ART Scientific | ASRM 2020 Review

Dynamic collaborations in reproductive medicine

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The Scientific Program at the first online ASRM Congress in October 2020 included talks on preimplantation genetic testing (PGT), Artificial Intelligence (AI), and back-to-basics approaches such as culture condition considerations, andrology and cryopreservation. It is my great pleasure to present key insights and reflections from our scientific team on some of the scientific content presented this year.

Plenary session

The opening Plenary Session was a lecture given by Jamie Metzl on a futurist's view of genetic engineering of humans, based on his book 'Hacking Darwin: Genetic Engineering and the Future of Humanity'.

He is a strong advocate of recent advances in genomics leading to better healthcare from birth. And he is right. Our genetic code has become readable, writeable and hackable leading to three major trends: precision medicine and healthcare, application of genetic technology to the nature of babies that are born, and genome editing of embryos.

Since competition between diverse societies will drive these advances, ethical and legal regulatory infrastructure will struggle to keep up with this exponential increase in science and technology. Therefore, reproductive healthcare specialists have a central role in patient education and treatment, and we need to become united globally to tell the story of a better future.

Advances in PGT

Many of the studies presented at ASRM demonstrated extended utility for PGT and were prominently featured in both oral presentations and posters.

M Viotti's (O-3) prize paper on the transfer outcomes of 1,000 embryos with mosaic results is the largest of such studies to date. The data found that embryos with segmental mosaic results were the most likely to result in an ongoing pregnancy (OP) or live birth (LB). However, they still had a significantly lower potential than euploids. Low-level mosaics performed better than high-level mosaics though even high-level, complex mosaics had a 13% OP or LB rate. Of the 20% of mosaic embryo transfers that resulted in miscarriage, 75% occurred before 6-8 weeks of pregnancy. Gestational length, birth weight, and neonatal assessments were not significantly different between babies from mosaic versus euploid embryo transfer.

J Buldo-Licciardi (O-170) reported improved clinical outcomes with the CooperSurgical PGTai℠ 2.0 platform. The single-center, retrospective cohort study of 24,000 biopsies and 1,174 embryo transfers found that this analytical tool identified significantly more euploid embryos and fewer aneuploid and mosaic embryos compared to previous methods. With the adoption of the PGTai 2.0 tool, live birth rates (LBR) increased from 61.65% to 70.32%, while biochemical pregnancy loss rates decreased from 11.80% to 4.64%. Both findings were statistically significant.

N Mohamed and colleagues (P-799) reported on their Egyptian clinic's experience with the CooperSurgical PGTai platform compared to manual PGT-A results calling. Analyzing 720 trophectoderm biopsies from 280 patients, the authors saw a relative increase in euploid embryos reported with PGTai, as well as a relative decrease in mosaic and aneuploid embryos reported compared to standard calling. They concluded that PGTai provides patients with more opportunities for embryo transfer.

D McCulloh (P-754) presented a poster assessing the success of PGT. Evaluating data from the US SART registry from 2014-2017 from over 303,000 IVF cycles, PGT versus no PGT results were compared. Although fewer embryos were transferred in the PGT-A group, the live birth per cycle was significantly greater in all age groups with PGT-A compared to the no PGT group. Miscarriage, multiple pregnancy and preterm delivery rates were all significantly greater in the no PGT group. These findings were even more striking when accounting for differences in age-specific diagnoses.

A Tiegs (O-73) reported on a multicenter non-selection study of PGT-A by next-generation sequencing. Tiegs and colleagues biopsied embryos but did not analyze the PGT-A results until after the embryos had been transferred based on morphological criteria alone. When they unblinded the PGT-A results, they determined that, of the 102 aneuploid embryos transferred, zero (0) resulted in sustained implantation (compared to 64% of euploid controls).

T Tan (O-75) focused on her center's non-selection study of low-level mosaic embryo transfer. The 112 low-level mosaic embryos transferred did not significantly differ from the 784 euploids controls in any of the outcomes measured, including LBR, miscarriage rate, or birthweight. These data add to the growing body of evidence that low-level mosaic results are correlated with better clinical outcomes.

Clinical genetics and genetic counseling

J Garbarini and colleagues (P-327) examined patients' motivations for pursuing PGT. The authors surveyed 169 PGT patients undergoing pre-test genetic counseling at a single PGT laboratory between April 2019 and May 2020. PGT-M and PGT-SR patients indicated PGT was a very

important factor in their decision to pursue IVF; they were also more likely to undergo IVF despite lack of insurance coverage than were PGT-A patients. Additionally, 93% of PGT-A patients, 88% of PGT-SR patients, and 84% of PGT-M patients reported their laboratory genetic counseling experience was useful or very useful.

C Ren (O-45) recounted the results from a survey of 97 IVF clinics' policies on the transfer of embryos with abnormal PGT results. Just over half did not have a policy, preferring to handle patient requests for abnormal embryo transfer on a case-by-case basis. However, 37% did have written policies, and 3% were actively writing policies at the time of the survey. The author noted that these types of patient requests are increasing. As such, clinic policies are prudent and thorough genetic counseling is essential to handle patient requests for abnormal embryo transfer.

Two studies investigated the PGT-A outcomes of a second IVF cycle after the first cycle yielded no euploid embryos. N Perlihy (P-791) assessed outcomes of 438 second cycles, while A Schickedanz (O-44) reviewed the treatment decisions and outcomes of 550 patients with no euploid embryos identified from the first IVF cycle. Both studies yielded similar results: approximately 50% of second cycles yielded at least one euploid embryo. Perlihy's study found that second cycle outcomes were not significantly different from expected age-related outcomes; Schickedanz found that patients with higher antral follicle counts and more mature oocytes retrieved had better second cycle outcomes. These results are reassuring for many patients who find themselves with no euploid embryos after their first IVF cycle.

M Kappy (O-261) utilized the 2014-2016 SART-CORS database to assess outcomes of 2,501 multiple euploid embryo transfers (MEET) compared to 14,408 single euploid embryo transfers (SEET). She found that nearly half of MEETs resulted in a multifetal pregnancy, compared to just 1.7% of the SEET group. MEET also resulted in substantially higher rates of preterm delivery <37 weeks, very preterm delivery <34 weeks, and low birth weight. The author and the live audience concluded that euploid embryos should be transferred one at a time in all but the most extenuating circumstances, and some called for regulatory guidelines mandating this practice.

B Hanson (O-5) in this prize paper session reported results with a clinically available niPGT assay. In this study, 166 blastocysts were tested concurrently via niPGT and traditional trophectoderm (TE) biopsy. It found that 37% of niPGT-A samples failed to amplify with 82% failure on day 5, 37% on day 6, and zero (0) on day 7. Euploid embryos were significantly more likely to fail amplification than aneuploid embryos. 40.4% of embryos had discordant results between niPGT and TE biopsy. Furthermore, the niPGT results did not correlate with the outcomes of 36 embryos that were transferred based on euploid TE results. Much work is still needed in this area.

Patient education

S Sehnert (O-66) presented survey data of 929 patients who completed the custom CooperSurgical PGT-A educational module via EngagedMD, before IVF treatment. 90% of patients stated they knew what to expect from PGT-A after completing the module, while less than half knew what to expect before the module. Over 98% of patients agreed the module was a helpful addition to consultations with their medical team, and nearly three-quarters of patients surveyed felt that the EngagedMD module made them more satisfied with their care experience. The researchers concluded that digital platforms are an effective means of patient education that can enhance the overall fertility patient experience.

Implantation

The ESHRE symposium focused on diagnosis and treatment options for recurrent implantation failure (RIF), about factors related to the mother as well as the embryo. T Strowitzki gave a comprehensive review of insights on different applied definitions for RIF, as well as the current literature. There are many variant definitions of RIF, the most accepted being failure to achieve a pregnancy following three completed fresh IVF-ET cycles and failure of ≥10 embryos to implant.

The diagnosis of RIF does not preclude spontenous pregnancy. In a group of patients diagnosed with RIF, afte a 5.5 years follow-up, the cumulative LBR was reported to be 49%. A sub-group of these patients deliveries (18%) resulted from natural conception, with nine months being the calculated median time to pregnancy.

Although there are a plethora of diagnostic and therapeutic interventions, there have been no recent studies on thrombophilia, despite similar patterns being observed in both RIF and recurrent pregnancy loss (RPL), and ESHRE recommends against routine screening.

Also, there are no studies on anti-cardiolipin antibodies in RIF, though ESHRE recommends screening for anti-phospholipid antibodies for RPL after two pregnancy losses. Uterine natural killer cell overexpression harms implantation, though a recent study was unable to demonstrate any significant difference between RIF and controls. There is no convincing advantage of personalized embryo transfer, a septate uterus should be surgically corrected in RIF, though a large retrospective study found no significant difference in LBR, miscarriage rate and preterm birth following surgery.

Recent Cochrane analysis of different diagnostic and therapeutic interventions such as adherence compounds, intrauterine hCG injection, endometrial scratching and seminal plasma reported insufficient, low to medium quality evidence of an effect upon LBR. In conclusion, there is no evidence to support almost all treatment options, most studies being of low or moderate quality; ie, RIF is a made-up disease treated with unproven therapies.

D Cimadomo reported results from an ESHRE questionnaire carried out by a special interest group that focused on implantation and early pregnancy. In total, 8,579 people from 6,916 IVF centers were surveyed (May-June 2020) of which: 96% treat RIF patients; 84% define RIF based upon the

number of failed embryo transfers and 64% on the number of embryos transferred (2, 3 or 4).

Generally, thresholds for treatment were significantly lower in the private sector, resulting in significantly more treatment. The response rate was low and there was a general lack of worldwide consensus, highlighting an urgent need for guidelines to protect patients from malpractice.

The interactive session 'Evidence-based embryo transfer: Maximizing Implantation' presented a good overview of the evidence available behind different techniques and practices for embryo transfer. The data reported showed insufficient evidence either way for analgesics, anesthesia and massage therapy for the preparation for transfer. There was fair evidence that acupuncture does not increase LBR and there are no proven benefits of using antibiotics at the time of transfer. In terms of transfer technique, ultrasound is a positive help, soft catheters are better than firm ones and there is insufficient evidence that blood on catheters affects results. The speed of injection may affect outcomes but there is too little evidence at this time. There was no information on laboratory techniques, however, this session did show the importance of this vital step.

T Roseboom presented an amazing lecture 'How the early environment shapes later health: the fundamental importance of a good start in life'. In her talk, she referred to humans as 'plastic' and responsive to the environment and that where you are born affects your life expectancy (this is predicted by the age of four).

She sited that relatively large population studies in New Zealand (4 million) and Denmark (4 million) have showed that 80% of healthcare costs are incurred by 20% of the population.

The early part of pregnancy is the time when the foetus is most susceptible to risk. Also, the environment in which a blastocyst is cared for affects cardiovascular health however little is known about the long-term effects of *in vitro* culture. Prenatal exposure to the 1918 Spanish flu pandemic led to an increased risk of cardiovascular complications in the offspring, and disasters during pregnancy, such as 9/11, have epigenetic effects.

The Queensland flood study showed that pregnant women who were able to maintain contact with their midwives experienced less stress and their offspring had fewer complications. Therefore, we must be particularly mindful of our youngest during the SARS-CoV-2 pandemic. In Roseboom's words, "Investing in a good start in life is the smartest investment anyone can make."

Back to basics

J Swain and K Go in their session 'Troubleshooting the Laboratory: Identifying issues and potential areas of risk' highlighted equipment, media, and cryopreservation as some key areas upon which to focus and how to minimize risk to protect the lab, clinic, and staff members involved .

J Swain focused on the importance of identifying risks proactively, collecting data, setting benchmarks (internal versus standard), and adjusting practices accordingly. He stated that labs must do their own risk assessments to take account of the unique mix of processes and workflows.

K Go focused more on three broad categories of errors: misidentification, incorrect technique or resources, and omissions. She discussed the value of using checklists and how to use root cause analysis prospectively to assess risks so effective corrective action can be developed and implemented.

E Forman and D Gardner, in an interactive session gave a review on culture media. Both speakers reviewed how culture media was first developed, fine-tuned, and how it continues to be an important topic for IVF laboratories. New culture media formulations were discussed and studies were reviewed alongside how all this information can be utilized to enhance wider knowledge in the field and provide a deeper understanding of different aspects within the IVF laboratory.

Meanwhile, the following presentations focused on how to improve clinical outcomes using various methods and protocols.

S Brouillet (O-108) presented data from a retrospective study. Embryos from 120 couples undergoing their first IVF cycle were continuously cultured at 5% O_2 from day 0 to blastocyst stage. For patients who did not achieve a pregnancy and returned for a second IVF cycle, their embryos were cultured at 5% O_2 until Day 3 of culture, then reduced to 2% until blastocyst stage. A significant improvement in the usable blastocysts rate and cumulative LBR were reported in this last group.

R Danis (P-13) cultured mouse embryos from the one-cell stage to the blastocyst stage in different oxygen tension. Embryos cultured at 1.5%, 2% or 3% O_2 showed the highest blastocyst formation than those cultured in <1% and 5% O_2.

I Insogna's (O-15) presentation examined if the overall proportion of usable (biopsied/frozen) embryos on day seven, that would have otherwise been discarded on day six, was age-dependent. Also, whether embryos failing to reach biopsy or freezing treatments on day six were more likely to meet these criteria if cultured in fresh media from day six to day seven.

It transpired that 9.9% of embryos otherwise discarded on day six met the criteria for biopsy and/or freezing on day seven. There was no benefit in refreshing media on day six for all patients, in particular those over 40 years of age. Women >43 years of age should be counseled that there is a slim to no likelihood that they will have a usable blastocyst on day seven after the culture of their embryo is deemed unusable on day six.

The usage of ICSI in couples with non-male factor infertility continues to be examined and debated. J Tozour (O-16) presented retrospective data spanning a four-year period and found that ploidy and mosaic rates are not significantly different following IVF and ICSI for non-male factor PGT-A cycles.

M Smith (O-4) presented data from a prospective randomized study set to determine if oocyte denudation and ICSI at 36.5 hours versus 39 hours post hCG influenced fertilization and blastocyst rates in good prognosis patients. The results showed that there was no impact on cycle outcome and that oocytes appeared to have a physiological tolerance for fertilization during a 2.5-5 hours time interval. Earlier ICSI could facilitate workflow in an IVF laboratory.

Two retrospective chart review presentations from Northwestern University looked at the relationship between semen parameters and paternal origin of aneuploidy, and paternal BMI and paternal aneuploidy rates. M Luck (O-71) reported no difference in aneuploidy by any semen parameters, even when comparing severe oligospermia or azoospermia. L Hughes (O-72) also reported no difference in aneuploidy rate or paternal aneuploidy between obese (BMI ≥ 30) and non-obese (BMI <30) patients.

In the cryopreservation section, E Osman (O-92), in a randomized blind trial, compared the post-thaw survival rate when blastocysts were cryopreserved with either conventional liquid nitrogen or a supercooled slush liquid nitrogen (LN2). The accelerated cooling rates with the slush LN2 seemed to overcome the vapor barrier surrounding the embryos at plunge, significantly improving survival rates in repeated vitrification trials.

J Yang (O-95) utilized a propensity score matching to show that oocyte vitrification can influence embryo development. Fertilization, embryo development and clinical outcomes were better for fresh oocytes compared to vitrified ones. The assessment of those oocytes donated to research offered the explanation that spindle-related aspects may be an explanation for the differences observed.

Innovations and late breaking news

Several presentations examined new approaches and techniques that can potentially impact the way we assess gametes and embryos.

S Alexandrova (O-91), in this prospective experimental research paper successfully produced normal euploid human blastocysts from sperm after partial freeze-drying, rehydration and ICSI. Liquid nitrogen was replaced with an ordinary freezer (-80˚C) with a Cryoprotectant (CP)-free solution.

A Parrella (O-69) investigated semen specimens from men with prior ICSI failure and screened for sperm chromatin fragmentation and double-stranded DNA breaks. It was determined that spermatozoa with an intact genome were associated with improved embryonic development and implantation rates in couples undergoing ICSI.

These tests were performed on raw specimens that were processed by density gradient centrifugation and microfluidic sperm selection (MFSS). Samples processed by MFSS yielded the highest portion of progressively motile spermatozoa that had the least amount of DNA damage.

S Cabello-Pinedo (O-13) presented data on a novel non-invasive metabolomics approach to screen embryos for aneuploidy. In the study, 7,523 metabolites were detected in spent media used for culture from day three to day five. Through dimension reduction techniques, 60 biomarkers were selected. Then, 40 samples were tested for concentration of the 60 selected markers against aneuploidy, which showed an accuracy of 97.5%.

M Venturas (O-81), using Fluorescent Life-Time Imaging Microscopy (FLIM), investigated the correlation of metabolic activity of cumulus cells to oocyte maturation and fertilization potential. Metabolic imaging was able to non-invasively detect significant metabolic variations in cumulus cells between mature and immature oocytes. These metabolic parameters were also associated with potential and clinically relevant factors, such as maternal age, but not BMI.

J Shah (O-185) also used FLIM to assess embryo metabolic state measure autofluorescence of nicotinamide adenine dinucleotide hydrogen (NADH) and flavin adenine dinucleotide (FAD) to identify significant metabolic differences between euploid and aneuploid embryos.

A Mokhtare (O-184) presented the development of a semi-automated oocyte denudation microfluid chip that uses a system like “speed bumps” to remove cumulus cells. Using murine cumulus-oocyte complexes, the technology showed less shear stress imposed on the oocyte with comparable denudation efficiency, fertilization and blastocyst formation rates.

Final word | SARS-CoV-2

Certainly, during this time, we cannot ignore the impact that SARS-CoV-2 is having on the field of assisted reproduction. We are concerned about its impact on patients and staff who work extremely hard to mitigate and prevent the spread of the virus so that clinics can continue to operate.

In an elegant study, M Viotti (O-270) showed that SARS-CoV-2 can enter the cells of human blastocysts. He demonstrated that embryonic cells have the ACE2 receptors and mechanisms that allow the virus entry in zona-free blastocysts. He clearly showed that SARS-CoV-2 entered blastocyst cells through the canonical Spike-ACE2 entry mechanism. Although unclear on what effects entry of the virus may have, and no data yet on the life cycle of the virus in embryonic cells or impact on implantation, miscarriage or neonatal health, this is one of the first pieces of evidence we have that shows the virus can enter embryos.

The take-home message is SARS-CoV-2 has definetely impacted the way we practice, communicate and support each other. Much is still to be learned when perfoming medical procedures, remembering that safety is critical in everything we do.