The Impact of ESHRE 2017 on Japanese Fertility Practice

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The Omnia-Prova Education Collaborative

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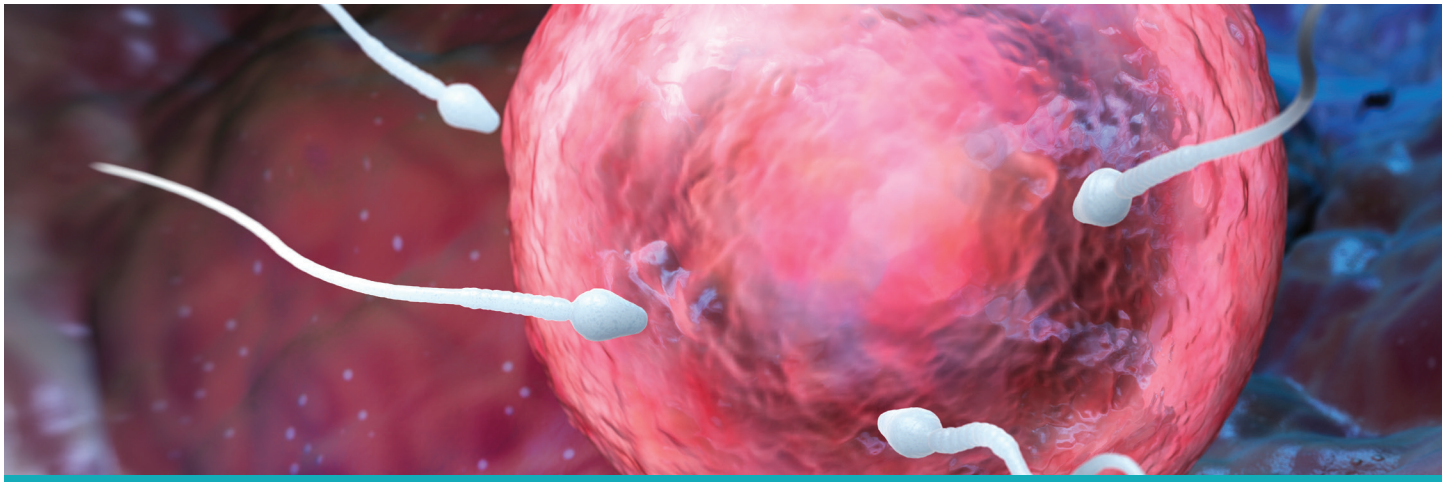

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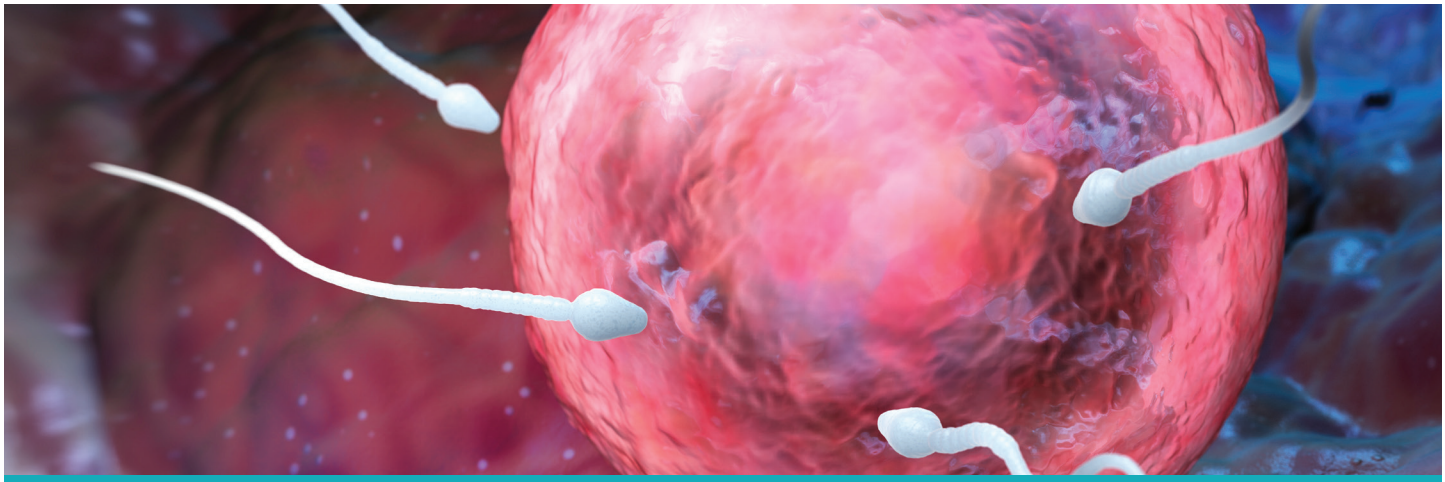
The GWHA was interested in the opinions of practicing clinicians and experts from Japan regarding some of the content presented at ESHRE 2017 and its impact on Japanese fertility practice. While at ESHRE 2017, thought leaders from throughout Japan, Drs. Kenichiro Hiraoka, Yukiko Katagiri, and Mitsutoshi Yamada, were each asked several questions pertaining to the sessions they attended. These included which presentations they found most interesting and which provided information most applicable to improving embryo quality and live birth rates in Japan following IVF procedures. The summarized collected responses are below:

Kenichiro Hiraoka
Senior embryologist
Kameda Medical Center

QUESTION:
Which presentation at ESHRE 2017 did you find the most interesting?

The oral presentation by Babariya and colleagues titled *Development and application of a novel strategy to explore blastocoel fluid and spent culture media as a source of embryonic DNA*, was quite interesting (O-028). As you are likely aware, PGS is prohibited in Japan, primarily due to it being invasive as well as the risk of damage during biopsy; however, there is little doubt it will help identify genetically normal embryos for transfer. This presentation asked if blastocoel fluid and/or spent culture media are a reliable source of embryonic DNA, and could they be potentially used for non-invasive preimplantation genetic testing. The short answer is yes; in fact, in this study blastocoel fluid and spent culture media both were found to contain embryonic DNA, and the sex of all the embryos was correctly detected from the spent culture media samples.

To accomplish this effort, the authors developed a novel method to amplify the minute quantities of DNA present in samples of blastocoel fluid and spent media. Of note, their preliminary analysis of spent media samples demonstrated a chromosomal copy number concordance of 95.65% with trophectoderm samples of the test embryos. While the test sample was relatively small, the findings suggest that this approach might be a noninvasive and precise source of critical information when assessing embryo quality. This is important in Japan, again where PGS is prohibited, as it may provide the information needed at reduced risk, and do so more economically than current standard biopsy approaches.



QUESTION:

Were there any other presentations at ESHRE 2017 that you found intriguing related to assessing embryo quality and involving use of spent culture medium?

Yes, the presentation by Vera-Rodriguez and colleagues (O-029) entitled *Non-Invasive PGS reveals the existence of complementary aneuploidy between DNA obtained from trophectoderm biopsy versus DNA in spent culture medium in the same embryo*. This presentation asked what are the aneuploidy concordance rates within the same embryo between trophectoderm biopsy (PGS) and DNA analysis of embryo spent culture medium (non-invasive PGS; NI-PGS). The exciting answer, in agreement with the Babariya and colleagues presentation I discussed previously, was that chromosomal diagnosis obtained by NI-PGS reveals complementary aneuploidies in gains or losses to findings obtained using PGS for a trophectoderm biopsy. They concluded that non-invasive PGS can be complementary with respect to gains/losses obtained using day-5 PGS.

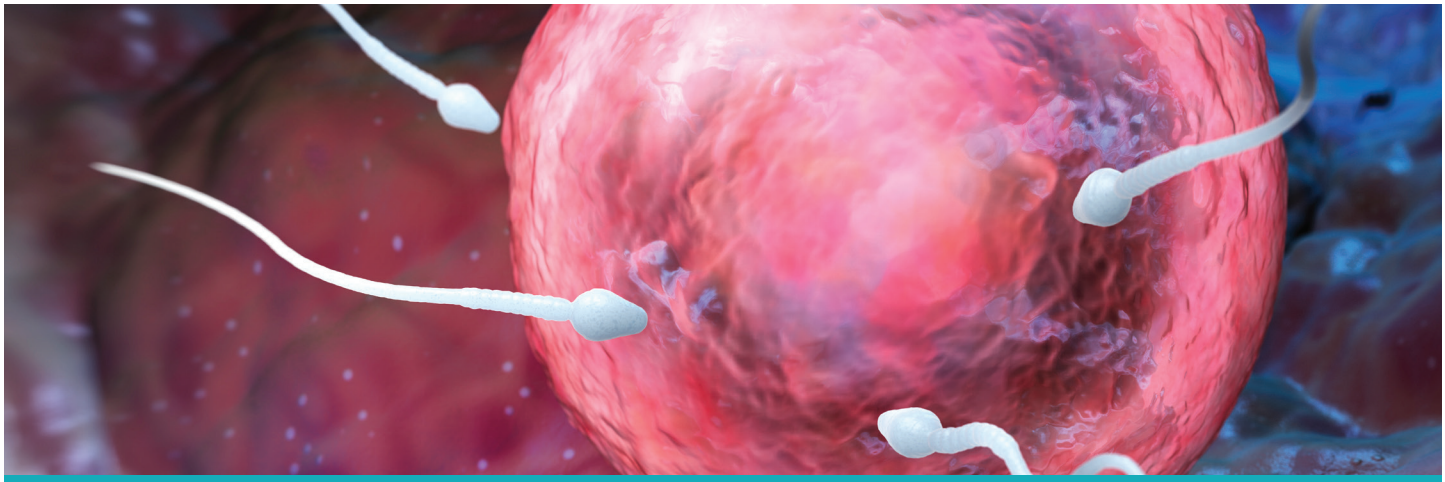
QUESTION:

What other information from ESHRE 2017 do you think is most applicable and important in Japan?

There were a number of presentations I found quite relevant for Japan. These included two presentations on laser-assisted hatching, both offered by authors Papatheodorou and Panagiotidis in conjunction with different colleagues. The first was titled *Laser assisted hatching before embryo transfer improves the clinical outcome in cases with vitrified oocytes from an egg donor cryobank: a prospective, control, randomized study (O-071); and the second was titled Laser-assisted hatching improves clinical outcomes of top grade, vitrified–warmed blastocysts developed in high prognosis oocyte donation cycles: a prospective randomized study (O-072)*.

The O-071 study asked if laser-assisted hatching is beneficial prior to embryo transfer when using embryos derived from vitrified/warmed oocytes in an oocyte donation program. The answer was that use of laser-assisted hatching provided a real and statistically significant improvement of implantation (p=0.0003).

The O-072 study asked if partially opening the zona-pellucida by laser assisted hatching affects the implantation potential of top quality, vitrified–warmed blastocysts. The finding was that the implantation rate of vitrified-warmed, top grade blastocysts was significantly increased 15% to 20% when a partial zona-pellucida opening occurs post-warming (p=0.003).



And, lastly, an excellent presentation from Japan nicely supported the Papatheodorou and Panagiotidis presentations. The Japanese contribution was entitled *Complete zona pellucida removal facilitates embryo attachment and outgrowth by upregulating the integrin 5 and 1 expression in human blastocysts: in vitro outgrowth model*, by K. Ezoe and colleagues (O-008). I am aware that my colleague, Dr. Yamada, agrees with my assessment of this presentation, so I will defer to his discussion of its merits.

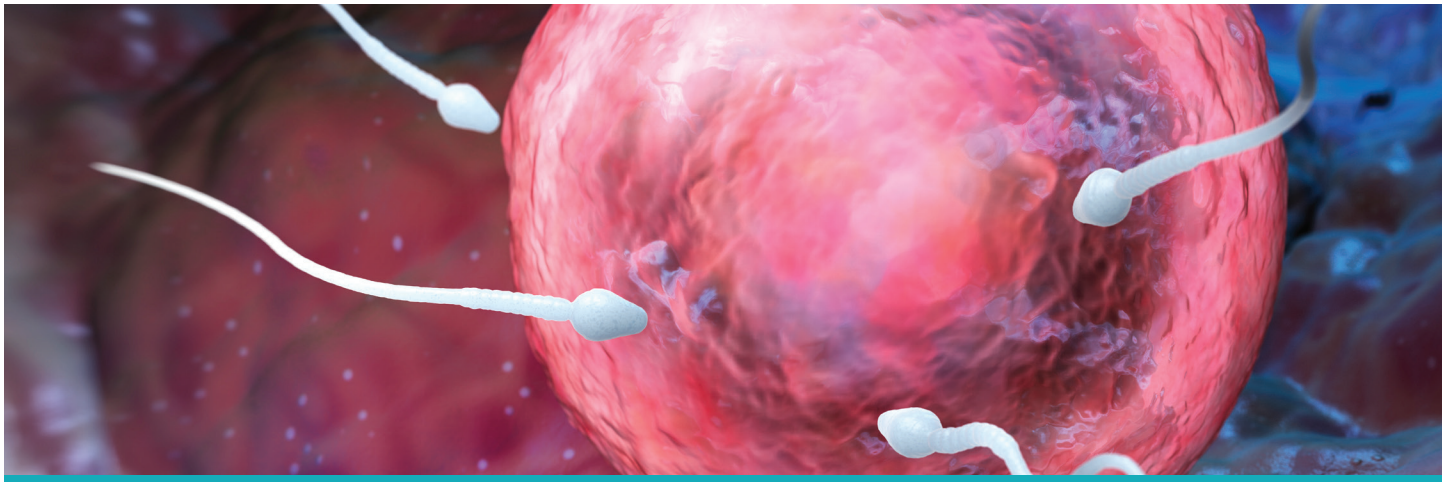
In the end, we as embryologists seek safe and effective applications that enhance embryo development and implant ability, all leading to improvements in live birth rates.

Yukiko Katagiri
Professor
Department of Obstetrics and Gynecology
School of Medicine
Faculty of Medicine, Toho University

QUESTION:
Which presentation at ESHRE 2017 did you find the most interesting?

Improving cumulative live birthrates is a very important goal in Japan. Two different presentations at ESHRE 2017 provided new insights that may assist us in that effort. The first was by Malchau and colleagues and titled *Impact of the number of retrieved oocytes on cumulative live birthrates after repeated cycles of assisted reproductive technology – A Danish national cohort study (O-126); and the second was by Tournaye and colleagues and titled A placebo-controlled, randomized, double-blind study of pregnancy and live birth rates after single oral administration of a novel oxytocin antagonist, nolasiban, prior to embryo transfer* (O-024).

Malchau and colleagues asked if the number of aspirated oocytes in the first assisted reproductive technology cycle is associated with the cumulative live birthrates in subsequent cycles. And they found that an increasing number of aspirated oocytes was in fact associated with higher cumulative live birthrates in subsequent cycles, and lower risk of the patient discontinuing treatment. Thus, initial treatment-response predicts outcome in subsequent cycles, so a good first cycle response should help affirm for the patient the value of continuing with additional cycles. The authors took data from the Danish National IVF-registry that included all ART treatments in public and private clinics since 1994. Further, the treatment-cycles were cross-linked with the Medical Birth Registry, identifying treatment-related births and spontaneous



conception births. The Danish National Cohort study included all women starting ART treatments with homologous eggs between 2002 and 2011, in total, 30,486.

The wider implications of their findings were reported as *emphasizing that the ovarian response to stimulation is an important prognostic factor, irrespective of age, for infertile couples entering ART programs, where the success is often based on repetitive cycles and combinations of both fresh and frozen-thawed embryo replacements*, a conclusion to which I am in complete agreement.

The second presentation, by Tournaye and colleagues, evaluated a novel oxytocin antagonist and its impact on pregnancy and live birth rates when administered prior to embryo transfer. This was a very exciting presentation. More specifically, they asked if oral administration of the oxytocin antagonist, nolasiban, prior to day 3 fresh embryo transfer improved pregnancy and live birth rates. They found that overall live birth rate was 40% after administration of different oral doses of nolasiban compared to 29% in the placebo group.

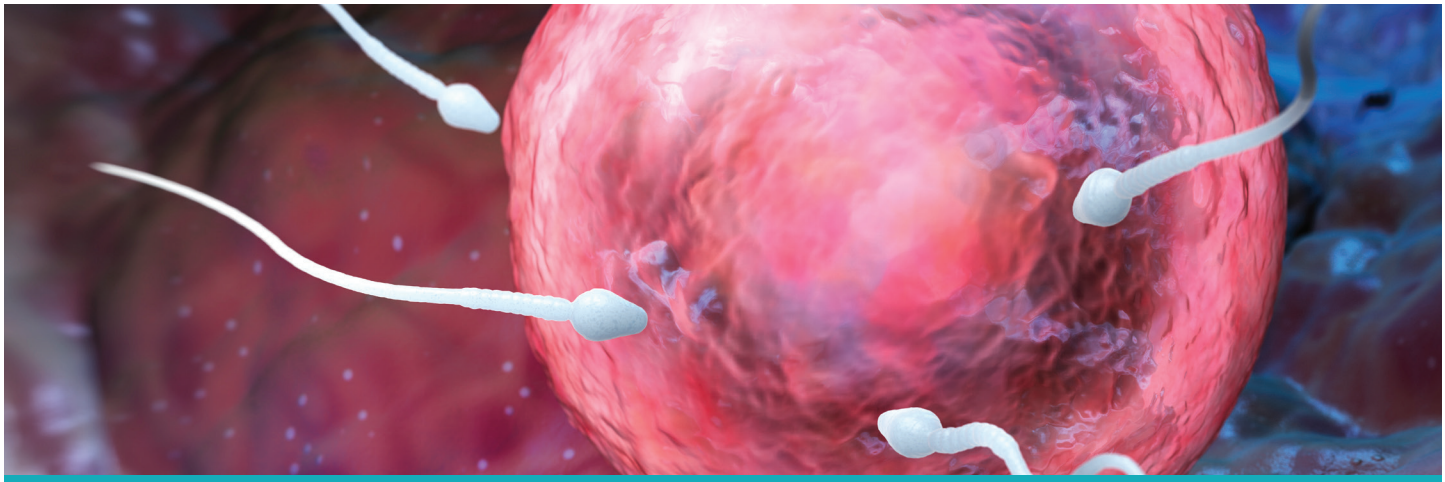
This was a multinational, prospective, double-blind, dose-finding, randomized, parallel group, placebo-controlled study assessing a single oral dose of 100, 300 or 900 mg nolasiban, or placebo, administered 4 hours before embryo transfer following IVF or intracytoplasmic sperm injection. There were 247 subjects, roughly 60 per arm. The authors explained that the scientific basis for the study derived from the hypothesis that antagonism of oxytocin and/or vasopressin V1a receptors expressed in uterus at the time of embryo transfer could enhance uterine receptivity and improve pregnancy rates, possibly by decreasing uterine contractions and by improving endometrial receptivity and perfusion.

Their overall conclusion was that there is *a potential 10 to 20% absolute increase in pregnancy and live birthrates compared to placebo after administration of a single oral dose of [an agent such as] nolasiban before embryo transfer*. And, I agree with their subsequent suggestion for the need to confirm this finding in larger prospective trials.

QUESTION:

What other information from ESHRE 2017 do you think is most applicable and important in Japan?

That is a more complicated answer, but I believe there was a continuing theme at ESHRE 2017 related to the value of single embryo transfer, or SET, with regard to perinatal outcomes—a broader view than just cumulative live births. I would remind you that Japan was at the forefront of guideline recommendations

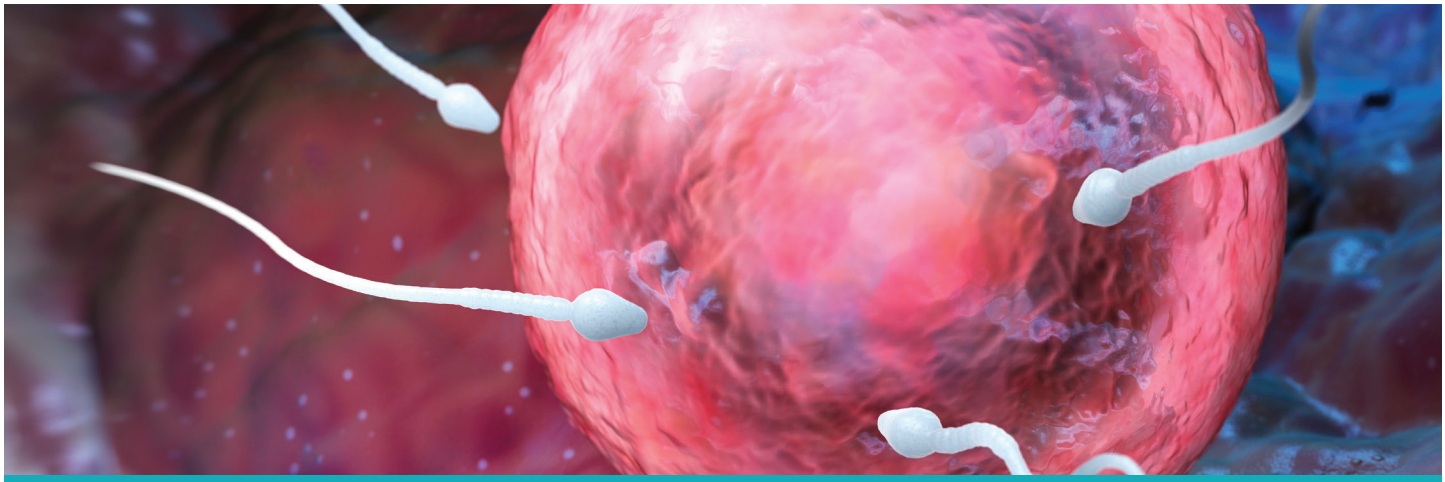


to employ SET in IVF implantation protocols. In 2008 the Japan Society of Obstetrics and Gynecology issued recommendations for SET for all cases except in repeated IVF failure or in patients greater than 35 years of age, where dual embryo transfer was acceptable. Numerous publications have validated those recommendations, one of the more recent by Takeshima and colleagues published in Fertility and Sterility in 2016 (see: *Fertil Steril*. 2016:105(2):337-346.e3).

With regard to presentations at ESHRE 2017, there were three that proved of interest. The first was by Magnusson and colleagues and were data from The Swedish National Quality Registry of Assisted Reproduction during 2007 to 2013. The title was *The number of oocytes retrieved during IVF: a balance between efficacy and safety* (O-038). They confirmed the value of SET, provided an upper number of retrieved oocytes, 20, that is a positive prognostic marker for attaining a live birth, and stressed the increasing utility of using frozen-thawed embryos for implantation.

The second presentation was from Roca and colleagues and addressed oocyte donation programs in Spain. It was titled *Single or double embryo transfer? Decision-making process in patients participating in an oocyte donation programme* (P-565). The strength of this poster presentation was that it provided absolute evidence that appropriate patient education regarding the likelihood of attaining a live birth is not improved with double embryo transfer as opposed to single embryo transfer. Critical is the understanding that there are more risks than benefit in double embryo transfer and, once understood, a more reasoned decision can be reached comfortably by the patient.

The third presentation is one that perhaps provides a prognostic marker for selecting an embryo and, if so, strongly supports use of single embryo transfer in that a high quality embryo will be selected for implantation. This was the poster by Geraldo and colleagues titled *MicroRNAs in day three embryo culture media as non-invasive biomarkers of implantation and live birth* (P-138). They asked if microRNAs are a useable biomarker of a high quality embryo more likely to implant and lead to a live birth, and found that microRNA signatures may be such a tool. While there are obvious limitations to this presentation, not the least of which is that implantation and attaining a live birth are complex process and that specific microRNA signatures in culture media are not likely to be prognostically definitive, they still may provide support when used with other more standard approaches to identifying quality embryos for transfer.



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Mitsutoshi Yamada
Department of Obstetrics and Gynecology
Keio University

QUESTION:
Which presentation at ESHRE 2017 did you find the most interesting?

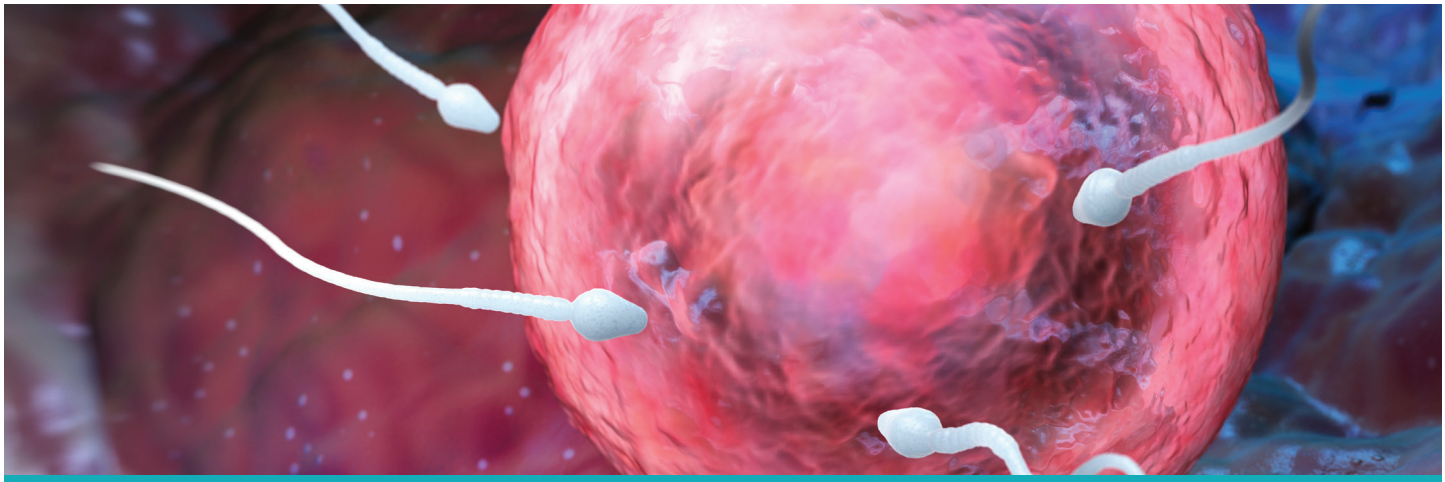
I have an interest in aneuploidies. As revealed by PGS, 70% of embryo meiotic aneuploidies originate from oocytes. Gamete origin of early pregnancy failure can be explained by abnormal meiotic divisions, abnormal spindle assembly, centromere reduction, telomere shortening and abnormal mitochondria function. In Japan, however, no clear consensus has yet been reached regarding now to best analyze aneuploid embryos. This area was nicely reviewed by Gianaroli (O-263) in a plenary session at ESHRE 2017.

Discussed in depth was that aneuploidy in human embryos is frequently due to malsegregation at meiosis and/or mitosis and that in natural pregnancies the great majority of aneuploidies can be traced to maternal chromosomes and are dependent on age. Data were presented that miscarriages that occur during the first weeks of gestation may go undetected and untested, suggesting that the incidence of aneuploid conceptuses may be higher from miscarriages or prenatal/postnatal diagnoses. Evidence was also presented related to sperm contribution to aneuploidy, and that aneuploidy is found in approximately 6% of sperm and even higher in severe male factor. Also suggested was existence of aneuploidy corrective mechanisms, which are corrective mechanisms that help restore development of a euploid conceptuses. Lastly, I should mention the use of polar body biopsy as a means to detect meiotic errors in oocytes as a valuable method to reduce abortion risk.

QUESTION:
Were there any other presentations at ESHRE 2017 that you found intriguing related to aneuploidy?

Yes, there were two presentations I found of great interest on this topic. The first was by Fragouli and colleagues titled *Factors affecting embryonic mosaicism* (O-110); and the second was from Munné and colleagues titled *Clinical results following the transfer of mosaic blastocyst—impact of different aneuploidy types to ongoing implantation rates* (O-290).

Fragouli and colleagues asked if differences in IVF procedures, such as culture medium, biopsy practitioner, incubator types, and patient characteristics influence the frequency of mitotic errors, leading to mosaicism.



The straightforward answer was that the type of medium used during embryo culture does affect mitotic malsegregation and rates of blastocyst mosaicism. Further, they suggested that certain patients generate an excess of mosaic blastocysts. The authors reviewed that mosaicism is the presence of chromosomally distinct cell lines within the same embryo, and that it is common in embryo development. Further, we now have next generation sequencing that allows accurate detection of mosaicism in trophectoderm samples.

A total of 19,719 embryos were examined in this study. Even so, Frangouli and colleagues believe that their study actually underestimates the true frequency of mosaicism. They further state that maintenance of genetic competence should represent a new focus for culture media development.

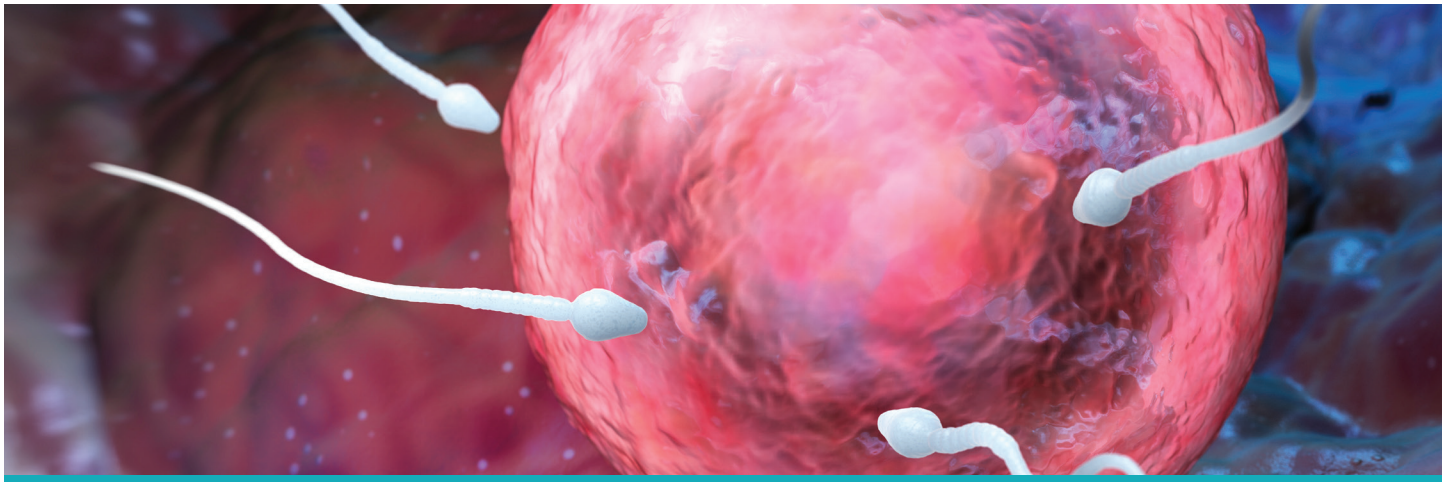
The presentation by Munné and colleagues addressed if the type of mitotic chromosome abnormality present in a trophectoderm biopsy affects the implantation potential of mosaic blastocysts. Their findings were very interesting in that while complex mosaics had lower implantation rates, the percent of abnormality, be it monosomy versus trisomy or full or segmental chromosome mosaicism, had no significant effect on pregnancy rates. This was a retrospective study comparing concurrent PGS cycles during which either 143 mosaic or 1045 euploid embryos were replaced in four fertility centers. This is important since the recent PGDIS (Preimplantation Genetic Diagnosis International Society) guidelines recommend that in the absence of euploid embryos, one should prioritize *replacing mosaic embryos with a low percentage of abnormal cells by trophectoderm biopsy, with monosomies over trisomies, and certain aneuploidies over others. However, to date, there has been little evidence to support this position*. In their study, Munné et al confirmed that pregnancy rate with mosaic monosomies (50%) was not statistically different from that of mosaic trisomies (41%).

Based on the author's findings that complex mosaic blastocysts have lower ongoing implantation rates than other mosaics, they suggest that mosaic blastocysts with 40% to 80% abnormal cells on trophectoderm biopsy samples will have similar ongoing implantation rates. However, mosaic monosomies perform as well as mosaic trisomies and mosaic segmental aneuploidies, providing support that current guidelines should be revised. The retrospective nature of this study is a limitation; nonetheless, the data are intriguing.

QUESTION:

What other information from ESHRE 2017 do you think is most applicable and important in Japan?

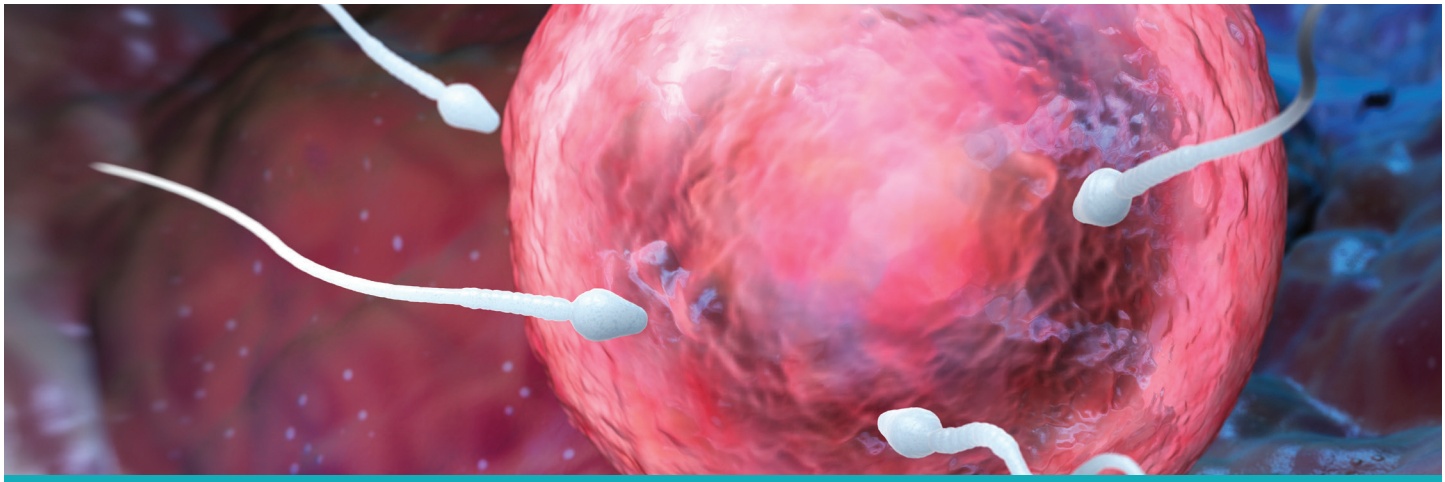
I believe that Wilkinson in an Invited Session asked and addressed a very important ethical question we will certainly face more frequently in the future. The question is, "*Should we edit the genomes of our future*



children?" (O-047). Clearly this is important not only in Japan, but also worldwide. Wilkinson correctly states that "*...this possibility raises various practical concerns, notably concerning safety.... It is argued that the ethics of genome editing depends, to a large extent, on what ends it is designed to achieve. There may, for example, be a huge difference between seeking to prevent painful and life-threatening diseases in future generations and seeking merely to satisfy prospective parent's aesthetic preferences.*" In August, a study published in Nature (Ma and colleagues, Nature 2017; see: https://www.nature.com/nature/journal/v548/n7668/full/nature23305.html#discussion) showed that by modulating the cell cycle stage at which the double strand breaks are induced, genome editing can be highly efficient without off-target mutation. But I personally think that genome editing is still an immature tool for clinical use, and also contains ethical problems. An international committee, composed of representatives from the National Academy of Science and the National Academy of Medicine in Washington, D.C., USA, issued a report concluding that additional studies are necessary and clinical trials can be performed but only under strict oversight. Importantly, the committee stated that genome editing should not be used for enhancement. Thus, at present, we need to focus on its possibility as therapy, keeping in mind that genome editing is not ready for clinical use.

Another important presentation came from Japan. This was offered by Ezoe and colleagues and titled *Complete zona pellucida removal facilitates embryo attachment and outgrowth by upregulating the integrin α 5 and α 1 expression in human blastocysts: in vitro outgrowth model* (O-008). They addressed if complete removal of the zona pellucida as a method of assisted hatching improved the adhesion and outgrowth of vitrified, warmed human blastocysts. They concluded that in fact complete zona pellucida removal increases the chance of blastocyst adhesion and promotes subsequent outgrowth by upregulating integrin 5 and 1 expression after the vitrification-warming procedure.

This was a study of 217 discarded cryopreserved human blastocysts that were donated for research by consenting couples, with the woman's age being 35.4 ± 0.3 years. The very relevant finding was that the complete zona pellucida removal as an assisted hatching method prevents hatching failure and is advantageous for blastocyst adhesion and its outgrowth as assessed in an in-vitro model. The authors state that when a blastocyst is chosen for vitrified-warmed blastocyst transfer, complete zona pellucida removal may help to increase the chance of blastocyst attachment. They also believe that further studies are required to explore the clinical efficacy of complete zona pellucida removal, a viewpoint I also share.



In closing, I would like to say that I am interested in embryo genome activation. Genome activation occurs at 8-cell stage of human embryos. Mosaicism is due to mitotic error, and seems to occur independently of genome activation. My personal focus is to identify the causes of embryo arrest and/or abortion through a better understanding of not only its cytology, but also through transcriptome analysis. My goal is to improve live birthrates by restoring these molecular dysfunctions.

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