Increased blastomere number in cleavage-stage embryos is associated with higher aneuploidy

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Objective: To evaluate the relationship between blastomere number and aneuploidy.
Design: Historical cohort study.
Setting: In vitro fertilization clinic.
Patient(s): Two hundred fifty-nine patients undergoing in vitro fertilization (IVF) in combination with comprehensive chromosomal screening of embryos.
Intervention(s): A total of 1,915 embryos were biopsied on day 3 and underwent comprehensive chromosomal screening with microarray-based comparative genomic hybridization.
Main Outcome Measure(s): Relationship between day 3 blastomere number, aneuploidy rate, and progression to the blastocyst stage.
Result(s): A number of day 3 blastomeres >9 was associated with significantly increased aneuploidy rates. Rapidly developing embryos were significantly more likely to blastulate regardless of their chromosomal status. Number of embryos per patient greater than 13 was independently associated with lower aneuploidy rates after controlling for maternal age. This trend was not significant with the use of a more clinically relevant threshold of greater than six embryos per patient.
Conclusion(s): Embryos with 6–9 cells at the cleavage stage should be considered for transfer over embryos with >9 cells. Day 3 blastomere number may be used in conjunction with extended culture to improve selection of euploid embryos, especially when supernumerary embryos are available. Further studies are needed to show if these selection criteria improve clinical outcomes. (Fertil Steril® 2015;103:694–8. ©2015 by American Society for Reproductive Medicine.)
Key Words: Cleavage-stage embryo, blastomere number, aneuploidy, comparative genomic hybridization, in vitro fertilization, embryo progression

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A major challenge of in vitro fertilization (IVF) is selection of embryos with the highest likelihood of being euploid. Uncertainty over embryo selection and transfer of multiple embryos significantly contribute to multiple pregnancy rates. A major obstacle to embryo viability is the high rate of aneuploidy in human oocytes and pre-implantation embryos, which strongly correlates with advancing maternal age (1, 2). Even young women (<35 years) have been found to have high aneuploidy rates, ranging from 20% to 44% according to quantitative polymerase chain reaction and up to 40%–70% according to comparative genomic hybridization (CGH) for chromosomal determination. Rates of aneuploidy progressively increase with age, reaching 50%–80% in women in their early 40s (3, 4). A major challenge has been to identify useful inexpensive and noninvasive criteria to guide embryo selection.

Two of the most commonly used methods for embryo selection are morphologic criteria and extended culture to the blastocyst stage. Morphologic criteria, including cell fragmentation and symmetry, have been used for embryo selection. Studies have found a weak association between better morphology and lower aneuploidy rates and improved clinical outcomes (5–7). Extended culture and blastocyst

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transfer have been found to have a small but significant increase in live birth rate compared with cleavage-stage transfer (8, 9). However, studies looking at chromosomal status of high-quality blastocysts still quote overall aneuploidy rates of 39%–57% (5).

Early developmental milestones, including timing of embryo cleavage, day 3 cell number, and time-lapse embryo imaging, have also been used to assess embryo quality and clinical outcomes. Early embryo cleavage 25–27 hours after insemination has been associated with a high rate of progression to the blastocyst stage as well as improved implantation and pregnancy rates (10, 11). Increased day 3 cell number has been associated with improved progression to the blastocyst stage, live birth rates, and embryo morphology (12–15). Studies have used time-lapse imaging to assess timing and coordination of early embryo development. Some studies have found synchrony, kinetics, and timing of early development to predict progression to blastocyst stage and to be associated with pregnancy success. However, data are still somewhat limited regarding clinical utility and increased cost (16–19).

Few studies have looked specifically at aneuploidy in relation to these early developmental milestones (20). Those that did were limited by the fact that chromosomal status was determined by means of fluorescent in situ hybridization (FISH), which fails to identify 20%–50% of abnormal embryos compared with CGH (5, 21). To the best of our knowledge, no studies to date have looked at the relationship between day 3 cell number and aneuploidy rates as determined by means of array CGH (aCGH).

The primary aim of the present study was to assess the relationship between day 3 cell number and aneuploidy rate. The secondary aim was to determine how day 3 cell number and chromosomal status predict developmental progression to the blastocyst stage and how these factors can be used to improve selection of euploid embryos.

MATERIALS AND METHODS

Patient Population

All patients included in the study had undergone IVF and preimplantation genetic screening with the use of aCGH at ART Reproductive Center in Beverly Hills, California from February 2010 to December 2011. Embryo biopsy and aCGH was performed in 29% of the IVF cycles during this time period. This technology was offered to patients regardless of diagnosis, with only 7.2% of screened embryos from patients with a diagnosis of recurrent pregnancy loss. There was no minimum number of embryos required to proceed with biopsy. The study was conducted after receiving Institutional Review Board approval. A total of 1,915 embryos from 259 patients were identified that had undergone chromosomal analysis with the use of aCGH and were included in the study. All patients undergoing IVF had received gonadotropins in conjunction with either a GnRH agonist or antagonist protocol. Ovulation was triggered with hCG 34–36 hours before oocyte retrieval.

Embryo Scoring and Chromosomal Analysis

Embryos were assessed for blastomere number and morphologic grade on day 3. Day 3 embryo assessment was performed 107–108 hours after hCG trigger administration. Morphologic grade A–D was assigned, with grade A being the highest grade and grade D the lowest. Major factors in determination of embryo grade included percentage of fragmentation and cell symmetry (22). Embryo biopsy was performed on day 3 of embryo culture with the use of the Zilos-tk noncontact infrared laser (Hamilton Thorne Biosciences), and one blastomere was removed for analysis. All embryos were then analyzed with the use of aCGH as previously reported (23, 24). Those embryos that were grade D or had <6 cells on day 3 were not biopsied and excluded from the study. Only those embryos determined by to be euploid based on a day 3 embryo biopsy were subsequently transferred.

Statistical Analysis

Analysis was performed with the use of a multivariate regression. The model assessed the relationship between day 3 cell number and aneuploidy rate. Patient age, paternal age, number of embryos per patient, morphologic grade, diagnosis of recurrent pregnancy loss, and nonindependence of embryos from the same patient were controlled for in the model. For all comparisons, a statistical significance was set at a P value of $<.05$. A threshold analysis (Z-score) was then performed to determine the best cutoff value for the relationship between aneuploidy and each significant continuous variable.

RESULTS

A total of 1,915 embryos from 259 patients were studied. The mean patient age was 37.5 years (range 21–47). The overall aneuploidy rate was 62%, and the majority (73%) of embryos were grade A or B. Embryos were stratified based on number of cells on day 3, with 84% of embryos with 6–9 cells and 15% of embryos with >9 cells. Embryos were further stratified by age, with 586 embryos in the ≤35 years age group, 563 embryos in the 36–40 years age group, and 471 embryos in >40 years age group (Table 1). The overall clinical pregnancy rate from single euploid embryo transfer of day 3–biopsied

TABLE 1

Embryo distribution by chromosomal status and day 3 cell number.

Characteristic		n (%)	
No. of patients		259	
Mean age (y)		37.5	
Total no. of embryos		1,915	
Overall aneuploidy rate, %		62	
Embryos per patient (n), mean		7	
Cells on day 3			
6–9		1,619 (84.5)	
>9		296 (15.5)	
Distribution by age group	<36 y	36–40 y	>40 y
6–9	586	563	471
Aneuploid	270 (46.1)	344 (61.1)	379 (80.5)
Euploid	316 (53.9)	219 (38.9)	92 (19.5)
>9	110	107	79
Aneuploid	59 (53.6)	74 (69.2)	67 (84.8)
Euploid	51 (46.4)	33 (30.8)	12 (15.2)

Note: Results presented as n (%) unless indicated otherwise.

Kroener. Blastomere number and aneuploidy rate. Fertil Steril 2015.

embryos during this time period was 40.4%. Pregnancy rates were further stratified by age, showing pregnancy rates of 54% for age <35 years, 54% for age 35–37 years, 40% for age 38–40 years, 29% for age 41–42 years, and 27% for age >42 years.

Aneuploidy rate was assessed as a function of day 3 cell number, which showed an increase in chromosomal abnormality as the number of cells on day 3 increased (Fig. 1). Based on a threshold analysis, 9 cells was the best cutoff value above which one would expect to find increased aneuploidy. A multivariate regression analysis was then performed looking at covariates that significantly affected the relationship between day 3 cell number and aneuploidy rate. For day 3 cell number >9, there was a significantly increased aneuploidy rate per embryo with an odds ratio [OR] of 1.39 (P=.0294). As expected, patient age was strongly associated with aneuploidy (P=.0005). Interestingly, number of embryos per patient greater than 13 was found to be associated with lower aneuploidy, independently from maternal age, with an OR of 0.70 (P=.0336). The diagnosis of recurrent pregnancy loss, paternal age, and embryo morphology/grade were nonsignificant covariates in predicting chromosomal status.

An increased number of embryos per patient was associated with a lower per-embryo aneuploidy rate. Patients with >13 embryos had the lowest rate of aneuploid embryos (OR 0.7; P=.0336). However, the number of individuals with greater than 13 embryos that underwent embryo biopsy was low. A lower, more clinically relevant analysis was done with the use of a threshold of greater than six embryos per patient. Patients who had greater than six embryos trended toward a lower aneuploidy rate compared with those who had fewer than six embryos, but the results were not statistically significant (OR 0.81; P=.1119). The analysis of patients with greater than six embryos was then stratified into three age groups: 1) <35 years; 2) 35–39 years; and 3) ≥40 years. There was a significant association between increased number of embryos greater than six and lower aneuploidy rate only for the youngest age cohort, <35 years (OR 0.57; P=.0327) and not for the older age groups.

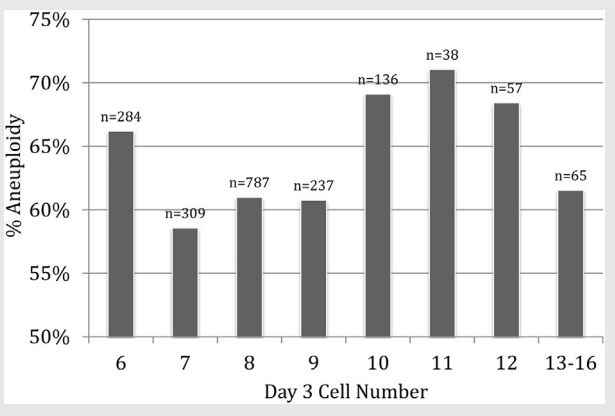
Blastocyst formation rate was assessed to evaluate the developmental potential of day 3 embryos with 6–9 cells compared with more rapidly developing day 3 embryos with >9 cells. The ability to blastulate was assessed as a function of number of cells on day 3 and chromosomal status. Rapidly developing embryos were significantly more likely to form blastocysts. Interestingly, this occurred regardless of whether the embryos were euploid or aneuploid (Fig. 2). Of those embryos that reached the blastocyst stage, normally developing embryos had a trend toward lower aneuploidy rates compared with rapidly dividing embryos (54% vs. 62%; P=.096).

DISCUSSION

Various methods are currently used in embryo selection with the goal to improve clinical outcomes in IVF. The most common clinically used noninvasive methods are morphologic characteristics and extended culture to day 5 for better selection of euploid embryos. Few studies have looked at the relationship between cleavage-stage development and clinical outcomes. These studies have been small and show inconsistent outcomes (12, 14). To our knowledge, this is the first study that looks directly at the relationship between chromosomal status, as determined with the use of aCGH, and early developmental milestones.

Blastocyst formation is associated with early timing of first cleavage and increased day 3 cell number (10, 11). Embryos that develop rapidly early on are more likely to continue developing at a rapid rate and form blastocysts. Our study confirms this finding, showing that rapidly dividing embryos with >9 cells on day 3 are more likely to become blastocysts than embryos with 6–9 cells on day 3. Interestingly, we found that rapidly developing embryos (>9 cells) were more likely to become blastocysts regardless of chromosomal status. When looking only at those embryos which progressed to blastocysts, a trend was seen between rapidly developing embryos on day 3 and

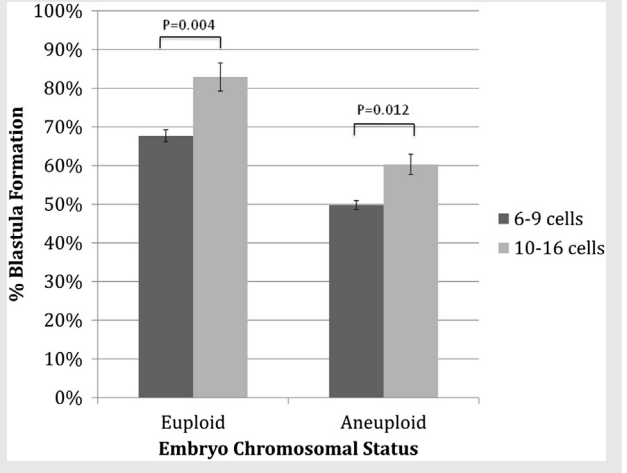
FIGURE 1



Day 3 cell number and aneuploidy rate.

Kroener. Blastomere number and aneuploidy rate. Fertil Steril 2015.

FIGURE 2



Rate of blastula formation according to day 3 cell number and chromosomal status.

Kroener. Blastomere number and aneuploidy rate. Fertil Steril 2015.

increased aneuploidy. When transferring blastocysts, evaluation of the cleavage stage may be considered along with morphology in embryo selection. This may be particularly useful in elective single-embryo transfer, because a high-grade blastocyst rising from a rapidly developing embryo may be more likely to be aneuploid than a blastocyst arising from a 6–9-cell embryo. The clinical outcome of these selection criteria could not be assessed in our cohort, because all of the embryos were selected for transfer based on known euploid status. A prospective study looking at the relationship between cleavage-stage progression and pregnancy rates in day 5 single embryo transfers without genetic screening would be the best way to validate these findings.

Interestingly, a predictor of aneuploidy was embryo number. If the number of embryos per patient was greater, the aneuploidy rate was lower. In particular, women <35 years old had an increased rate of embryo aneuploidy if they had fewer than six embryos resulting from the cycle. These results suggest that poor response may be an independent predictor of increased aneuploidy, especially in younger poor responders in whom poor ovarian response is less often encountered. Earlier literature has been variable as to whether poor ovarian response is associated with a higher rate of aneuploidy independently from age. Although one recent paper did not find a higher rate of aneuploid embryos in patients with poor ovarian reserve (25), multiple other studies, including a recent prospective trial, have demonstrated that overall poor responders are at higher risk of aneuploidy, which is consistent with our findings (26–28).

One limitation of the present study is that biopsy was performed on day 3. Embryo biopsies for chromosomal screening performed with the use of FISH on day 3 have less reproducibility compared with day 5 embryo biopsies (29). This is due, at least in part, to the incidence of embryo mosaicism and decreased accuracy of a day 3 biopsy when sampling only one or two blastomeres (30). However, literature suggests that aCGH has a high sensitivity rate and a low error rate compared with FISH, even with day 3 embryo biopsies (23). Additionally, day 3 embryo biopsy has been show to decrease reproductive potential of cleavage-stage embryos and could potentially affect embryo developmental progression to the blastocyst stage (31). One could hypothesize that biopsy itself could have more of an impact on embryos with fewer cells at the time biopsy, potentially skewing results. These limitations could be alleviated by the use of day 5 biopsies, which is becoming more standard for chromosomal screening. Finally, a larger sample size would have further powered our study.

Despite these limitations, this study provides guidance in the selection of embryos for transfer that is low cost and does not necessitate additional equipment. A more rapidly cleaving embryo on day 3 has traditionally been identified as a better embryo; however, embryos with >9 cells are more likely to be aneuploid compared with embryos with 6–9 cells. Therefore, there may be an advantage to selecting cleavage-stage embryos with 6–9 cells over more rapidly dividing embryos. Day 3 cell number, in conjunction with morphology and extended culture, may help to improve euploid blastocyst selection at the time of transfer, particularly when preimplantation genetic screening is not readily available or is not performed.

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