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COMMENTARY

A cautionary note against embryo aneuploidy risk assessment using time-lapse imaging

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Abstract Preimplantation genetic screening (PGS) for embryo aneuploidy using embryo biopsy is a widely available technique used to select embryos for transfer following IVF for certain patient populations. Since its introduction, there has been an ongoing search for a non-invasive technique to perform PGS. Such an advance would revolutionize the field of IVF enabling PGS to be used universally as a routine embryo selection tool with the potential to significantly increase pregnancy rates and decrease poor outcomes such as miscarriage. Recent publications illustrating the development of an algorithm using time-lapse imaging of IVF embryos have claimed to have done just this. We believe that the statements made in these articles, which include the proposed ability to increase pregnancy rates by determining embryo aneuploidy risk by time-lapse imaging, are premature and to this point unsubstantiated by the published data. We provide evidence from existing publications and from our own data that suggests that the statements recently made are misleading. We make the point that further investigation is needed either in the form of a larger, age-adjusted data set or preferably in a randomized controlled trial.

KEYWORDS: aneuploidy, blastocyst, embryo implantation, morphokinetics, preimplantation genetic screening, time-lapse imaging

We have read with great interest the two recent articles by Campbell et al. (2013a,b) that describe an algorithm using time-lapse imaging for aneuploidy risk classification of human preimplantation embryos. On the basis of their results, the authors postulated that by using specific morphokinetic markers they could reliably select euploid embryos for transfer. Thus, increasing pregnancy rates and reducing miscarriage rates due to aneuploidy. Although we believe that the authors have identified developmental time points — time from insemination to blastulation (t_{SB}) and time of insemination to full blastocyst (t_B) — that can be used to establish an embryo's implantation potential in their culture system, we believe that the study is underpowered and that maternal age rather than specifically embryo aneuploidy is likely to be the causative factor.

First, it is our opinion that there may be no need to analyse both t_{SB} and t_B since these time points appear to be highly linked, as shown by the authors in Figure 2 (Campbell et al., 2013a). The apparent linear relationship between the two variables, from the point of insemination, suggests that using only one of the variables would likely result in similar statistically significant findings. We conclude that the two articles by Campbell et al. are based on the premise that faster developing blastocysts are more likely to be euploid and hence implant and result in a pregnancy.

From what is known in the published literature and based on the analysis of our data, we do not agree that faster developing blastocysts have a greater implantation potential due to lower level of aneuploidy than those with slower developmental rates. Kroener et al. (2012) clearly show that delayed blastulation is not associated with increased aneuploidy rates. In line with this publication, a logistic regression analysis adjusted for maternal age performed on our data showed that faster growing embryos

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(day-5 biopsy blastocysts) had the same euploidy rate (298/639, 46.6%) compared with slower growing embryos (day-6 biopsy blastocysts) (117/294, 39.8%; $P > 0.05$; Capalbo et al., unpublished observations). Based on these data, we believe that there is no evidence to suggest that day-6 blastocysts have a higher rate of aneuploidy and lower rate of implantation when compared with day-5 blastocysts (at least when comparing embryos within the same IVF cycle or from an age-matched population), rendering any screening test for early blastocyst formation impractical as a risk assessment for embryo aneuploidy.

We therefore postulate that the observed difference in implantation in the author's data set could be attributed to factors other than chromosome copy number. A recent meta-analysis of data from the Society for Assisted Reproductive Technologies in the USA highlighted the significant difference in success rates of IVF when treating patients of differing age groups (Cohen et al., 2012). It is well established in human IVF that implantation potential of embryos from patients of advanced maternal age are reduced when compared with younger patient groups (Scott et al., 2012) and that blastocyst formation and hatching are negatively correlated with maternal age (Porter et al., 2002). From our data, a logistic regression analysis of 956 biopsied blastocysts showed that female age is positively associated with a delay of embryo development to the blastocyst stage ($P < 0.01$). The mean female age for day-5, -6 and -7 blastocyst formation was 36.1, 38.5 and 39.4, respectively ($P < 0.01$; Capalbo et al., unpublished observations). It is well established that embryo aneuploidy levels increase with advancing maternal age (Munné et al., 2007). Thus, in a non-age-controlled study population, there should always be higher aneuploidy rates in slower developing blastocysts.

In the paper by Campbell et al. in which they modelled the risk classification (Campbell et al., 2013a), the reader was informed that 25 couples were enrolled in the study group, with an age range of 31–47 years. No data was provided about maternal age of the 97 analysed embryos that fell within the three aneuploidy risk classifications (low, medium and high). As no maternal age data of the embryos within each risk classification was provided, it is logical to assume (from the evidence presented above) that the differential of embryo development and rates of aneuploidy between younger and older patients have created a bias in the findings. This is a confounding factor in the study and a serious statistical flaw resulting in potential bias in the results. It is therefore possible that the algorithm is predictive of maternal age alone. In order for this retrospective study to rule out an age-related affect, the authors needed to perform a logistic regression analysis adjusted for maternal age to see if the algorithm was predictive of aneuploidy alone. We conclude that using their algorithm, the authors are in fact able to predict the chromosome copy number of any one particular embryo from their study group as a whole. However, the authors have failed to demonstrate whether the algorithm has the same predictive value of aneuploidy and implantation potential when applied within a cohort of embryos from a single IVF cycle or a controlled patient population.

Likewise in the retrospective implantation analysis paper (Campbell et al., 2013b). No age data were presented for the embryos that were within the three risk classification groups. We propose that, although the implantation rates between the three classification groups were found to be significantly different, the large maternal age range of patients enrolled in the study (from 25 to 47 years) creates a bias. Maternal age must be considered as a potential confounder of these observed differences. Again, as we know that embryos from younger patients have a greater change of implantation, the authors should provide age data for the classification groups. This is the only way to rule out the possibility that they are not merely separating their study group into three age classifications (low, medium and high).

It should be also noted that both studies – the one in which the algorithm was developed (Campbell et al., 2013a) and the one in which it was applied retrospectively (Campbell et al., 2013b) – are based on a relatively small sample size (97 and 88 blastocysts respectively) with significant overlap within the low and medium classification groups. In the retrospective analysis, the only group without overlapping was the high-risk group where only four embryos were included, making this classification totally underpowered to be of statistical and clinical relevance.

It is apparent that the authors have identified two novel morphokinetic parameters for identifying embryo implantation potential in their culture system. We agree with the author's suggestion that such a tool could be used in conjunction with PGS in their laboratory as an additional selection tool provided the results are confirmed from a larger data set or independent evaluation. We respectfully disagree with the author's statements that their algorithm has the ability to screen out embryos with the highest risk for aneuploidy and could be offered to patients as an alternative to PGS with potentially as much as a 3-fold increase in implantation rate. Making such conclusions are misleading without data from a larger, age-matched study group or a prospective, randomized controlled trial to confirm their findings.

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