Letters to the Editor


Han X, McShane M, Sahertian R, White C, Ledger W. Pre-mixing samples with assay buffer is an essential pre-requisite for reproducible anti-Müllerian hormone (AMH) measurement using the Beckman Coulter Gen II assay (Gen II). Hum Reprod 2013;28(Suppl 1):0–185.


Loh JS, Maheshwari A. Anti-Müllerian hormone: is it a crystal ball for predicting ovarian aging? Hum Reprod 2011;26:2925–2932.


Christine A. Clark1,*, Carl A. Laskin2 and Kenneth Cadesky3
1Mt. Sinai Hospital and LifeQuest Centre for Reproductive Medicine, Toronto, ON, Canada
2University of Toronto and LifeQuest Centre for Reproductive Medicine, Toronto, ON, Canada
3LifeQuest Centre for Reproductive Medicine, Toronto, ON, Canada
*Correspondence address. E-mail: cclarksolo@aol.com
doi:10.1093/humrep/det413
Advanced Access publication on November 13, 2013

Time-lapse parameters could not predict pregnancy: a hasty conclusion?

Sir,

Kirkegaard et al.’s (2013) prospective study provides additional evidence that timing of early cleavage events can predict blastocyst development, first established using cryopreserved, donated embryos (Wong et al., 2010) and recently validated in a large-scale multi-center prospective study with 1233 embryos from 160 patients (Conaghan et al., 2013). Three specific time-lapse parameters: duration of (i) 1st cytokinesis, (ii) 2-cell stage and (iii) 3-cell stage were identified in retrospective studies and prospective trials as enhancing blastocyst prediction (Conaghan et al., 2013; Kirkegaard et al., 2013). Although Kirkegaard et al.’s model used direct cleavage (DC) to 3-cell instead of duration of 2-cell stage, the authors acknowledged DC as ‘derived from duration of the 2-cell stage’ and concluded that duration of 2-cell stage ‘holds predictive value’. Since reproducibility is a cornerstone of scientific and clinical research, these prospective studies demonstrate consistency in the predictive value of early time-lapse parameters for blastocyst development.

However, I wish to discuss several limitations and other conclusions from Kirkegaard et al.’s study. The authors tested whether these timing parameters differed between implanted and non-implanted embryos concluding that ‘time-lapse parameters could not predict pregnancy’. I believe this conclusion is premature. First, the authors state that their sample size (n = 84) is ‘sufficiently large to test up to five parameters using the targeted logistic regression approach’, an overstated assertion since they failed to consider the low prevalence of a positive outcome (n = 26 implanted embryos; 31%). According to the well-established 1-in-10 rule (Harrell et al., 1996), at least 50 implanted embryos are needed to test five parameters. In contrast, blastocyst prediction analyses were based on a sample size of 571 embryos with 140 high-quality blastocysts. Since the authors conclude that time-lapse parameters cannot predict pregnancy from an absence of statistical significance, one must consider type II statistical error (i.e. failure to identify a difference when one exists). While the authors identified small sample size as a key study limitation, this does not justify their overstated conclusion. The emphasis in both the title and in the discussion on
prediction of pregnancy outcome is unwarranted given the lack of sufficient power to detect a difference. Secondly, this study only included Day-6 blastocysts, a population which is undoubtedly different from embryos selected for transfer on Day-2, 3 or even 5. Any conclusions based on blastocyst transfers in general and Day-6 transfers in particular may be irrelevant in many IVF clinics’ routine practice, thereby missing the potential of time-lapse enhancing embryo selection when cleavage-stage transfer is preferred. The limitations in sample size, population and transfer day contribute to an inadequate study design for testing the ability of time-lapse imaging to predict implantation.

On the basis of the work of Conaghan et al. it appears that a combination of traditional embryo selection and time-lapse may provide a more robust algorithm, something Kirkegaard et al. did not consider and which may have yielded better selection of embryos with potential to implant. In Conaghan et al.’s study, addition of quantitative time-lapse information to traditional Day-3 static morphology assessment significantly improved the ability of experienced embryologists to select which embryos would develop to blastocysts, and reduced variability among embryologists. Further studies on embryo selection with time-lapse will likely include a combination of embryo morphology and time-lapse, particularly for studies performing transfers at the cleavage stage.

Kirkegaard et al. and Conaghan et al. represent important milestones toward larger well-designed randomized controlled trials evaluating the clinical value of time-lapse versus current embryo selection standards. While Kirkegaard et al.’s data support previously published blastocyst prediction models, their study was not powered to test the utility of time-lapse for predicting implantation potential, making their final conclusions unsupported and potentially misleading.

Conflict of interest
A.T. acts as a consultant (scientific advisor) for Auxogyn, Inc.

References

Alan Thornhill*
Consultant Clinical Research Scientist, Assisted Conception Unit, 11th Floor, Tower Wing, Guy’s Hospital, Great Maze Pond, London SE1 9RT, UK
*Correspondence address. E-mail: arthornhill@aol.com
doi:10.1093/humrep/de394
Advanced Access publication on October 30, 2013

Reply: Time-lapse parameters could not predict pregnancy: a hasty conclusion?

Dear Sir,
We read the above letter with great interest and thank Dr Thornhill for his comments. We welcome the opportunity to further discuss this important subject.

Dr Thornhill expresses relevant concerns regarding the number of positive outcomes in the study. On the presumption of a type II error, he suggests that ‘time-lapse parameters could not predict pregnancy’ is a hasty conclusion. We entirely agree that both the number of positive outcomes and embryo transfers represent an important limitation to our study, which we believe is thoroughly acknowledged in the paper. In consistency, we desist from drawing any conclusions on the ability of time-lapse parameters to predict pregnancy in general, as implied in the letter. As correctly referred, we identified three parameters that predicted development to high-quality blastocysts. The parameters included in the analysis were selected from events that had previously been identified as potential predictors of viability and which occur within the first 2 days of development. Recognizing that blastocyst development serves only as a surrogate end-point of pregnancy, we tested the strength of the prediction by evaluating the identified parameters in a logistic regression analysis with pregnancy as the end-point. As one of the time-lapse parameters (direct cleavage to 3 cells) could not be included, only two time-lapse parameters were tested in the model together with one potential confounder at a time (Table VI of our original article). With 26 pregnancies, two to three parameters may be tested according to the rule of 10 events per predictor. As written in materials and methods, this is what we did. Even so, we should like to point out that the rule of 10 events per predictor is only a rule of thumb, and it has been shown that problems with CI coverage, type I error or relative bias are uncommon even with only five to nine events per parameter (Vittinghoff and McCulloch, 2007).

In contrast to age, the two time-lapse parameters did not hold predictive power with regard to pregnancy. We therefore consider our final conclusion, that ‘the parameters predicting development to high-quality blastocysts do not predict pregnancy in this population’ trustworthy, with the cautions discussed in the paper. As the parameters tested consisted of events occurring only within the first 2 days of development, we do, however, entirely agree that our data do not support a general conclusion of time-lapse parameters not having the potential to predict pregnancy, and consider this contention an oversimplification of our final conclusion. Regarding the elective Day 6 transfer policy, which concerns Dr Thornhill, any potential impact of the delayed transfer would apply to all embryos in the study, and thus not cause any bias affecting the internal validity, as Day 6 transfer was a general strategy. How this affects the external validity is a relevant question that we, in our opinion, have addressed appropriately in the discussion, which is why we limit our final conclusions to the population studied. We would, however, like to repeat our main point that prediction of development, may it be on Day 3, Day 5 or 6, is a surrogate end-point and any parameters predicting such end-points should be confirmed by their ability to predict pregnancy.