, the implantation rate was 44% (49/111) and was significantly different from the implantation rate for nonblastocysts (Fisher's exact test, $P = 0.043$). The implantation rate of embryos biopsied on day 3 when blastocysts were transferred (44%) was not significantly different (according to Fisher's exact test) from the implantation rate of embryos undergoing trophectoderm biopsy, vitrification, rewarming and transfer (58%, 45/78).

Discussion

These observations provide evidence to support three important ideas: (i) extended culture enables selection of embryos with a greater chance of implantation and live delivery; (ii) selection of embryos that achieve the blastocyst stage enriches for euploid embryos; and (iii) extended culture does not lead to a significant loss of euploid embryos capable of implantation.

The present observations indicate that among euploid embryos biopsied on day 3, those that become blastocysts by the time of transfer are more likely to implant than those that do not become blastocysts by the time of transfer. It is clear that transferred euploid blastocysts selected on day 5

have greater potential for implantation than euploid embryos that had not reached the blastocyst stage at transfer. This suggests that there is a qualitative component of the embryo that is associated with ability to implant but that is unassociated with embryo ploidy. This qualitative component may simply be the embryo's potential to implant or a more complex embryo–uterus synchrony issue. This confirms Munne's results on discarded embryos and morphology along with also confirming day-5 embryo transfer yielding higher pregnancy and implantation rates (Gardner and Lane, 2003; Grifo et al., 2007). Extended culture enables selection of embryos with a greater chance of implantation and live delivery.

Our data demonstrate that embryos biopsied at the blastocyst stage (trophectoderm biopsy) have a higher euploidy rate than embryos biopsied as cleavage-stage embryos (embryo biopsy). Extending culture and performing biopsy at the blastocyst stage also results in lower aneuploidy rates as compared with day-3 biopsy. The selection process that occurs in the Petri dish eliminates the need to biopsy those embryos not competent to advance beyond the cleavage stage and supports the concept that selection is occurring in the laboratory. Biopsying at the blastocyst stage identifies only those embryos with the best chance for being chromosomally normal and developmentally competent. This is potentially the reason that extended culture provides higher pregnancy, implantation and live birth rates. Selection of embryos that achieve the blastocyst stage enriches for euploid embryos.

A distinction should be made between euploid embryos and euploid blastocysts in the blastomere biopsy group where some of the biopsied embryos found to be euploid failed to progress to the blastocyst stage by the time of transfer (day 5) or cryopreservation (day 6). This attrition accounts for the difference between numbers of euploid embryos and euploid blastocysts in the blastomere biopsy group. In the trophectoderm biopsy group, only blastocysts were biopsied so there was no difference between the number of euploid embryos and the number of euploid blastocysts. The enrichment in euploid embryos obtained by selection of blastocysts for biopsy was achieved with no significant loss of euploid embryos since neither the number of euploid embryos per patient nor the number of euploid blastocysts per patient was significantly different when comparing the two biopsy groups. Excluding the embryos that failed to progress to become blastocysts from testing had no impact on the patient other than to reduce the number of embryos biopsied. Extended culture does not lead to a significant loss of euploid embryos capable of implantation.

The advantages of trophectoderm biopsy over blastomere biopsy are not only selection, but also with trophectoderm biopsy more cells can be removed, compared with one blastomere at day 3 (Schoolcraft et al., 2010). This permits a more robust amplification and aneuploidy assessment. Although there are more cells removed for testing, a lesser percentage of the embryo is removed and the inner mass cells destined for fetal development are not compromised, presumably avoiding damage to the tissue that becomes the fetus. Along with the advanced development at the blastocyst stage, there is evidence of less mosaicism found compared with that of day-3 embryos (Grifo et al., 2007).

Advances in cryobiology, specifically vitrification, have allowed for cryopreservation and very high post-warming survival of the blastocysts post biopsy. Trophectoderm biopsy would not be universally feasible without reliable cryopreservation techniques. The ability to vitrify blastocysts with reliable, excellent warming results, allows time for analysis (Kuwayama, 2007) and minimal loss of precious, euploid embryos. Also there is evidence that an unstimulated cycle provides a potentially superior uterine environment as compared with conventional fresh embryo transfer (Shapiro et al., 2013). This allowed for transfer of warmed embryos in a programmed warming cycle. Transfer of vitrified–warmed euploid blastocysts was certainly not inferior to transfer of fresh euploid blastocysts in the current study centre. In addition, the implantation rate of vitrified, euploid blastocysts was significantly greater than implantation rate of fresh transferred embryos selected solely based on euploidy. It has been suggested that the uterine environment after exogenous oestrogen and progesterone may be less prone to detrimental effects thought to occur during ovarian stimulation. The success with this approach demonstrates that single warmed euploid embryo transfer is an extremely effective method for achieving euploid pregnancy.

Numbers of embryos per patient undergoing extended culture (beyond day 3) and the number of cells in embryos (on day 3) were not significantly different. In contrast, from this similar number of embryos with similar quality, a significantly lower number of blastocysts and blastocysts (stage 2 or greater) arose in the blastomere biopsy group compared with the trophectoderm biopsy group. The number of euploid embryos averaged 1.9 ± 2.3 per retrieval following blastomere biopsy. This number declined to 1.2 ± 1.6 euploid blastocysts on day 5 following blastomere biopsy due to failure of biopsied embryos to progress to the blastocyst stage. The performance of day-3 biopsy is one remarkably different feature that stands out when comparing these two groups of embryos. This suggests that day-3 biopsy itself is responsible for poorer development to the blastocyst stage and supports the contention of Scott et al. (2012, 2013) that blastomere biopsy on day 3 is detrimental to embryo development, although this study cannot exclude the possibility of simple, inherent poor quality of some of the day-3 euploid embryos. The number of euploid blastocysts averaged 1.7 ± 2.8 following trophectoderm biopsy. The lack of significance between the numbers of euploid blastocysts with embryo versus trophectoderm biopsy suggests that there was no significant loss of euploid embryos or blastocysts in association with extended culture without biopsy. This lack of significance gives confidence in delaying biopsy to the blastocyst stage.

The limitations of this study are that it is retrospective and that the study was performed longitudinally. The intent of this paper was to describe this study centre's experience with implementing trophectoderm biopsy and some notable observations about the success of trophectoderm biopsy relative to blastomere biopsy. Despite its longitudinal nature, the patients in the two study groups were quite similar as indicated in **Tables 1–3**, which show that the patients' ages, ovarian reserves, diagnoses, treatment parameters and responses were not significantly different. The

similarity between study groups demonstrates that comparisons of outcomes are meaningful.

In conclusion, by extending embryo culture to the blastocyst stage, a selection process occurs that enriches for euploid embryos. This further elucidates why higher pregnancy, implantation and live birth rates can be achieved with blastocyst transfer in unscreened embryos compared with day-3 transfer. This is accomplished by eliminating those embryos not competent enough to develop to the blastocyst stage in addition to enrichment for euploidy. However, trophectoderm biopsy adds a further tool by being able to screen out blastocysts (viable embryos) that are aneuploid, thereby improving chances of implantation by a normal embryo and delivering a healthy, chromosomally normal baby. Many morphologically normal blastocysts are aneuploid and selecting only euploid embryos lowers miscarriage rates (Hodes-Wertz et al., 2012). Combining extended culture, trophectoderm biopsy and aneuploidy assessment by aCGH and subsequent vitrification can provide a highly efficient means of achieving singleton euploid pregnancies in IVF.

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