#5

Fertility Newsletter 2021

ANNUAL EDITION ABOUT THE NEWS AND ACTIVITIES OF EMBRYOLAB ACADEMY

Fertility Newsletter #05

About us

Embryolab Academy is a non-for-profit foundation, focused on education, training and research in assisted reproduction and reproductive medicine. Embryolab Academy organises and hosts international workshops and seminars, focused on state of the art assisted reproduction techniques.

Issue participants

Alexia Chatziparasidou
MSc, PMI-RMP, Clinical Embryology Consultant, Director at Embryolab Academy, Co-founder of Embryolab

Nikos Christoforidis
MD, MSc, FRCOG, Obstetrician/Gynaecology Surgeon, Scientific & Clinical Director at Embryolab, Co-founder of the Embryolab Academy

Marina Dimitraki
MD, MSc, M.H.A., PhD, EFOG-EBCOG, Assisted Reproduction Gynaecologist, European Fellow of Reproductive Medicine ESHRE/EBCOG, Embryolab Associate Scientific Director

Achilleas Papatheodorou
PhD, M.Med.Sc., Senior Clinical Embryologist (certified by ESHRE), Director of the Embryolab Laboratories

Michalis Kyriakidis
MD, MSc, Assisted Reproduction Gynaecologist, Embryolab Associate Scientific Director

Marianna Papadopoulou
BSc, Clinical Embryologist (ESHRE certified)

Mary Karagianni
BSc, MSc, Biologist, Clinical Embryologist

Chara Oraipoulou
BSc, MRes. Biologist, Sr. Clinical Embryologist (ESHRE Certified)

Evi Kalouta
Psychologist

Issue edition

Vasso Petridou
M.Sc., Embryolab Marketing and Communications Manager

Find us

173-175 Ethnikis Antistaseos st.,
55134 Thessaloniki, Greece
T. +30 2310 473001
info@embryolab-academy.org

www.embryolab-academy.org

Embryolab Academy: Eight years of International Progress and Innovation!

Alexia Chatziparasidou
MSc, PMI-RMP, Clinical Embryology Consultant, Director at Embryolab Academy, Co-founder of Embryolab

The rapid advances in scientific knowledge over the last years have been boosting our techniques and practices daily, resulting in the ongoing improvement of the level and effectiveness of the services we offer to our patients.

Nowadays, a modern assisted reproduction unit must constantly adapt to changes and advancements, while continuing to operate and safely offering its valuable services to its patients. In this unique environment, systematic education and effective management of change seem to turn into the main components for the operation of a modern and successful assisted reproduction unit.

Since 2013, the Embryolab Academy, true to its founding values, has been organizing educational seminars for all modern assisted reproduction lab techniques. Our mission is to educate the scientists in this field, promote the science of assisted reproduction, offer training in the new lab techniques and introduce them to the labs worldwide.

Our Academy continued its mission in the last 2 years, despite the adverse conditions imposed by the pandemic. It participated in online seminars held by renowned organisations in Greece and abroad.

In April 2021 it had the honour of hosting its own global online virtual event on: Safe Practices in Assisted Reproduction. The seminar was very well received, with 1,081 people registering for the event from 80 countries around the world! The comments we received were full of praise, both for the content and for the ground-breaking hosting of the event.

In October 2021, supported by pharmaceutical giant Merck, it held a two-day training seminar on: Preimplantation Genetic Diagnosis Practices. The event was addressed to our African colleagues, who were seeking ways to train on new techniques, such as preimplantation genetic diagnosis, so as to introduce them to their countries and labs, and offer more to their patients!

Our Academy has been treading this planet for 8 years, bringing together scientists from every corner of the globe, and promoting training and education that advance assisted reproduction practices through our internationally recognised work.

Since 2013, the Embryolab Academy, true to its founding values, has been organizing educational seminars for all modern assisted reproduction lab techniques.

Speckle-Tracking Technology

All the latest in the Ultrasonic Assessment of Uterine Contractility

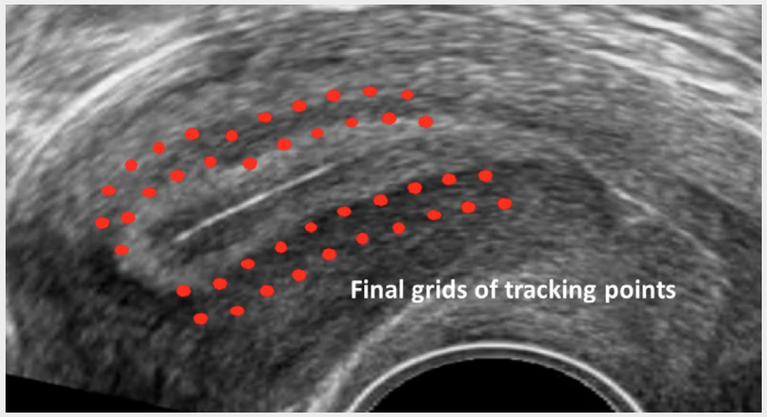
Nikos Christoforidis

MD, MSc, FRCOG, Obstetrician/Gynaecology Surgeon, Scientific & Clinical Director at Embryolab, Co-founder of the Embryolab Academy

Uterine contractility is turning into a significant additional factor affecting the chance of successful foetal implantation in IVF treatment. While normally some uterine contractile activity is observed, depending on which stage of her cycle a woman is, this activity is affected both by the hormonal treatment followed in various assisted reproduction protocols and by abnormal conditions of the uterus, such as the presence of uterine fibroids or adenomyosis.

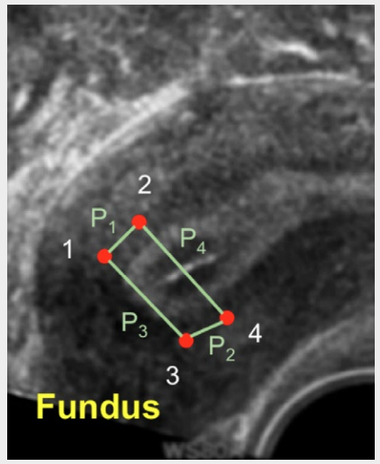
Until recently, the diagnosis of uterine contractility was mainly based on ultrasound techniques, with several difficulties encountered in the objective interpretation of uterine activity recordings. With the introduction of speckle-tracking technology in modern ultrasounds, we are now able, on the one hand, to record myometrial activity with greater accuracy and precision and, on the other hand, to objectively assess this activity and correlate it to the receptivity of the endometrium.

> With the introduction of speckle-tracking technology in modern ultrasounds, we are now able to record myometrial activity of the endometrium with greater accuracy and precision.



Prompted by the research activity of F. Sammali in ultrasound speckle-tracking technology in 2018, in partnership with the Eindhoven University of Technology and Catharina Hospital, Embryolab initiated a research protocol to investigate the correlation between uterine contractility and the outcome of the assisted reproduction treatment cycle. Initially, our aim is to investigate the cases where uterine contractility reduces the chance of successful implantation. In the future, we will examine ways to alter uterine contractility, such as through scheduled cryopreservation and embryo transfer strategy or, later on, through administration of pharmaceutical products that are associated with a decrease in uterine contractility, such as progesterone, oxytocin antagonists, etc.

Ideally, ultrasound examination of uterine contractility before embryo transfer will be the last, yet extremely significant, factor for the successful completion of assisted reproduction treatment.



Effectiveness of Mild Stimulation Cycles and Embryo Collection in women with Diminished Ovarian Reserve

Michalis Kyriakidis

MD, MSc, Assisted Reproduction Gynaecologist, Embryolab Associate Scientific Director

Major strides have been made over the last decades to render assisted reproduction methods more successful and more patient-friendly. It is now evident that the effectiveness of IVF is greatly associated with ovarian stimulation response and the number of recruited eggs.

These days, the most common approach is the pharmaceutical stimulation of the ovaries, based on the rationale that response depends on the dose of the pharmaceuticals administered. Evidently, the aim is to increase the likelihood of success by increasing the number of eggs collected. Indeed, it seems that the ideal result is produced when a couple collects an adequate number of eggs and embryos.

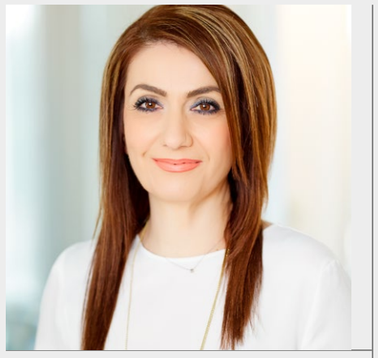
In spite of this, a high percentage of women present diminished ovarian reserve and a low number of eggs. This group of women will not respond excessively to ovarian stimulation, despite the high doses of pharmaceutical treatment that may be used. The best alternative for these women is to combine mild stimulation cycles with embryo collection.

The term “mild stimulation” refers both to the low-dose pharmaceutical treatment and the restriction of the hormonal burden during the treatment cycle. During these cycles, pharmaceutical treatment depends on the special traits of each woman and may include anything-from stimulating pills to injections. For almost the past two decades, literature has linked mild-stimulation protocols to the collection of better quality eggs and higher pregnancy rates. It is possible that a homogeneous good-quality group of eggs may be due either to the best natural selection by the ovary or to the lowest exposure to stimulating drugs. The benefit of mild-stimulation protocols is not only limited to the characteristics of the eggs, but also extends both to the hormonal environment of the uterus and to the implantation process. There are adequate data available presently for the clinical effectiveness of mild stimulation at levels comparable to conventional ovarian stimulation. However, the small number of available embryos is a restricting factor which does not allow the couple to maintain a stock.

The daily use of new technology nowadays, such as pre-implantation diagnosis, has created the need for a larger stock of embryos. Therefore, this limitation of mild protocols may be addressed with the collection of embryos during different cycles of the woman. Women with diminished ovarian reserve or high reproductive age will be especially benefited by the systematic collection of eggs or embryos. Being aware of the significant developments in cryobiology and the safety of cryopreservation methods, it becomes evident that embryo collection is a viable alternative.

So, combining mild-stimulation protocols with the collection and cryopreservation of embryos is quite a successful strategy these days. Of course, it must be adapted to the special traits and wishes of the couple. However, it remains an effective alternative and a useful tool in achieving our goal to create a family.

Changes in the Menstrual Cycle after COVID-19 infection or vaccination



Marina Dimitraki

MD, MSc, M.H.A., PhD, EFOG-EBCOG, Assisted Reproduction Gynaecologist, European Fellow of Reproductive Medicine ESHRE/EBCOG, Embryolab Associate Scientific Director

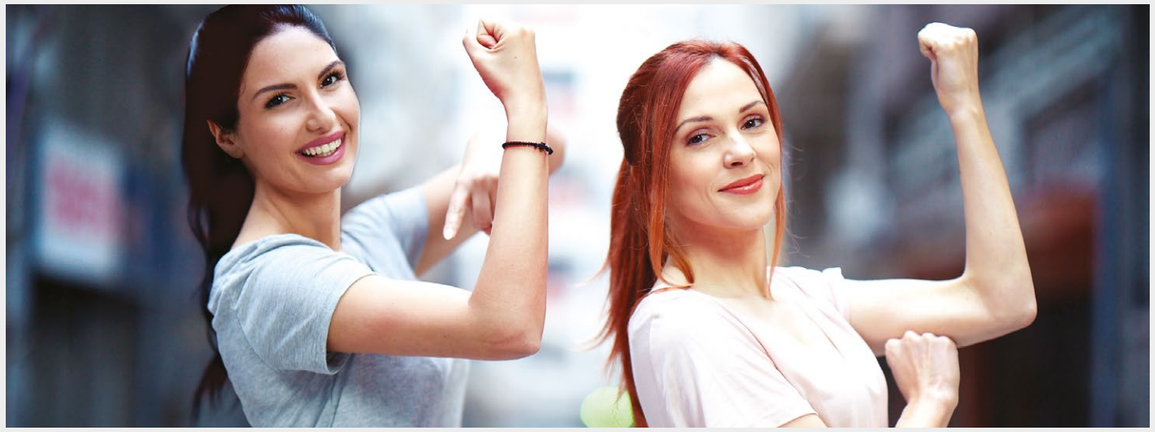
As we are nearing two years into the COVID-19 pandemic, there is increased interest in determining and understanding the post-infectious consequences of SARS-CoV-2 infection as well as the adverse reactions of the vaccines against the virus. More and more data are demonstrating that both SARS-CoV-2 infection and vaccines, as well as the increased emotional stress associated with coming into contact with the virus can affect the women's menstrual cycle. As a matter of fact, with regard to the vaccines, the menstrual disorders recorded were not associated with the type of vaccine (meaning whether it was an mRNA or adenovirus-vector vaccine). The most frequent menstrual disorders were mainly late menstruation and less blood volume, while irregular cycles and loss of great blood volume were reported less frequently. In 99% of the cases, the disorders subside after 1-2 months following infection or vaccination.

The menstrual cycle includes a series of biological events and complex interactions between various tissues, hormones and systems. Consequently, it is sensitive to internal and external factors, including infections and lifestyle changes. It seems that the activation of the immune system in response to some virus may affect the endocrinological events of the cycle. It has been proven that certain viruses affect the reproductive endocrine system. Disorders of the menstrual cycle are often observed in HBV and HCV infection, while HIV has been associated with early menopause.

The biological mechanisms behind the correlation of the SARS-CoV-2 infection or vaccine to the menstrual disorders include hormonal and immunological causes.

The proper function of the hypothalamic-pituitary-ovarian axis results in stable and regular ovarian function and, consequently, cycle. In cases of extreme stress – both physical and emotional – hypersecretion of stress hormones (ACTH, cortisol, prolactin, oxytocin, epinephrine, norepinephrine, vasopressin) is observed, which suppress the production of pituitary hormones, resulting in menstrual disorders. Besides, it is a well-known fact that when the body is experiencing intense stress, as is the case in an acute infection, the ovarian function is suppressed so as to preserve more important body functions, resulting in absence of ovulation and disorders of the menstrual cycle.

> The response of the immune system to the virus seems to also play a key role in the etiopathogeny of menstrual disorders.



The response of the immune system to the virus seems to also play a key role in the etiopathogeny of menstrual disorders. The relationship between oestrogens (produced mainly in the 1st phase of the cycle) and immunological response has been studied. The cytokines – inflammatory cells – play a significant role in the function of the menstrual cycle, affecting the hormones of the reproductive axis, as well as in the changes and functionality of the uterine interior, the endometrium, both during the cycle and during the first stages of pregnancy.

Treatment for COVID-19, which often includes corticosteroids, may also be considered a cause of disorders in menstruation, affecting the regularity of the cycle the mechanisms mentioned above.

In addition, COVID-19 seems to potentially directly affect the ovary, since, as arising from a study on animals, the receptor through which the virus enters the cells, the angiotensin-converting enzyme 2 (ACE2), is expressed on the granular cells of the ovary (the high expression of this enzyme on the testicles is also known).

Note that the menstrual cycle seems to play a protective role against COVID-19, as younger women of a reproductive age present decreased morbidity and mortality compared to men of the same age. In this case, it is oestradiol that plays a protective role, as it regulates the cytokines, the key proteins of the immune function.

Apart from everything else, every medical intervention and treatment must also be examined and studied with regard to its potential impact on the menstrual cycle. Further studies will provide additional information on the effect of COVID-19 infection or vaccination on the hormonal profile and menstrual cycle for specific groups of women. Our team is actively participating and showing keen interest in studying the effect that the contact with the specific virus or the vaccination against it may have on the hormonal events of the menstrual cycle and the endocrinological profile of women of a reproductive age.

Useful articles

Li K, Chen G, Hou H, et al. *Analysis of sex hormones and menstruation in COVID-19 women of child-bearing age.* Reprod Biomed Online 2021; 42:260-7.

Morris RS. *SARS-CoV-2 spike protein seropositivity from vaccination or infection does not cause sterility*. FS Rep 2021.

Orvieto R, Noach-Hirsh M, Segev-Zahav A, Haas J, Nahum R, Aizer A. *Does mRNA SARS-CoV-2 vaccine influence patients' performance during IVF-ET cycle?* Reprod Biol Endocrinol 2021;19:69.

Medicines and Healthcare Products Regulatory Agency. COVID-19 vaccines: updates for August 2021. https://www.gov.uk/drug-safety-update/covid-19-vaccines-updates-for-august-2021

Bentov Y, Beharier O, Moav-Zafrir A, et al. *Ovarian follicular function is not altered by SARS-Cov-2 infection or BNT162b2 mRNA Covid-19 vaccination.* medRxiv 2021:2021.04.09.

Safrai M, Rottenstreich A, Herzberg S, Imbar T, Reubinoff B, Ben-Meir A. *Stopping the misinformation: BNT162b2 COVID-19 vaccine has no negative effect on women's fertility.* medRxiv 2021:2021.05.30.

Eunice Kennedy Shriver National Institute of Child Health and Human Development. Item of interest: *NIH funds studies to assess potential effects of COVID-19 vaccination on menstruation.* 2021.

Karagiannis A, Harsoulis F. *Gonadal dysfunction in systemic diseases.* Eur J Endocrinol. 2005; 152:501-13

Monin L, Whettlock EM, Male V. *Immune responses in the human female reproductive tract. Immunology.* 2020; 160:106-15.

Psychological support

Psychological effects of Covid-19 Pandemic in couples starting ivf treatment

Evi Kalouta

Psychologist

This research is carried out by the Clinical Department of Embryolab Fertility Clinic and specifically by the Psychological Support section. It aims to study the effects of Covid-19 Pandemic on the psychological state of people who organize their ivf therapy in the present circumstances.

It's a quantitative research and the data are collected through a questionnaire, which is completed individually by men and women. Participation is voluntary. Questions are about emotional impact of the process of assisted reproduction, the fact that it begins in the midst of the pandemic and also of the pandemic itself.

Through this research, the Clinic's target is to understand to a great extent the impact of the pandemic on couples' psychology and thereby, adapt support more efficiently through a holistic care model.

Implementation period: May 2021 – December 2021

Applying Artificial Intelligence (AI)

Optimise the selection of the most suitable embryo for transfer in Assisted Reproduction Cycles

Achilleas Papatheodorou

PhD, M.Med.Sc., Senior Clinical Embryologist (certified by ESHRE), Director of the Embryolab Laboratories

It is widely and globally accepted that the fertility of couples diminishes over time. The World Health Organization (WHO) has defined infertility as a "disease" of the reproductive system. Infertile couples resort to assisted reproduction treatments to find a solution to their problem. The science of assisted reproduction is relatively new and keeps progressing year by year. Despite all the clinical, laboratory and technological advancements in this field, the effectiveness of these treatments remains low. Only 1/3 of the couples who resort to In Vitro Fertilization (IVF) will manage to have a child. There is an imperative need to find effective tools that will improve the clinical result. Essentially, it is a social need.

IVF today

The IVF process includes the implementation of a clinical protocol, which results in the couple undergoing treatment having many embryos available for transfer into the woman's uterus. The legislation but also responsible clinical practice ideally demand transferring maximum one or two embryos into the woman's uterus. The limitation to this number exists so as to avoid multiple gestation, which, in some cases, may actually prove dangerous for the expectant mother.

The most crucial stage in IVF treatment is the selection of the most suitable embryo for transfer. If this is done correctly, the couple will have a positive result and will not need to resort to new treatment, which alleviates the emotional, physical and financial stress.

Embryo assessment

The traditional approach to embryo assessment is performed manually. It includes the assessment of the embryos by trained scientists, known as clinical embryologists, who use light microscopes to observe specific visual specifications. At the end of the assessment, the embryologist assigns a description/score to each embryo, trying to place them in an order of preference. The embryo with the highest score is considered the most suitable for transfer. The grading system most commonly used by embryologists is the Gardner system, which checks and grades certain areas of the embryo on the 5th day of its development, using alphanumeric scores: the inner cell mass (ICM), the trophectoderm (TE) and the rate of development of the embryo's distinctive cavity at this stage.

Unfortunately, embryo assessment carries a high degree of subjectivity and, as a result, great variations in grading are observed both between different laboratories and within the same laboratory. So it is clearly evident that it is hard to achieve standardisation in embryo assessment within one laboratory, all the more at an international level.

How can technology assist in embryo assessment?

The use of new technology in assisted reproduction attempted to provide better prospects for selecting suitable embryos, aiming to increase the effectiveness of IVF treatments. The embryos were placed in time-lapse (TL) technology incubators, meaning chambers with micro cameras, so they would be under constant observation. It was believed that this practice would assist embryologists to select the most suitable embryo and that it would be easier for the couple to eventually achieve pregnancy. Many couples were benefited by this technology, but a recent review of international literature revealed that there is room for improvement in the effectiveness of these treatments. Besides, even in this approach, the embryologists still select the embryo for transfer in a subjective manner. On many occasions, they may do so under emotional pressure. Additionally, this technology is so expensive that just 5% of laboratories globally were able to incorporate it into their day-to-day practice.

What is artificial intelligence?

"Artificial intelligence" (AI) is the field of computer science that designs and develops IT systems that simulate elements of human behaviour which imply at least basic intelligence: learning, adaptability, extraction of conclusions, context-based understanding, problem-solving, etc.

AI offers to machines the ability of understanding their environment, solving problems and acting towards achieving a specific goal. The computer receives data (readily available or collected via sensors, e.g. a camera), processes them and responds based on them.

The AI systems are capable of adapting their behaviour, to a certain extent, analysing the consequences of previous actions and solving problems autonomously. An impressive feat of their nature is that they learn from their mistakes and they self-improve.

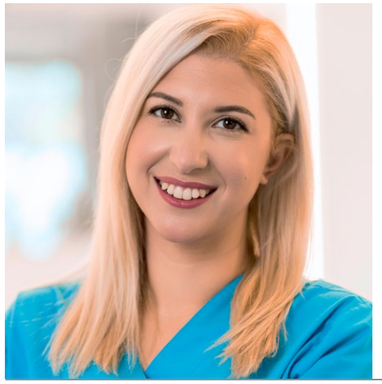
AI in assisted reproduction

The latest developments in the field of AI have helped optimise medical procedures. What we are expecting in assisted reproduction in the coming years is the development of certain IT systems – "machines" – that will be able to process and analyse a multitude of data relating to a couple (e.g. demographic, hormonal, genetic, information about the couple's laboratory attempts, etc.) and eventually link them to the videos of the embryos in pre-implantation development created using the time-lapse incubator technology explained above. Then, again with the help of AI, these machines will be trained to show us which embryo is the most suitable for transfer. This new scientific and technological approach aims to reduce to a minimum the time needed for an infertile couple to achieve pregnancy.

Use of AI in Embryolab

In the last two years, Embryolab has been participating in the development of an AI system, a non-invasive tool, that will feature automations and will indicate which embryo is most suitable for producing a much-wanted pregnancy. This technological product is the result of the partnership between Embryolab and AiVF, an Israeli tech and innovation company specialising in the development of AI systems for the field of assisted reproduction. The software this machine uses has been trained by tens of thousands of time-lapse videos with in-vitro embryo development footage and, guided by the Embryolab embryologists, has learned to perceive the stage of development the embryos are at and successfully grade them. In addition, this "machine" is able to perceive hundreds of morphokinetic events in embryo development and combine them with a positive or negative result. This is a continuous learning process that allows the system to self-improve. So, as time goes by, we are expecting that this system will develop the kind of AI technology that will allow us to identify the most suitable embryo for transfer not just based on the obvious features of an embryo, but also based on data the human eye cannot perceive. So we are aspiring for these innovative systems to increase the effectiveness of IVF treatments and bring about change in the field of assisted reproduction in general.

This new scientific and technological approach aims to reduce to a minimum the time needed for an infertile couple to achieve pregnancy.



Multinucleated Blastomeres

Embryos with or without Multinucleated Blastomeres in Preimplantation Genetic Diagnosis (PGD) cycles to test for Aneuploidy: Is there a difference?

Marianna Papadopoulou

BSc, Clinical Embryologist
(ESHRE certified)

The findings of a recent study by Embryolab, which were announced at the 37th virtual conference held by ESHRE, could become useful tools in the hands of embryologists in selecting the most suitable embryos for transfer. The study evaluated specific events observed during embryonic development and how these could influence the embryo itself, as well as the IVF outcome.

Specifically, when the first cell of the embryo, called the zygote, starts to divide, two new cells are expected to arise, similar both to each other and to the original cell. However, in some cases, some of these new cells have more than the one nucleus that would normally be expected. The presence of multinucleated blastomeres in embryos cultivated as part of IVF is a phenomenon that always raised concerns among clinical embryologists. For several years, the observation of such cells was random and sporadic. However, it has become much more detailed with the new-technology time-lapse incubators. This is happening because it is now possible to observe embryonic development in great detail 24/7.

> The study findings indicated that the presence of multinucleated blastomeres within one IVF cycle does not affect embryonic development.

With this innovative equipment available at our Unit, we were able to complete a retrospective research study on the presence of multinucleated blastomeres in embryos and how this correlates with the chromosomal composition of embryos, but also the possible effect on the clinical outcomes of IVF.

The study included 97 IVF cycles, where PGD for aneuploidy was performed from May 2017 to December 2020. For the purposes of the study, all the embryos that had been cultivated in a time-lapse incubator were split into 2 groups, depending on the presence or not of multinucleated blastomeres during their development.

The study findings indicated that the presence of multinucleated blastomeres within one IVF cycle does not affect embryonic development, as to the likelihood of blastocyst formation, or the pregnancy and birth rates, which did not present significant difference between the two groups upon comparison. Furthermore, the presence of multinucleated blastomeres did not seem to be associated with a specific type of chromosomal anomalies.

However, the findings revealed that embryos arising from one IVF cycle and which had presented multinucleated blastomeres at some stage of their development tend to carry chromosomal anomalies more frequently. This finding may be used as a tool for the selection of embryos for transfer, provided, of course, that several embryos are available.

Artificial collapse-AC

Artificial Collapse (AC) of Human Expanded Blastocysts protects the quality of embryos during the vitrification/warming procedure

Mary Karagianni

BSc, MSc, Biologist,
Clinical Embryologist

Embryology is a rapidly developing science both on a clinical and an embryological level. A typical example of how the clinical management of IVF incidents has changed is the use of the Freeze All strategy. In the past, each new IVF cycle that started with oocyte retrieval ended up in embryo transfer in 90% of cases. From the moment it was established that hormonal stimulation influences the receptivity of the endometrium, and thanks to the simultaneous development of cryopreservation (vitrification) methods, this rate changed. These days, 80% of cases will not end up in embryo transfer, but in the selective freezing of all embryos, some of which will then be thawed and transferred to a next cycle of endometrial preparation. On an embryonic level, the technology of cultivated material production has progressed by leaps and bounds and, coupled with the use of new-generation incubators, it will allow us to cultivate embryos in ideal laboratory conditions, resulting in the development of more, larger (expanded) and better quality blastocysts! Therefore, we are living at a time when we have all the more in-vitro embryos which we can freeze at any time, to use when the conditions allow us to do so.

However, expanded blastocysts appear to be more sensitive and susceptible to cryo-injury during vitrification, due to the large quantity of fluid in the blastocoel, which may cause insufficient dehydration. This is where artificial collapse (AC) comes into play. This method is applied before cryopreservation, whereby the blastocyst is collapsed and as much as possible fluid is removed from the blastocoel. As a result, the contact time of the blastocyst with the cryoprotectants is sufficient to allow adequate dehydration of the blastocyst cells.

At Embryolab we conducted a prospective, randomised study, which included 94 cases of good prognosis with more than 4 expanded blastocysts. The patients were randomised and assigned to either the study group, where artificial collapse was applied to 1 or 2 expanded blastocysts with the use of laser before vitrification, or the control group, where the corresponding embryos were frozen without artificial collapse. A total of 171 embryos were analysed and cryoinjury was classified depending on the presence of degenerated cells in the inner cell mass (ICM) or the trophectoderm (TE).

According to the study findings, cryoinjury in the AC group was significantly lower compared to the control group ($p<0.05$). The difference in the pregnancy rate (positive beta-HCG), but also in the clinical pregnancy rate, was not statistically significant between the two groups under study.

In conclusion, artificial collapse with the use of laser does not pose a threat to the quality of blastocysts. On the contrary, it appears to reduce the likelihood of injury during freezing. What remains to be examined in larger-scale studies is whether it can also improve the pregnancy rate.

At Embryolab we conducted a prospective, randomised study, which included 94 cases of good prognosis with more than 4 expanded blastocysts.

The path to clarifying the Genetic Basis of Azoospermia starts in Greece!

Alexia Chatziparasidou

MSc, PMI-RMP, Clinical Embryology Consultant, Director at Embryolab Academy, Co-founder of Embryolab

Azoospermia is defined as the condition where no spermatozoa are identified in 2 or more ejaculation samples under microscopic observation of the samples. Around 1 in 10 infertile men suffer from azoospermia.

Up until 1995, azoospermia diagnosis was tantamount to sterility. Due to the advancements in the science of assisted reproduction, the 1st child after intracytoplasmic sperm injection using testicular spermatozoa was born in 1995, a feat that gave hope to millions of couples for the first time.

In the years that followed, the IVF method, combined with testicular biopsy, was the leading choice for men with azoospermia, and thousands of children have been born around the world to date thanks to it. Despite the very important advancements all these years, there are significant gaps in knowledge as to the genetic basis of azoospermia, as well as the ability of effective diagnosis and treatment to restore fertility in azoospermic males. Today, unravelling, the genetic basis of azoospermia seems to be the most promising path for advancing our knowledge and our diagnostic and treatment options for these men. In 2019, the Embryolab Assisted Reproduction Unit and the Embryolab Academy, in partnership with the BIOZ Laboratory of the Department of Biochemistry and Biotechnology, University of Thessaly, submitted Spermogene, a ground-breaking research proposal, to the Research-Create-Innovate initiative, on the topic of clarifying the genetic basis of male infertility. The proposal was selected among thousands of others and received funding.

Since then, hundreds of semen and blood samples have been collected, with the support of volunteers of the Spermogene project.

The blood and semen samples undergo detailed genetic analysis using new and advanced genetic analysis tools. With the help of bioinformatics, the data arising are then analysed to identify the markers that are more directly linked to male infertility. A special group of samples is that of azoospermic males. In this patient group, we aim to:

1. Identify genetic markers that will allow us to predict whether spermatozoa will be found or not in the testicles of azoospermic males.

2. Identify genetic markers that will allow us to accurately determine the functional adequacy of testicular spermatozoa and their ability to lead to a healthy pregnancy.

3. Clarify the inherited mechanisms associated with the various types of azoospermia.

More than 30,000 genes have been studied to date and their translated products (RNA molecules) have been identified and quantitatively classified in the various types of azoospermia, to identify the underexpressed and overexpressed genes in men with azoospermia compared to men with normal spermatogenesis. The data available to date for men with azoospermia confirm the existence of significantly different genetic expression profiles, which, in turn, greatly affect the biological paths directly or indirectly involved in the process of spermatogenesis.

This research project is a very important partnership among organisations and scientists from different fields of study, who join forces and, with the most modern genetic analysis and bioinformatics tools in their arsenals, create the best possible conditions for clarifying the genetic basis of azoospermia.

Testicular Tissue

Effect of Testicular Tissue Quality and Cryopreservation on laboratory and clinical outcomes

Chara Oraipoulou

BSc, MRes. Biologist, Sr. Clinical Embryologist (ESHRE Certified)

Non-obstructive azoospermia (NOA) is marked by absence of spermatozoa during ejaculation due to pathologic spermatogenesis. Nowadays, a significant percentage of men with NOA can have children, since the likelihood of locating spermatozoa following testicular biopsy is close to 50%. The question is whether the laboratory and clinical outcome differs depending on the quality of the testicular tissue from where the spermatozoa used for microfertilisation (ICSI) originate. The answer to this question was examined as part of an Embryolab study, also taking into account the effect of possible testicular tissue cryopreservation.

Testicular tissue quality following biopsy is determined by three key parameters: the spermatozoa concentration in the testicular sample, the presence of motile spermatozoa and the testicular spermatozoa morphology. Two groups were used to conduct the study: a) good-quality and b) low-quality testicular tissue samples. The aim was to investigate their effect on the fertilisation rate, the embryonic development up to day three, the positive beta-HCG rate and the birth rate. In addition, the effect of testicular tissue cryopreservation was assessed against the above rates.

According to the Unit's published findings (https://doi.org/10.1111/and.14040), the quality of testicular tissue does not affect the pregnancy and birth rates (Table 1). However, it is interesting that the low-quality testicular samples are associated with lower fertility rates and produce fewer good-quality embryos on day three. Consequently, investing time to find suitable testicular spermatozoa for fertilisation is of essence even in low-quality testicular samples. Despite all this, the experience of the operator in determining the time frame for searching for spermatozoa plays an important part, since it has been proven that when the search time exceeds a certain limit, the fertilisation and pregnancy rates drop in cycles with fresh eggs.

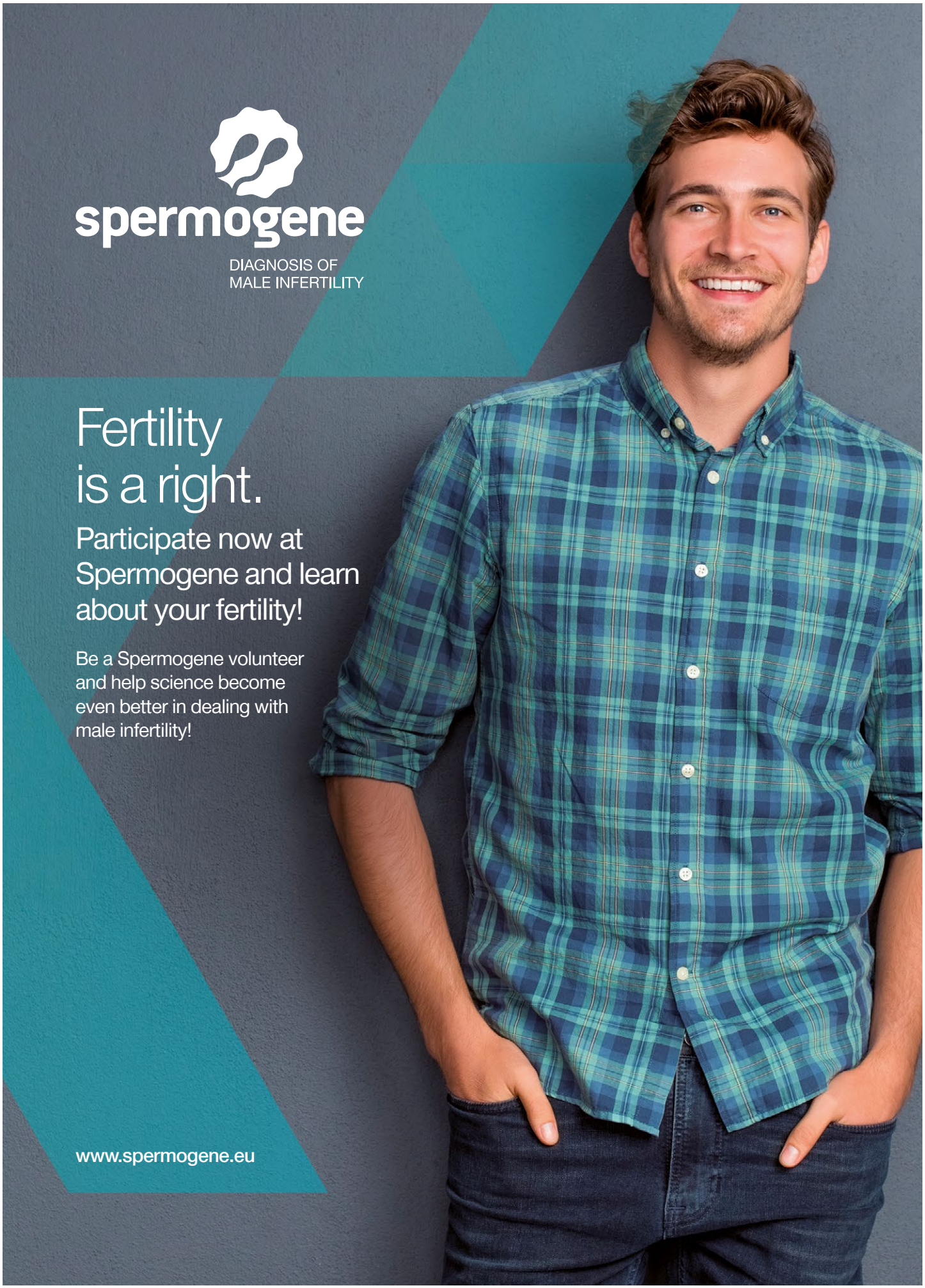
The cryopreservation of testicular tissue does seem to influence the clinical outcome, since there is no statistically significant difference in the pregnancy and birth rates among fresh and frozen testicular spermatozoa (Table 1). Nevertheless, low-quality fresh testicular samples seem to lead to better outcomes in terms of day three embryo formation, compared to frozen testicular samples of similar quality.

To date, the possibility of predicting the ability of testicular spermatozoa to support embryonic development is limited. These data, coupled with additional studies, could assist in creating a model for predicting the reproductive potential of testicular spermatozoa, so as to improve the treatment of men with non-obstructive azoospermia.

Table 1

Statistical results from the comparison of the two groups of testicular samples (Group A: good-quality testicular samples, Group B: low-quality testicular samples) to determine their effect on the fertility rate, the number of good-quality day 3 embryos, the positive beta-HCG rate and the birth rate.

	Fertilisation rate	Number of good-quality embryos on day 3	Positive beta-HCG rate	Birth rate
Group A vs Group B	64% vs 52% **p=.036**	66% vs 50% **p<.0001** (Group A: 1.56 additional embryos)	42% vs 36.9% **p=.600**	20% vs 22.6% **p=.764**
Group A, fresh vs Group A, frozen	69% vs 63% **p=.388**	65% vs 62% **p=.461**	30% vs 50% **p=.323**	20% vs 20% **p=.932**
Group B, fresh vs Group B, frozen	55% vs 50% **p=.470**	57% vs 47% **p=.0007** (Group B, fresh: 1.57 additional embryos)	27.8% vs 35.7% **p=.317**	22% vs 23.2% **p=.565**


spermogene
DIAGNOSIS OF
MALE INFERTILITY
Fertility
is a right.
Participate now at
Spermogene and learn
about your fertility!
Be a Spermogene volunteer
and help science become
even better in dealing with
male infertility!
www.spermogene.eu