

AETE

Association Européenne des Technologies de l'Embryon

Association of Embryo Technology in Europe

December 2021

AETE Newsletter Issue 56

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PRESIDENTS LETTER

Dear friends.

Another year is coming to its end; a year without any spectacular event, full of "working from home" and "meeting online through a digital environment". It was a year during which a "staycation" was the best we could get. No international scientific meetings, no social events with colleagues and friends, no handshakes or hugs, no plane travels overseas. It felt a bit like the "reset button" of our modern life had been pushed.

For our international society it was a challenge to move forward, to foresee content and provide an alternative for our cancelled physical meeting in Dublin. I am very proud and grateful for the decision that was taken by the board in early spring this year to come up with a completely new idea. A series of webinars replaced the physical meeting as all board members agreed that a full digital alternative for our cancelled meeting was not the best way to go.

Editor: Roger Sturmey

Our webinar series provided us with an ideal opportunity to strengthen our relationship with the National Embryo Transfer Societies in Europe by inviting them to cohost our webinars. After many emails and preparative online meetings, the AET-d (Association of Embryo Technologies of German speaking countries) was the first to step on board. They organized a very attractive webinar program about the new European regulations concerning assisted reproduction and trade of germinal products and embryos within Europe and with third countries. In October AETE and IETS cohosted a webinar about Emergencies in the IVF lab. This was also a very attractive and interactive program was offered and many people registered. Our last joint webinar was organized in collaboration with the Italian Embryo Transfer Society (SIET) covering the topic of the application of ART in a dairy cow setting. Many practicalities at the level of the donor, recipient but also at the level of the culture well were discussed in detail. Attendees were even given the chance to list their questions and to send them to the speakers after the meeting. In total, about 700 people registered for our webinars, originating from more than 60 different countries. All webinars were recorded and placed on the AETE YouTube channel. Everybody who registered received a password to watch the recordings. Today we count more than 330 views further illustrating the great interest.

Of course, as you know, AETE does more than just informing practitioners. Science is an important aspect of our society. This year we generated a platform for our Master's and PhD students to present their work and to compete for the best oral presentation prize. We organized two student webinars and so two oral presentation prizes were provided. Daniel Angel-Velez (Webinar 6-9-21) and Alba Perez-Gomez (Webinar 13-9-21) were chosen by the attendees as the best speakers. They were awarded €250 and a free registration to our next annual meeting in September 2022.

So yes, as I write this, there will be a next Annual meeting and we hope (we are almost sure) that this meeting will take place in a physical format. Both Jan Detterer and I had opportunity to visit Utrecht. Netherlands and to meet with the local organizing committee, chaired by Dr Hilde Aardema. All LOC members are very enthusiastic and provided us already with many great ideas for our next event. The city of Utrecht is a perfect location and also the venue will be optimal to house our meeting and to stimulate interaction. Final budget calculations are made, contracts are being signed and the scientific program is set. In the name of all the members of our Society I like to thank Hilde and her team for the excellent work they have done so far already. Our 38th Annual meeting will take place on Thursday and Friday 15 and 16 of September 2022.

I hope you all agree with me when I say that AETE is in good shape. All board members worked very hard and aided in the difficult transition process during the past Covid years. I am proud of our team and of what we achieved together and it is an honour to collaborate with them. Thank you very much. Dear friends, take care of yourself and your beloved ones. I wish you a healthy and happy 2022. Looking forward to meet you in person in Utrecht, The Netherlands next year in September.

Jo Leroy, President of the AETE

December, 2021

AETE BOARD MEMBERS

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REFLECTIONS ON THE AETE 2021 WEBINARS

During the past summer and autumn, AETE organized five webinars. In two of these webinars, held in September, ten selected abstracts out of all those submitted in 2021 were presented. The other three webinars covered practical topics, delivered by invited world experts, interesting for a wide group of our members, with a special focus on practitioners.



The webinar series started on June 17th with a topic "EU regulation for livestock breeding in the Europe and third countries", organized in collaboration with the German speaking embryo transfer society AET-d. This event was chaired by Christine Wrenzycki. Bernhard Polten presented the European animal breeding legislation; the history and basics, the goals, development and finally the status quo. Erik Mullaart described how the regulations are implemented in the standard procedures of embryo production in CRV, The Netherlands. Hanna Grothmann described how some of the regulations affect the certification of embryos in Germany. And finally Hélène Quinton described the practical consequences of some important points in the new Animal Health Law from the perspective of Evolution, France.

Emergencies in the IVF lab: lessons from human ART, October 6th, was cohosted with the IETS. The main speaker was Rita Vassena with a title "When good things go bad: technical failures and human errors in the IVF lab". She covered a wide range of non-conformances in IVF labs as well as quality control systems and standard operating procedures to control these. Daniel Le Bourhis' topic "How to prevent pathogenic contaminants in a bovine IVF lab" covered lab facilities, labware and equipment media as and possible source of а contamination. Lotte Stroebech presented a procedure of troubleshooting in the IVF lab: "IVF laboratory emergency rescue plan when blastocyst rate equals 0". Finally, we heard a sponsor talk by Vetoquinol and Peter May, who

introduced the newly founded British Embryo Technologies Association.

The final session of the webinar series was held on October 27th, in collaboration with the Italian Embryo Transfer Society SIET. Daniela Demetrio's presentation "Practical aspects of ART in the dairy cow business" included experiences and results from her daily ET-practice on a large dairy farm in California, RuAnn Genetics, where her team does excellent work and achieves superior results. Her talk was inspiring for all those who work in the bovine in vitro embryo production.

There were altogether 700 participants from 60 countries online during these webinars. AETE wants to thank all the presenters and the audience and the co-hosts AET-d and SIET. Special thanks go to Roger Sturmey, who was responsible for the technical implementation of all webinars.

Contributed by Marja Mikkola

HANDS ON TRAINING

COURSE - BOVINE IVF

In collaboration with the Advanced Breeding Team at Tyndale Veterinary Practice Ltd. U.K., IVF Bioscience recently hosted delegates from the EU and the UK, to be trained in the skills to successfully develop *in vitro* produced (IVP) bovine embryos; from immature oocytes right up to the blastocyst stage.

Tyndale Veterinary Practice have invested in their state-of-the-art bovine IVF laboratory and aspire to become a Centre of Excellence for bovine IVP in the UK.

Attendees were invited to experience first-hand the day-to-day running of a successful commercial bovine OPU-IVP provider and received hands-on training covering each aspect of the IVP protocol, as well as some theoretical classroom sessions.

Course Structure:

Firstly, attendees were given a tour of the facilities and introduced to Quality Assurance and laboratory management. Slaughterhouse ovaries were then aspirated before the oocytes were searched and cultured in maturation medium overnight.

Following overnight culture, oocyte maturation was assessed via cumulus cell expansion and viscoelasticity. Sperm quality (from both conventional and sex-sorted straws) was also assessed for motility and concentration, before being washed and introduced to the matured oocytes, for overnight fertilisation.



Meanwhile, blastocysts that had been cultured one week prior to the course were assessed and graded in accordance with the IETS guidelines.

Attendees were then introduced to the importance of record keeping and labelling for commercial IVF embryos, to comply with UK regulations. The blastocysts from the previous week were loaded into straws, so that they could be slow-frozen prior to being stored in liquid nitrogen.

Presumptive zygotes from the previous day were assessed for fertilisation rates and denuded of their cumulus cells prior to being cultured in a non-sequential culture medium for seven days.

Outcome:

The interactive workshops facilitated the exchange of knowledge between attendees from varying backgrounds and specialising in assisted reproductive technologies (ART) in different species.

The purpose of the bovine IVF training course was to introduce the basic aspects of IVP, upskill attendees' laboratory skills and improve their confidence in handling gametes and embryos in the IVF laboratory.

The course was well received and both attendees felt that the sessions equipped them

with the knowledge and skills to further their understanding of OPU-IVP and support the use of these technologies in their respective countries. One attendee stated, "The whole IVF laboratory course offered was extremely useful for us" and both delegates rated the course as "Excellent" in terms of organisation, content, and overall satisfaction.

As the demand for bovine IVP training increases, IVF Bioscience is looking forward to hosting training courses in collaboration with Tyndale Veterinary Practice in the future.

In addition to the bovine IVP training sessions, IVF Bioscience has collaborated with Drs Chelsey Leisinger and Carlos Pinto at Louisiana State University, to deliver a very successful equine OPU-ICSI training course in the USA. This hands on equine IVP course was the first of its kind to be held in the USA.

Contributed by Jake Silcock, IVF Bioscence

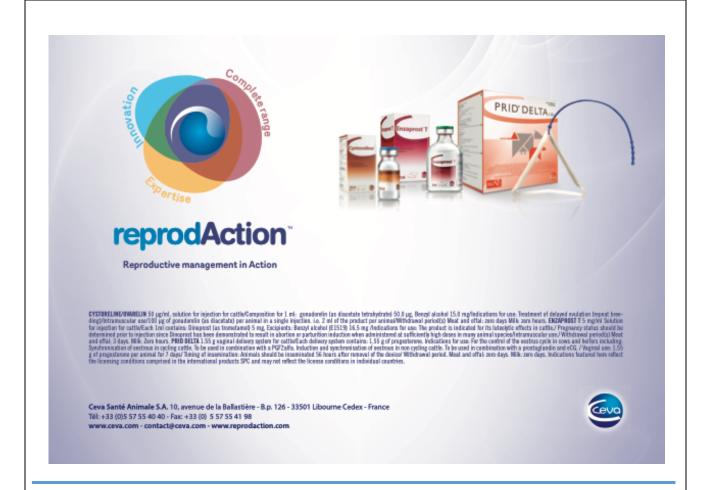
TRAVEL GRANT

The AETE would like to remind all members of the AETE travel grant. The purpose of the grant is to make a partial financial contribution to student presenters attending our annual conference. The number of grants available will be announced on Feb 15 each year, on both our website and our Facebook page. If awarded, the student will receive the bursary after the conference using electronic money transfer. In order to maximize the number of granted awards, the precise value of the award will depend on the estimated expenses and demonstrated/perceived needs recipients, up to a maximum of 250€ per awardee. The award can be used to cover costs associated with travel, hotel and registration for our AETE conference.

The grant is for students wishing to present their work as a first author during one of the lecture/poster sessions at the next AETE conference. It is mandatory that the student is the first author of the abstract.

Applicants will need a letter of support from their supervisor (or chair of department) and you will also need to write a letter explaining your motivation for the application.

Full details and full eligibility criteria can be found on our website: https://www.aete.eu/students/travel-grant/



PRACTITIONERS! GET INVOLVED...

A central mission of the AETE is to encourage the involvement of practitioners. With this ins mind we have a series of exciting new initiatives planned for the 2022 meeting in Utrecht.

First, the board decided to create a NEW CATEGORY FOR ABSTRACT SUBMISSION specially addressed to practitioners. We have called it "Practitioner's and clinical reports". In this category our members will be able to present case reports about original results for example.

Secondly, we will hold a practitioners forum, similar to the event in Murcia in 2019. During this forum, some selected case reports will be presented and practical interesting topics will be discussed. We will ask our sponsors to choose speakers with experience on the field to talk together about 3 possible topics:

- How to deal with nutrition and/or young animals for embryo production?
- What are the different used protocols of hormonal stimulation for IVD and IVP
- 3. Technical aspects (different possibilities of material to perform embryo collection, OPU...)

And finally, a Booth Tour will be organized during coffee breaks and poster sessions. Through this tour, sponsors will give an overview on their booth each in turn to present a new product, service or idea. An agenda will be given to all members to organize their own tour.

We hope this conference in Utrecht will attract and satisfy our practitioner members and encourage them to invite other practitioners for next AETE events."

Contributed by Helene Quinton

AETE 2021 PRIZES

BEST PRESENTATIONS AS VOTED BY ATTENDEES

Comparison of sugars as nonpermeating cryoprotectant for immature equine oocyte vitrification

Daniel Angel-Velez^{1,2}, Tine De Coster¹, Nima Azari-Dolatabad¹, Osvaldo Bogado-Pascottini^{1,3}, Ann Van Soom¹, Katrien Smits¹

^{1:} Department of Reproduction, Obstetrics and Herd Health, Faculty of Veterinary Medicine, Ghent University, Merelbeke, Belgium; ^{2:} Research Group in Animal Sciences - INCA-CES, Universidad CES, Medellin, Colombia; ^{3:} Department of Veterinary Sciences, Gamete Research Center, Veterinary Physiology and Biochemistry, University of Antwerp, Wilrijk, Belgium

Oocyte cryopreservation in horses would be an important complement to the growing clinical ICSI programs, but the efficiency is not satisfactory yet. Sugars are non-permeating cryoprotectants that exert an osmotic effect during vitrification. However, the influence of different sugars during vitrification and warming of equine oocytes on subsequent embryo development has not been evaluated. Therefore, we aimed to determine the effects of three sugars on the developmental competence of equine oocytes. Cumulus-oocyte complexes (COCs) were obtained slaughterhouse-derived ovaries. The cumulus cells were removed by pipetting until around 4 layers remained. Then, COCs were vitrified immediately in three groups: sucrose (S; n=155), trehalose (T; n=160) and galactose (G: n=153). Oocytes were equilibrated for 25 seconds in base solution (BS; TCM 199 with Hanks' salts and 0.4% (w/v) BSA) with 10% (v/v) ethylene glycol (EG) and (DMSO), dimethyl sulfoxide 10% subsequently transferred to a vitrification solution (BS with 20% EG, 20% DMSO and 0.5 M sugar), loaded onto a custom-made minimal volume (<1 μL) cryo-device (Equine Vet J. 50(3). 391-397. 2018) and plunged into liquid nitrogen within 40s. For warming, the cryo-device was placed directly into a 0.5 M sugar solution and incubated for 5 min, then COCs were transferred and washed in BS. All procedures were performed on a thermal plate at 39°C. Once warmed, COCs were matured in 500 µl M199 with Earls' salts and 10% FBS at 38.5°C in 5% CO₂ in air for 28 hours. Oocytes with an extruded polar body were injected by piezo assisted ICSI and presumed zygotes were cultured in 20 µl droplets of DMEM-F12 with 10% FBS under oil for 7 – 10 days at 38.2°C in 5% O₂, 5% CO₂ and 90% N₂. A control group (n=173) with non-vitrified oocytes was included in every replicate (5 replicates). The effects of the sugars

on maturation, cleavage, and blastocyst rate were fitted in generalized and linear mixed-effects models and results are expressed as least square means with standard errors. Galactose tended to result in a lower maturation rate compared to trehalose (p = 0.060) and the control group (p=0.069), but there was no significant difference in maturation rate among sugars (S: 52.4±4.1%; T: 57.4±4.1%; G: 43.1±4.1%), and $(56.9\pm4.0\%; P > 0.05)$. Cleavage rates were not different between treatments (S: 53.2±5.6%; T: 61.8±5.2%; G: 73.4±5.5%), but the cleavage rate after vitrification with sucrose was significantly lower than that of the control (75.3±4.8%; p=0.02) and tended to be lower than that of galactose (p=0.066). Finally, blastocyst rates for all vitrified groups (S: 5.0±2.5%; T: 4.3±2.2%; G: 7.6±3.4%) were significantly lower compared to the control group (26.5±5.7%). Nevertheless, galactose, a monosaccharide tested for the first time in equine oocyte vitrification, resulted in the highest blastocyst rates after vitrification, as well as in equal cleavage rates compared to the control. Therefore, galactose should be considered as an alternative sugar for future optimization of vitrification protocols for eguine immature oocytes.

Pre-hatching exposure to N2B27 medium improves post-hatching development of bovine embryos in vitro

Alba Pérez Gómez, Priscila Ramos-Ibeas, Leopoldo González-Brusi, Alejandra C Quiroga, Pablo Bermejo-Álvarez

INIA, Spain

The developmental competence of in vitro produced (IVP) embryos still remains a matter of concern, as pregnancy rates are often reported to be lower for IVP embryos compared to their in vivo derived counterparts. Following embryo transfer and prior to implantation, the hypoblast must proliferate and migrate to cover the inner surface of the trophoblast, whereas the epiblast should form the embryonic disc from which the fetus will be developed. Several in vivo and in vitro evidences suggest that epiblast development is the most critical process to ensure embryo viability shortly after hatching, and we have previously observed that, in contrast to conventional embryo culture media, N2B27 medium supports epiblast development in Day 9 IVP bovine blastocysts (Ramos-Ibeas et al. Reproduction 2020). The objective of this study has been to determine if earlier exposure to N2B27 enhances subsequent development. As N2B27 does not support bovine embryo development from the zygote stage, all the IVP presumptive zygotes used in this study (1626) were initially cultured in SOF medium and then moved to N2B27 medium on days 5 (group N5), 6 (group N6), 7 (group N7) or 9 (group N9) postfertilization. The number of total (DAPI), trophectoderm (CDX2+), hypoblast (SOX17+) and epiblast (SOX2+) cells were analyzed by immunohistochemistry (IHC) on 64, 30 and 38 Day 8 (D8) blastocysts from N5, N6 and N9 groups, respectively. Post-hatching development was assessed on Day 12 (D12) embryos by IHC using the same lineage markers than on D8 in 61, 35, 32 and 84 embryos from N5, N6, N7 and N9 groups, respectively. Blastocyst rates on D8 were similar in all groups (22.7±2.4 vs. 20.9±1.4 vs. 22.3±3.4 vs. 25.1±11.3 %; mean±s.e.m., for N5, N6, N7 and N9, respectively, ANOVA p>0.05). Total cell number on D8 was significantly higher in N5 vs. N9 (Total: 158.4±14.7 vs. 136.9±13.8 vs. 109.7±7 for N5, N6 and N9, respectively, ANOVA p<0.05), whereas SOX2+ and SOX17+ cell number was significantly higher in N5 and N6 vs. N9 (SOX2+: 53.9±6.4 vs. 41.5±6.6 vs. 25.5±2.3; SOX17+: 41±6.6 vs. 33±6.7 vs. 16.8±2, for N5, N6 and N9, respectively, ANOVA p<0.05). All groups displayed a similar number of CDX2+ cells (89.6±12.3 vs. 83.8±9.9 vs. 64.9±7.1, for N5, N6 and N9, respectively, ANOVA p>0.05). Survival rate (not-collapsed structures) from presumptive zygotes to D12 was significantly higher in N5 group vs. N9 (29.8±4.2 vs. 18±2.5 vs. 21.3±3.1 vs. 20.2±1.5, for N5, N6, N7 and N9 groups, respectively, ANOVA p<0.05). Finally, Complete hypoblast migration rate at D12 was significantly higher in N5, N6 and N7 groups compared to N9 (30/61 vs. 16/35 vs. 17/32 vs. 20/84, for N5, N6, N7 and N9, respectively, z-test<0.05). Similarly, significantly more embryos developed embryonic disc structure in N5, N6 and N7 groups compared to N9 (25/61 vs. 16/35 vs. 15/32 vs. 16/84, for N5, N6, N7 and N9, respectively, ztest<0.05). In conclusion, culture of bovine embryos in N2B27 from Day 5 onwards enhance the development of hypoblast and epiblast lineages, whose survival is hampered by prolonged culture in SOF medium.

Support: StG-757886 from ERC, and AGL2017-84908-R and RYC2018-025666-I from MINECO

CONGRATULATIONS to both of our winners who each receive €250 and a free registration to our 2022 Annual meeting.

INVITATION TO UTRECHT

2022

Dear Colleagues,

On behalf of the Association of Embryo Technology in Europe (AETE) you are cordially invited to attend the 38th Annual Meeting of the (AETE), which will be held in Utrecht, The Netherlands, on September Thursday 15th and Friday 16th 2022! With the location of the AETE 2022 being the City Center of Utrecht, it creates a unique atmosphere for the conference setting.

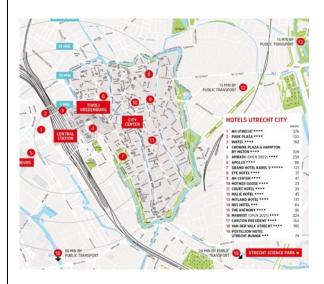


We foresee a great conference in a medieval city; Utrecht celebrates its 900 anniversary in 2022, with an excellent atmosphere! Please note that days slightly differ from the traditional Friday and Saturday, which gives you an extra day to catch a glance of this iconic venue and to enjoy what Utrecht offers!



The AETE annual meeting is an excellent opportunity to meet most of the European experts in the field of animal reproduction. The Board has put together an exciting programme and all invited speakers have accepted the invitation (see www.aete.eu) for the 2022 meeting, which has exciting new information for both scientists and practitioners. The central theme of our 2022 meeting will address the role of the "father" in the determination of embryo quality and postnatal health. The meeting not only focuses on science and embryo technology applications, but puts a large emphasis on interaction and networking at our practitioner and sponsor forum. This is further stimulated with a very attractive conference venue and an attractive social program!

The conference venue is located in the City Center of Utrecht with everything in direct reach so that you can get the maximum out of your stay in Utrecht.



The city of Utrecht has a wide range of classified hotel accommodation offering a total of 3.543 rooms in 2022. It will be very easy to book (and pay) hotel accommodation in Utrecht and surroundings in the 3-, 4- and 5-star category via Hotel service Utrecht on the AETE website.

Last, but not least, Utrecht is easily accessible via Schiphol Airport in Amsterdam with every 15 mins trains to Utrecht Central station by which you will reach Utrecht in 30 mins.



WE HOPE TO MEET YOU AT THE AETE IN UTRECHT IN 2022!

On behalf of the local organizing committee of AETE Utrecht,

Hilde Aardema, Faculty of Veterinary Medicine, Utrecht University

UPCOMING EVENTS

FERTILITY 2022 5-8 January 2022

Virtual

https://fertilityconference.org

IETS – 48[™] ANNUAL MEETING 10-13 January 2021

Savannah, Georgia, USA

https://www.iets.org/Meetings/2022-IETS-Annual-Meeting

ICAR 2020+2 26-30 June, 2022

Bologna, Italy

http://icar2020.org/2022/

SOCIETY FOR THE STUDY OF REPRODUCTION 26-29 JULY, 2022

Spokane, Washington

 $\underline{https://www.ssr.org/events/event-description?CalendarEventKey=6c2ae7f2-3e95-4fe9-84a1-\underline{da75ea002940\&Home=\%2fnews-events\%2fupcoming-events}$

AETE

15-16 September 2022

Utrecht, The Netherlands https://www.aete.eu

ESDAR

28 September-2 October 2022

Thessaloniki, Greece

https://www.esdar.org

AETA & CETA/ACTE JOINT ANNUAL CONVENTION 27-29 October 2022

Louisville, Kentucky, USA

https://www.aeta.org/mtg future.asp

PLEASE NOTE – ALL DETAILS SUBJECT TO CHANGE...

PRELIMINARY PROGRAMME - AETE 2022



38th Scientific Meeting

UTRECHT CONVENTION BUREAU
Utrecht, The Netherlands

PROGRAMME

15th and 16th September, 2022

WEDNESDAY, September 14th 2022

18.30-20.00: Registration

20.00-22.00: Welcome Reception

THURSDAY, September 15th 2022

08.00-17.00: Registration
08.45-18.00: Opening meeting
SESSION 1: First invited lecture:

Marcella Milazzotto, Center of Natural and Human Science of Federal University of ABC, São Paulo, Brazil

Erasing gametes to write blastocysts: metabolism as the new player in epigenetic reprogramming.

Second invited lecture:

Adam Watkins, Division of Child Health, Obstetrics and Gynaecology, University of Nottingham, UK

Defining the male contribution to embryo quality and offspring health in assisted reproduction in farm animals

POSTER SESSION 1 and coffee break

Short oral communications: Student Competition Sponsor presentation

Lunch

SESSION 2: Third invited lecture:

Inmaculada Parrilla, Department of Animal Medicine and Surgery, University of Murcia, Murcia, Spain

Boar seminal plasma: overview and current insights on its potential role in assisted reproductive technologies in swine

Short oral communications/Sponsors and practitioners forum (Parallel session)

POSTER SESSION 2 and coffee break

Workshop I: Preparing semen for practical use in and out the IVF-lab chaired by Sean Fair, Department of Biological Sciences, University of Limerick, Ireland

20.00-02:00: Gala Dinner

FRIDAY, September 16th 2022

08:00-18.00:

SESSION 3: Fourth invited lecture:

Albert De Vries, Department of Animal Sciences, University of Florida, USA *Economics of assisted reproduction in farm animals.*

Short oral communications

POSTER SESSION 3 and coffee break

General Assembly

Lunch/Student Lunch

SESSION 4: Short oral communications

Fifth invited lecture

Hilary Dobson, School of Veterinary Science, University of Liverpool, Liverpool, UK

Understanding the trade-off between the environment and fertility

Pioneer award 2022 - Hilary Dobson AETE Medalist Presentation introduced by Martin Sheldon (UK)

POSTER SESSION 4 and coffee break

Workshop II: Rigor in the IVF laboratory. chaired by Ana Sousa Lopes, Esco Medical, Denmank

Closing session:

Student Competition results and invitation to the AETE Conference 2023

20.00-24:00: Farewell party

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