SIRT EDUCATION INITIATIVE

Sperm Morphology Course

A one-day course was held on Saturday 12th November at the Monash Medical Centre, in the Education Program in Reproduction and Development (EPRD) laboratories within the Monash University Department of Obstetrics and Gynaecology. Delegates came from as far afield as Hong Kong, New Zealand and Tasmania, and ranged from relative novices to senior clinical scientists. However, they all had one thing in common, the desire to improve their skills in assessing sperm morphology.

Sally Catt and the team at EPRD provided the excellent laboratory facilities and provided help and guidance throughout the day, and Olympus kindly provided a full set of microscopes for the delegates. Phill Matson (Principal Scientist at Fertility North) started off the day explaining how the criteria of normality for sperm morphology has changed over the years. De-Yi Liu (Andrology Scientific Director, Melbourne IVF) shared a vast experience in the use of WHO criteria of sperm normality, Emily Zuvela (EQASRM Programme Manager and Laboratory Manager at Fertility Specialists of WA) showed how external quality assurance programmes can be used to best effect in monitoring one's own laboratory performance.

The course was enjoyed by all and there was definitely an increased awareness and general improvement in morphology assessment as the day went on. The results for each slide analysed were discussed at length by the group, and those with outlying results were able to revise their techniques. The delegates were then able to take away with them a WHO 5th Edition manual and a set of reference slides so they could continue to improve and incorporate things learned in to their every day work.

So, all in all a successful day. Hopefully SIRT will continue its educational efforts in the future.

to the CRISPR/Cas9 technology and highlighted why this is such a game changer for scientists in many fields and also how researchers from China and the UK have begun to experiment with this technology to modify human embryos.

Next Sarah Long gave an excellent presentation on the technologies available and currently used for prenatal testing.

The day came to a close with a counselling presentation of the implications and controversies in cross border reproductive care by Iolanda Rodino. This topic is becoming ever more prevalent in our clinics and Iolanda provided an insightful perspective on the issues we may encounter.

THE EFFECT OF ABNORMAL CLEAVAGE ON BLASTULATION USING TIME LAPSE VIDEOGRAPHY

WRITTEN BY MELISSA VITORINO

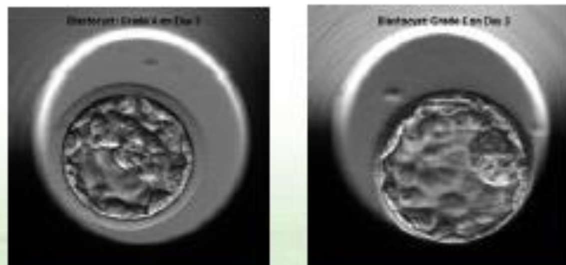
Embryo selection in human IVF has certainly come a long way in recent years and as Embryologists our methods for selecting embryos with the greatest chance of implantation have definitely evolved. The purpose of culturing embryos is to monitor their growth and development to aid the embryologist to select the ones with the highest implantation potential. In the earlier years of IVF, embryos were selected for transfer at the cleavage stage using conventional static morphology scores as the main criteria however there was a high proportion of selected embryos that did not implant. Three main strategies have emerged in recent years for weeding out the poorer quality embryos; extended culture, PGS and time-lapse videography, and more recently with metabolic analysis on spent culture media of embryos showing encouraging results. Certainly there are many reasons why a limited time in culture is advantageous. Workflow in the laboratory is much simpler having embryos for only three days rather than up to six. Embryologists spend a lesser number of days observing the embryos and certainly the financial benefits of a reduced need for space in incubators, IVF lab wear and culture media are apparent. Then there are also the epigenetic reasons where reducing the potential effects that the laboratory environment can have on the resulting progeny. At Fertility North, we use a time-lapse incubator and our Scientific Director, Dr Yanhe Liu, has developed a cleavage stage algorithm which when applied to the embryos development on day 3 increases the predictability of implantation. The full version of this has been published in Fertility & Sterility 2016 (105) 656-662 and using the algorithm allows for day 3 embryo selection with our latest implantation rates of highest graded embryos reached above 60%.

The time-lapse technology has completely changed the way we view embryos and what they get up to whilst we aren't watching. With time-lapse technology we have identified a specific group of embryos that have good conventional morphology (e.g. 8 cells on day 3 with <10% fragmentation) that have significantly poor implantation rates. These embryos are those that exhibit abnormal cleavage events such as reverse cleavage and direct cleavage. Our clinic data from 2015-2016 has shown that these embryos have a 2.4% implantation rate. Without time-lapse technology we would have never been able to separate these embryos from the group with implantation rates of 62% (based on 2015-2016 data). There are many different groups looking into developing highly specific algorithms that use quantitative measures of timings for embryos to reach certain milestones. Most however, have been shown to be inconsistent when trying to transfer the algorithm between laboratories because embryos grow at different rates depending on the lab culture environment (CO_2, O_2, temperature and culture media), stimulating protocols and patient profiles. While using such qualitative measures (abnormal cleavage) seems to have little transferability issue since they are independent of absolute cell cleavage rates. By first excluding all embryos that exhibit abnormal cleavage as a first method of deselection the ability to choose the embryo with the highest implantation rate will improve significantly. It is interesting to know that abnormal cleavage occurs much more regularly than you would think – around 30% of best morphology Day 3 embryos (selected from the cohort based on conventional scores) exhibit abnormal cleavage up until the 8 cell stage. This is a significant proportion of your conventionally good morphology embryos that have a highly impacted potential.

This discovery also posed a new problem for our laboratory because suddenly we were faced with a group of embryos that previously before time-lapse we would have frozen or transferred without a thought. At first we were still freezing these embryos on day 3 and selecting them last for transfer in FET cycles. Often we found that patients with one of these embryos left in storage were starting to opt for a fresh cycle rather than use them based on the low potential and doctors were questioning their viability too. So rather than decide to completely rule these embryos out we moved to extended culture for these embryos instead of freezing them. We figured that based on their low pregnancy rate they probably wouldn't form blastocysts and then

we would be able to discard them knowing we had given them the as much of a chance as possible. What happened next was by far the greatest surprise.

The embryos in the abnormal cleavage group were forming blastocysts at a rate of 70%! Of most interest to us was that it posed the question - is blastocyst formation really a good indicator of embryo potential? Here we have embryos with an implantation rate of 2.4% forming a blastocyst most of the time. This could perhaps be also useful for clinics who are running a complete blastocyst program, by using abnormal cleavage as a deselection tool between blastocysts. Two blastocysts with similar appearance but derived from a Grade A and Grade E embryo (abnormal cleavage) on Day 3 respectively, can be seen in the photographs below.



To understand more about this group of embryos we looked into when the abnormal cleavage was occurring in development and categorised it by cell stage (1 cell, 2 cell, 4 cell stage). From here we analysed if there was a stage dependant effect of abnormal cleavage on blastulation rate. Indeed, there was a significant impact on blastocyst formation for those embryos that had abnormal cleavage earlier in development. This made logical sense too, given that if at the one cell stage an abnormality occurred then 100% of the embryo was affected compared to the four cell stage where only one cell meant just 25% of the embryo. The next question that arose was - have the embryos that form blastocysts 'fixed' the error and what does this mean for their implantation potential going forward?

A year has passed and I am still trying to collect FET data for these embryos. Given the success of our algorithm in selecting embryos with the higher implantation rates first we often see our patients falling pregnant before they need to try these abnormal cleavage blastocysts! So the data I have is limited and the numbers are very low but certainly we are seeing a trend starting to emerge. The pregnancy rates for the abnormal cleavage blastocysts is much lower than those of our non-abnormal cleavage blastocyst group. Certainly it is not as low as the cleavage stage implantation rates which might be attributable to much of these embryos not reaching blastocyst and not being frozen. The challenge now is to collect enough data to analyse which type of abnormal cleavage and at which cell stage does it render an embryo completely deselected for transfer or freezing on day 3. It would also be interesting to look into PGS/PGD outcomes of such affected embryos, which have a certain proportion of cells displaying abnormal cleavage, since the inconsistent genetic characters of different cells may lead to detected and undetected abnormalities or mosaicism following embryo biopsy.