AETE
Association Européenne de Transfert Embryonnaire
European Embryo Transfer Association

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PRESIDENTS LETTER

Dear Friends, dear Colleagues,

2018 is coming to an end and it is an honour for me to have the opportunity to prepare these short President's words for you. I feel fortunate and grateful to be the elected President of the Association of Embryo Technologies in Europe since September 2018. My AETE time started way back in 2004 when I was presenting my PhD research in the Student's Competition session during the society meeting in Lyon. The family-atmosphere struck me right away and I appreciated the way that young researchers were given the chance to present their work. For this reason, I was happy to receive the opportunity to become involved in the "student affairs" when I was voted onto the board during the General Assembly in Saint-Malo in 2012, already 6 years ago. With a lot of support of the other board members we have been able to work further on this student focus, implementing a student lunch, extra prizes for the best oral and poster presentations, a new abstract reviewing process and the publication of the abstracts in the Web of Science cited journal "Animal Reproduction" in collaboration with the Brazilian Society of Embryo Technologies, SBTE. For the AETE 2018 meeting we had about 22 authors submitting their work in the Student competition, an absolute record! So please, keep on stimulating your students to use the AETE meetings as an ideal platform to network, to present their work, to develop their communication skills and of course ... to become a member of our close AETE family.

Dimitrios Rizos asked me a couple of months ago to take over his job as the new President. I was happy to accept this responsibility, knowing it would be a challenge to keep up with the fantastic way he has led the society over the past years. All board members agreed on this. He was a great leader of the board and always prioritized the perfect atmosphere on our meetings. Thanks Dimitrios, my good friend, for everything you did for our Society. Luckily you promised to stay "consultable" for difficult issues. And yes, I used that offer already several times!

Of course, many other important changes occurred in our board. **Marja Mikkola** accepted to be the

vice-president, bringing in the necessary practitioner's voice next to the President. **Lotte Stoebech** was elected during the General Assembly meeting to be the new board member. In the name of the entire society, we warmly welcome you to our board where you are asked to take over my former responsibilities of "student affairs".

The meeting in Nantes was, I am sure you all agree, a great success. The LOC composed by members from INRA, Allice, Oniris and Evolution and chaired by Daniel Lebourhis did a fantastic job. We already knew in advance that the AETE2018 organisation would be in very good hands. The venue, the atmosphere, the food, ... were all magnificent. Also, the social program was outstanding. I will never forget the Elephant from Nantes and how CEVA made it possible that about 40 members could climb this wonderful beast. Thank you, Daniel for your enormous work and commitment until the very last moment and for closing this giant AETE2018 project with a balanced budget.

But of course, we should not forget, we had a very interesting scientific program with a line up of fantastic speakers. We were fortunate to have **Prof Marcelo Seneda**, the president of the SBTE, giving an excellent talk on ovarian follicle counts in relation to fertility in cows. I will never forget Marcelo's words during the farewell party saying that he never had a meeting before which such a nice family-atmosphere. Thanks Marcelo for these nice words, it meant a lot to me.

If you like, the rest of the scientific program can be overviewed at http://www.aete.eu/index.php/meetings/155-34th-scientific-meeting-preliminary-programme/file. It will help you to remember the great talks the other invited speakers gave. And don't forget, the invited papers are all published in Animal Reproduction and can be cited accordingly. You can download the Special Issue of the Journal right from our website.

This year's Pioneer Award was given to **Dr. Patrice Humblot** (Swedish University of Agricultural Sciences, Uppsala, Sweden) and the laudatio was kindly provided by his good friend Prof Hilary Dobson (from the UK). Patrice received the award of the AETE due to his distinguished contributions worldwide in the field of reproductive physiology and reproductive technologies (AI, ET, embryo sexing, *in vitro* production). I do not know many researchers who are perfectly able to combine academic fundamental knowledge (the "omics") with the necessary statistical insights and the constant search for applications for the benefit of farmers and the breeding industry. He is an established and internationally recognized expert in the field of animal reproduction and reproductive technologies. In the name of all members of our society, I wish you all the best for your future professional work but also with your sailing activities ... on safe waters.

And of course we had some great winners during our meeting! Congratulations to **Bea Rodríguez-Alonso** (INIA, Spain) providing deep insights on the local embryo effect on the transcriptomic response of oviductal epithelial cells. Her abstract was shortlisted after a very strict preselection before the meeting together with the 4 other student nominees. She convinced all jury members during the conference with her excellent talk starting with a nice "open-ocean metaphor". She provided a summary of her research further in this Newsletter. Congratulations Bea with your 750 euro award and your complementary registration for our next meeting in Murcia.

The best oral presentation was won by Karolien Desmet (University of Antwerp, Belgium) speaking about the effect of metabolic stress during oocyte maturation and the consequence on Day 14 embryo development. Karolien has a lot of speaking experience as she won the Student competition prize during our AETE2017 meeting. The best poster presentation was won by Charles Banliat (Inra, France). He convinced the jury with his intriguing and nicely presented poster on the lipidomic profile of the oviductal environment. Congratulations Charles.

The venue in Nantes was, so to say, ideal. Ideal for the AETE, because the setting stimulated maximal contact between delegates and the sponsors.

Sponsors are an indispensable part of our society. We had a lot of room for the sponsor boots combined with the coffee breaks. But, we have to be honest: during and after the meeting we got some feedback from practitioners and sponsors that our program sometimes tend to be too scientific. For some of you there is a lack of applied content, hands-on workshops and courses. We, as a board, do take this comment very seriously and are already working very hard to further improve:
- we are inviting sponsors to actively participate in advising on the program and to organise demo sessions or workshops.
- we asked all our invited speakers to provide clear take home messages and to pay enough attention to translation for the day to day practice
- we will adapt and further improve the workshops within the program to make them more interactive and hands-on.
-we are brainstorming within the board how to attract more practitioners. We spoke already with many different people on this subject. If you have an opinion or an idea, please let us know. Your voice and opinion are important for us.
-I put this topic on the agenda of the Affiliated Societies meeting at the IETS2019 conference. This concern is on the top of the agenda as also other societies have similar issues.

To end with, only good news. We go to Murcia, Spain next year! Jan Detterer and I were fortunate to travel south and do a pre-visit in October! And I can tell you already now: AETE2019 is in fantastic hands, the hands of the LOC chaired by Rakel Romar (University of Murcia)

The budgets are approved, the conference venue is booked, the swimming pool is reserved, the beach is ready to have us for the farewell party, the menu is decided and some major sponsors are already on board. Please follow us on Facebook and our website for the latest updates. I am also very proud to say that all our keynote speakers accepted our invitation, promising us to bring a scientific program that is very attractive for the day to day practice. Vétoquinol was eager to bring in and support **Professor Peter Hansen** from the University of Florida to speak about heat stress. We are very grateful for this opportunity and the board decided to make heat stress the central theme on our next meeting as also an entire workshop will focus on the topic (organised and chaired by **Dr. Zvi Roth**). Due to the changing climate, heat stress is a major factor in our ET business so it deserves centre stage.

Finally, allow me to thank via this way all the board members of our Society. They are working very hard as a team, behind the scenes, to make AETE to what it is today and to what it will be tomorrow. It is, believe me, an everyday commitment and the list of points to deal with the coming months is still long.

Anyway, stay with us, check our website regularly and submit your work and abstracts before the **1st of May 2019**! Murcia will be a feast and I hope to welcome you there.

Merry Christmas and a happy and healthy New Year!

Jo Leroy

President of the AETE

AETE Board Members

President Jo Leroy; Belgium
Jo.leroy@ua.ac.uk

Vice President Marja Mikkola; Norway
marja.mikkola@geno.no

Treasurer Jan Detterer; Germany
j.detterer@vost.de

Secretary Teresa Mogas; Spain
teresa.mogas@uab.cat

Student Affairs Lotte Stroebech; Denmark
Lotte.stroebech@etbiotech.com

Newsletter Editor Roger Sturmey; United Kingdom
roger.sturmey@hyms.ac.uk

Web site Hilde Aardema; The Netherlands
h.aardema@uu.nl

Annual Statistics Helene Quinton; France
helene.quinton@evolution-xy.fr

Scientific Committee Jane Morrell; Sweden
jane.morrell@slu.se

Representative of ET Industry & French Foundation Daniel Le Bourhis; France
Daniel.lebourhis@allice.fr

A.E.T.E. Secretary
Teresa Mogas
Animal Reproduction Unit
Veterinary Faculty
Autonomous University of Barcelona
Spain

Tel: + 34 93 581 10 44
email: info@aete.eu

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Workshop Report – Sanitary and regulatory considerations for ET

Francis Fieni

The first speaker, Dr **Pascale Chavatte-Palmer** from the French National Institute of Agronomical Research (INRA), past chair of HASAC and vice-president of IETS, explained the missions of HASAC and how the three HASAC sub-committees (research, regulatory and manual subcommittees) worked together to issue proposals that are submitted to the approval of the IETS board of governors. Once approved, these proposals are transferred to the OIE (World Organization for Animal Health) and submitted to vote for relevant amendments on the terrestrial code.

Following this intervention, Dr **Etienne Bonbon**, president of the OIE terrestrial animal health standards commission, organized the presentation of two movies explaining the work of OIE delegates.

The third speaker was Dr **Stephan Zientara**, head of the joint Virology Research unit dedicated to animal and veterinary public health (French Agency for Food, Environmental and Occupational Health & Safety) and investigating animal viruses responsible for epizootics or with potential for zoonotic transmission and/or emergence. He presented a thorough update on current viral emergence in Europe and consequences for the risk of disease transmission via in vivo derived embryos, demonstrating the importance of IETS HASAC contribution to the establishment of national and international legislations and stressing the need for more research to be funded on this topic.

The end of the workshop focused on practical applications in embryos and in semen trade. Dr **Helene Quinton** from EVOLUTION breeding organization for livestock farmers, presented the constraints for a registered Embryos Transfer Team concerning bovine embryos importation and exportation. Finally, Dr Olivier Gérard, director of the international affairs of ALLICE (union of cooperatives which unites all the French animal selection and reproduction companies and also a Belgian selection company) spoke about the consequences of sanitary issues (diseases outbreaks) on bovine semen exportations from Europe to third countries, giving a historical perspectives and an update of the current situation.

Altogether, this workshop raised a lot of interest both during the session itself but also during subsequent sessions where several questions were raised on the sanitary aspects of new procedures involving embryos. This was a very nice example of how IETS and AETE collaborate and hopefully brought more awareness about the need to maintain funding for research on how embryo transfer may prevent the transmission of emerging diseases.

AETE 2018 workshop on Sanitary and Regulatory considerations for embryo transfer
From left to right: Prof. Francis Fieni, Dr. Pascale Chavatte-Palmer, Dr. Hélène Quinton, Dr. Stephan Zientar and Dr. Olivier Gérard

Workshop II: Preservation of IVP Embryos

Teresa Mogas

The combined use of reproductive technologies such as trans-vaginal ovum-pick up and *in vitro* embryo production has great potential for enhancing genetic selection and optimizing crossbreeding schemes in beef and dairy cattle production systems. Because of the worldwide economic importance of cattle, large numbers of embryos are generated through *in vitro* technologies, and the cryopreservation of these embryos has become important for the best use of supernumerary embryos. However, while 62.1% of *in vivo*-derived embryos transferred in 2016 were frozen only a 27.1% of in vitro produced (IVP) embryos transferred were frozen. Because of the lower quality of cryopreserved IVP embryos compared with fresh IVP-produced embryos, pregnancy rates after embryo transfer are still lower. In fact, the low numbers of IVP embryos subjected to cryopreservation worldwide have been associated with these low rates of pregnancy after thawing. This has meant intense research efforts invested in designing a procedure for the effective cryopreservation of IVP bovine embryos together with the development of an standard methodology for embryo cryopreservation that facilitate its use in field conditions

After a short introduction, researchers representing key actors in the field of cryopreservation of in vitro produced embryos

(**Bruno V. Sanches,** Cogent IVF, Oregon, USA. *Cryopreservation of IVP bovine embryos;* **Cristina Cuello**, University of Murcia, Spain. *The challenge of vitrifying in vitro-produced porcine embryos;* **Christine Wrenzycki**, Justus-Liebig-University Giessen, Germany: *Effects of (cryo)preservation on the quality of in vitro produced embryos)* presented the most recent approaches on effective cryopreservation procedures for IVP bovine and porcine embryos to decrease embryo cryoinjuries during the cryopreservation process and increase survival and pregnancy rates. Dr. Sanches gave a very thorough overview about the cryopreservation of Bos taurus and Bos indicus IVP embryos. He mentioned that despite the advantages of IVP, cryopreservation represents a challenge for commercial laboratories and adaptations are necessary for each practice. He mentioned the need for the development of an effective cryopreservation technique that simplifies logistics and is practical for the transfer of vitrified embryos in the field. Although the cryopreservation technique predominantly used for IVP embryos is vitrification, he showed that the process of thawing and the direct transfer (DT) of embryos to cows make the slow freezing protocol – a method previously described in vivo embryos – could be efficient for IVP commercial use. The DT strategy has recently been performed by commercial laboratories, providing good embryo viability after thawing. He also showed some results about IVP embryos with sexed semen that could be directly transferred with similar conception rates to vitrified embryos.

Referring to cryopreservation of porcine IVP embryos, Dr. Cuello pointed out the significant progress in pig embryo cryopreservation due to the development of vitrification as an alternative to slow-freezing. Currently, high in vitro survival rates and promising reproductive performance after ET can be reached with vitrified in vivo-derived porcine morulae and blastocysts. Although piglets have been obtained from vitrified in IVP embryos, their vitrification ability is still far behind that of their in vivo-derived counterparts. Dr. Cuello mentioned embryonic stage, the length of the in vitro culture, the high incidence of polyspermic penetrations in porcine IVF, the concentration and type of cryoprotectants, vitrification devices or the equilibration temperatures as possible factors that could affect vitrification outcomes regardless the origin of the embryo. However, she specified two main aspects that could make the IVP embryos particularly sensitive to vitrification: their high lipid content and a much poorer quality compared to the in vivo-derived ones. In this regard, she mentioned good results after transfer of IVP embryos subjected to delipidation by micromanipulation or embryos where lipolysis was stimulated by the addition of chemicals agents such as forskolin or L-ascorbic acid to protect IVP embryos against oxidative stress during in vitro culturing and vitrification-warming procedures. She also pointed out the importance of selecting the monospermic embryos previously to vitrification in order to discard polispermic embyos and so, increase survival rates after warming.

Dr. Wrenzycki referred to morphological and functional damages suffered by bovine IVP embryos during cryopreservation. She also mentioned differences in re-expansion and hatching rates after vitrification/warming depending on the development stage, origin of the embryo or the applied technique (slow freezing and vitrification). So, as strategies to increase the outcomes of cryopreservation of IVP embryos, she mentioned the use of cytoskeleton stabilizers, antioxidants, or drugs that decrease the lipid content of the embryos. However, she emphasized the need to assess the quality of the embryo after cryopreservation. The best assessment is to perform embryo transfer but it is time consuming. So, two main methods could be distinguish: invasive techniques (take a biopsy and evaluate chromosomal alterations, apoptosis, gene expression, methylation profile,…) or non invasive measurements (assessment of morphology, morphogenetic, development competence or metabolism of the embryo). Another alternative would be to look at the oocyte quality and the surrounding cumulus together with the use of an IVM/IVF/IVC system that ensures a good embryo quality with a higher cryotolerance. When her group compared slow freezing and vitrification techniques for the cryopreservation of IVP bovine embryos, they observed differences in gene expression depending on the technique used. Also, they attempted to preserve *in vivo*-derived embryos in the fridge and they obtained good survival and hatching rates of both non- or biopsied Day 6 morulae preserved in the fridge with media supplemented with 25% FBS.

The workshop concluded that the outcomes of the cryopreservation of IVP bovine and porcine embryos are somewhat more promising in that post-thawing embryo survival rates and subsequent pregnancy rates have been acceptable. However, it is essential that further research efforts focus on improving IVM/IVF and/or IVC system/s to increase embryo quality together with the development of an effective cryopreservation procedure that will simplify logistics and be practical for the transfer IVP embryos in field conditions.

AETE 2018 Workshop on Preservtaion.
From left to right: Christine Wrenzycki; Cristina Cuello, Bruno Sanches, Teresa Mogas

2018 PRIZE WINNERS

STUDENT COMPETITION

Chosen by Members of the AETE Board

Winner: Beatriz Rodríguez-Alonso
University College Dublin and INIA, Madrid
Email: Jessie.DeBie@uantwerpen.be

LOCAL EMBRYO EFFECT ON THE TRANSCRIPTOMIC RESPONSE OF THE OVIDUCTAL EPITHELIAL CELLS RESULTS FROM *IN VIVO* AND *IN VITRO* APPROACHES

B. RODRÍGUEZ-ALONSO[1,2], M. HAMDI[2], J.M. SÁNCHEZ[1], A. GUTIERREZ-ADAN[2], P. LONERGAN[1], D. RIZOS[2]

[1]University College Dublin, Dublin, Ireland; [2]Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria (INIA), Madrid, Spain.

Based on previous data, the presence of a single 8-cell embryo does not alter the transcriptome of the cells of the oviduct, although this apparent lack of response might be due to a local effect at the precise position of the embryo which is missed if the whole oviduct is studied. Thus, we aimed to study the local embryo effect on the transcriptomic response of the epithelial cells of the oviduct *in vivo* and *in vitro.* For the *in vivo* experiment, 15 cross-bred beef heifers were synchronized, artificially inseminated and slaughtered on Day 2.5 after estrus. The oviducts from each animal were isolated, trimmed free of tissue and divided between ampulla and isthmus. The ipsilateral isthmus was then divided into smaller sections (2 cm). Each section was sequentially flushed until the embryo was located (n = 4; three at 2-cell stage and one at 8-cell stage), opened and scraped longitudinally to obtain the epithelial cells. Cells were snap- frozen in liquid nitrogen for gene expression analysis. The *in vitro* approach consisted of the co-culture of fifty *in vitro* produced embryos (2- to 4-cells) on a bovine oviductal epithelial cells (BOEC) monolayer. BOEC from the ampulla and isthmus of ipsilateral oviducts collected during the early luteal phase were mechanically harvested and separately cultured with TCM-199+10% FCS in 5% CO_2 in air at 38.5°C for 7 days until confluence. *In vitro* 2- to 4-cell embryos were produced in parallel. A day before co-culture, BOEC medium was replaced with SOF+5% FCS. The groups were: Ampullary BOEC co-cultured with (A+) and without (A -) embryos; isthmic BOEC with (I+) and without (I -) embryos. After 24h of co- culture, BOEC were recovered from each group and snap frozen for gene expression analysis (5 replicates). Ten transcripts previously reported to be differentially expressed between the isthmus of pregnant and cyclic heifers (*Maillo et al. Biol Reprod. 2015. 92: 144*) were analysed in BOEC recovered from both experiments: *STK32A*, *SLC26A3*, *KERA*, *QRFPR*, *MCTP1*, SOD3, *PRELP*, *VAT1L*, *SOCS3*, *CCL20*. Data were analysed using one-way ANOVA and t-test. The results from *in vivo* samples revealed that 6 out of 10 transcripts (*STK32A, SLC26A3, QRFPR, MCTP1, SOCS3* and *CCL20*) were different between the segment where the embryo was collected and other locations within the ipsilateral oviduct which suggested the presence of an embryo site-specific signal. Comparison between the ipsilateral embryo site with the contralateral site revealed only one transcript different (*VAT1L*). Regarding the *in vitro* BOEC co-culture, 3 out of 10 genes (*SLC26A3, KERA* and *QRFPR*) were not expressed. For the remainder of the genes analysed, no differences were detected. In conclusion, under our experimental conditions, *in vivo* the embryo elicits site-specific signals in the oviduct, while *in vitro* evidence for these signals were not observed neither by the presence of the embryo, nor by the spatial differences of the bovine oviduct.

EU, Horizon 2020 Marie Sklodowska-Curie Action, REPBIOTECH 675526; Spanish MINECO AGL2015-70140-R & AGL2015-66145-R.

Bea wins €750 plus free registration to our next annual meeting.

BEST ORAL PRESENTATION

Winner: Karolein Desmet
Gamete Research Centre, University of Antwerp, Wilrijk, Belgium

Karolien wins €250 plus free registration for our next annual meeting

BEST POSTER PRESENTATION

Winner: Charles Banliat
INRA, Plate-forme de Chirurgie et d'Imagerie pour la Recherche et l'Enseignement (CIRE), Pôle d'Analyse et d'Imagerie des Biomolécules (PIAB), CHRU de Tours, Université de Tours, 37380 Nouzlly, France

Charles wins free registration for our next annual meeting

USE OF EMBRYO TRANSFER TO INCREASE FERTILITY IN THE REPEAT BREEDER COW

Peter J Hansen

Use of embryo transfer to increase fertility in the repeat breeder cow

Peter J Hansen, Dept. of Animal Sciences, University of Florida

Reasons why embryo transfer bypasses some causes of the repeat breeder syndrome

Many things can go wrong in an inseminated cow to prevent conception and establishment of pregnancy. Sometimes the cow is not really in estrus at the time of insemination or ovulation failure occurs. In some situations, either the oocyte released at ovulation or the sperm deposited in the reproductive tract are not capable of fertilization. Even if fertilization does occur, defects in the gametes can result in an embryo with reduced capacity for sustained development. The reproductive tract also plays an important part in determining whether the outcome of insemination is successful. An inadequate environment in the oviduct or uterus could inhibit fertilization or lead to early embryonic mortality. Given all these potential roadblocks to pregnancy, it is not surprising that the proportion of females that become pregnant after artificial insemination (AI) is only around 45-60% for heifers and 25-40% for lactating dairy cows.

Some female cattle, particularly lactating dairy cows, experience prolonged periods of infertility characterized by multiple inseminations without successful establishment of pregnancy. Termed "repeat-breeders", these animals are usually defined as females that experience more than three inseminations without successful pregnancy. There are multiple causes of the repeat-breeder syndrome including anatomical disorders, ovarian dysfunction and infectious disease.

As shown in Figure 1, ET allows pregnancy in some repeat-breeder cows by overcoming ovulation failure, poor oocyte quality and an inadequate reproductive tract environment during the first 7 days of gestation (embryos are typically transferred at Day 7 after ovulation). Embryo transfer would not, however, prevent embryonic or fetal losses occurring because of a poor uterine environment existing after Day 7.

Table 1

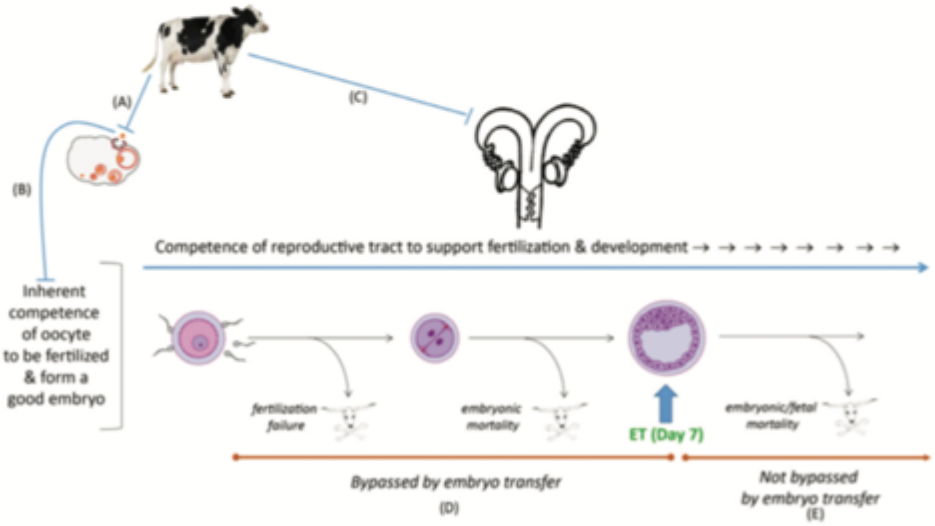
Effectiveness of ET for improving fertility in repeat-breeder cows and heifers as compared to AI.

Study number[a]	Location	Animal type	Treatment	n	Pregnancy rate, %
1	USA-Pennsylvania	Holstein cows, non-lactating, 4-11 previous services	AI at estrus	22	50
			MOET (fresh) at estrus	23	70
2	South Korea	Holstein cows, lactating, 2 or more previous services	AI at estrus	27	19
			TAI	13	8
			TET, MOET (frozen)	13	54
3	USA-Florida	Holstein cows, lactating, 3 or more previous services, hot weather	TAI	33	21
			TAI, IVP embryo (fresh)	25	60
			TAI, IVP embryo (vitrified)	31	29
4	Brazil-São Paulo	Holstein cows, lactating, 4 or more previous services, all year	AI after estrus	5,693	18
			MOET after estrus	3,858	42

a Study 1: Tanabe *et al.* (1985); Study 2: Son *et al.* (2007); Study 3: Block *et al.* (2010); Study 4: Rodriques *et al.* (2007).

Figure 1

Schematic diagram illustrating how ET can circumvent specific causes of infertility in repeat-breeder cows. Some cows repeatedly fail to become pregnant because ovarian function is disrupted and either ovulation does not occur (A) or the oocyte produced is not competent to be fertilized or to form an embryo capable of sustained development (B). Another cause of infertility is a reproductive tract environment inadequate to support fertilization and embryonic development (C). Transfer of a blastocyst-stage embryo into a repeat-breeder cow would bypass any pregnancy failure causes by ovulation failure, release of a dysfunctional oocyte or inadequate reproductive tract environment existing before the day of transfer (D). Embryo transfer would not, however, overcome pregnancy losses in repeat-breeder cows caused by embryonic or fetal death caused by an inadequate uterine environment existing after transfer (typically Day 7 after ovulation) (E).



Evidence that embryo transfer improves fertility in repeat-breeder cows

Results of studies to test the effectiveness of ET for increasing pregnancy rates of repeat-breeder cows are summarized in Table 1. In each study, the percent of cows pregnant after receiving an embryo was compared to percent of cows pregnant after AI. Most of these studies involved low numbers of cows (no more than 33 per group). In each of these small experiments, though, substantially more repeat-breeder cows were pregnant after ET than after AI. This was true whether cows received a fresh (Study 1) or frozen (Study 2) embryo produced by MOET or a fresh embryo produced by IVP (Study 3). Embryo transfer provided no benefit over AI, however, when an IVP embryo that had been vitrified was transferred (Study 3). Such a result reflects the difficulty in cryopreserving embryos produced *in vitro*.

One large-scale study conducted in Brazil confirms the improvement in fertility in repeat-breeder cows through use of ET (Table 1). In that study (Study 4), 42% of 3,858 repeat-breeder cows receiving an embryo produced by MOET were pregnant versus only 18% of 5,693 repeat-breeder cows that were inseminated.

Two groups of investigators have compared fertility for repeat-breeder females that were inseminated and then received an embryo vs for repeat-breeder females that were inseminated only. Canu *et al.* (2010) analyzed farm data from the United Kingdom and observed that pregnancy rate for dairy cows at the fourth insemination that also received an embryo produced *in vivo* 7 days (n=114) was 53% vs a pregnancy rate of 30% for similar-type cows that were inseminated only (n=56). An experiment in Japan compared the pregnancy rate for Holstein cows and heifers classified as repeat-breeders (3-21 previous inseminations) that received both AI and ET (using an IVP embryo) vs those that received an embryo but were not inseminated (Dochi *et al.*, 2008). The total number of animals was 532. Surprisingly, pregnancy rates were higher for females that were both inseminated and received an embryo (43%) than those that received an embryo only (23%). Despite this outcome, results summarized in Table 1 would indicate that ET without AI is sufficient to achieve acceptable pregnancy rates in repeat-breeder cows.

Embryo transfer will not reverse all infertility associated with the repeat-breeder syndrome

As illustrated in Figure 1, ET will not reverse all sources of infertility that are present in a population of repeat-breeder females. Disorders in the recipient that persist after ET are likely to compromise establishment and maintenance of pregnancy.

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Thus, for example, an inadequate uterine environment caused by uterine infection of inadequate progesterone support could disrupt pregnancy after ET. Indeed, pregnancy rates for repeat-breeder cows used as ET recipients can be lower than for cows not classified as repeat-breeders. In one commercial facility, for instance (Hasler, 1991), the percent of cows pregnant after transfer of frozen/thawed MOET embryos for cows classified as normal was 53% (n=706) whereas the percent of repeat-breeders pregnant was 32% (n=94).

Does ET improve fertility in lactating cows not classified as repeat-breeders or exposed to heat stress?

Given the ability of ET to reduce sources of pregnancy wastage such as anovulation, fertilization failure and early embryonic mortality (Figure 1), one might expect pregnancy success after ET to generally surpass that possible using AI. One potential target for ET would be the lactating cow because it experiences lower fertilization rates and higher early embryonic mortality than non-lactating cows. Unfortunately, as least as ET is practiced currently, this is not usually the case. Consider for example a study by Sartori *et al.* (2006). Using embryos produced by MOET and transferred either fresh or after freezing, it was found that there was no difference in pregnancy success between lactating cows that were inseminated by TAI vs those that received an embryo using TET. For cows with one corpus luteum, the percent of cows pregnant after TAI was 29% (n=157) vs 32% (n=174) for cows after ET. For cows with more than one corpus luteum, values were 43% for TAI (n=28) and 25% (n=28). Perhaps, improvements in the procedures used to produce and transfer embryos will allow realization of the potential ET offers for improving fertility in cattle through bypassing sources of pregnancy wastage.

Synopsis: Recommendations for use of embryo transfer for repeat-breeder cows

Experiments with the repeat-breeder cow indicate that ET can be used to get repeat-breeder females pregnant. The effectiveness of ET as a fertility treatment for repeat-breeders will likely depend on the particular causes of infertility present in the population. Therefore, practical experience on results obtained using ET in a particular group of repeat-breeder females will be informative as to whether the procedure should be continued. Ultimately, the decision to use ET with repeat-breeder cows will depend on the increase in pregnancy success, the value of a pregnancy and the costs of embryo production. ❑

REFERENCES

BLOCK J, BONILLA L, HANSEN PJ. Efficacy of in vitro embryo transfer in lactating dairy cows using fresh or vitrified embryos produced in a novel embryo culture medium. *J Dairy Sci* 2010; 93:5234-5242.

CANU S, BOLAND M, LLOYD GM, NEWMAN M, CHRISTIE MF, MAY PJ, CHRISTLEY RM, SMITH RF, DOBSON H. Predisposition to repeat breeding in UK cattle and success of artificial insemination alone or in combination with embryo transfer. *Vet Rec* 2010;167:44-51.

DOCHI O, TAKAHASHI K, HIRAI T, HAYAKAWA H, TANISAWA M, YAMAMOTO Y, KOYAMA H. The use of embryo transfer to produce pregnancies in repeat-breeding dairy cattle. *Theriogenology* 2008;69:124-128.

HASLER JF. Using frozen bovine embryos to impregnate problem breeders. *Proc Ann Conf Am Embryo Transfer Assn* 1991; 72-75.

RODRIGUES CA, AYRES H, FERREIRA RM, TEIXEIRA AA, MANCILHA RF, OLIVEIRA MEF, SOUZA AH, BARUSELLI PS. Transferência de embriões em vacas Holandesas de alta produção repetidoras de serviço. *Acta Sci Vet* 2007; 35 (Suppl 1):1255 (abstr).

SARTORI R, GÜMEN A, GUENTHER JN, SOUZA AH, CARAVIELLO DZ, WILTBANK MC. Comparison of artificial insemination versus embryo transfer in lactating dairy cows. *Theriogenology* 2006;65:1311-1321.

SON DS, CHOE CY, CHO SR, CHOI SH, KIM HJ, HUR TY, JUNG YG, KANG HG, KIM IM. A CIDR-based timed embryo transfer protocol increases the pregnancy rate of lactating repeat breeder dairy cows. *J Reprod Dev* 2007; 53:1313-1318.

TANABE TY, HAWK HW, HASLER JF. Comparative fertility of normal and repeat-breeding cows as embryo recipients. *Theriogenology* 1985;23:687-696.

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ACHIEVE MORE TOGETHER

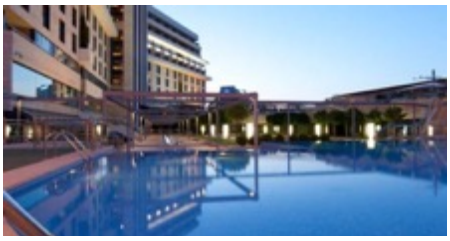
INVITATION TO MURCIA 2019!

On behalf of the European Embryo Transfer Association, the local organizing committee cordially invites you to the 35th scientific meeting in Murcia, Spain, from the 13th-14th September 2019.

The Local Organizing Committee will be chaired by **Dr. Raquel Romar**, University of Murcia, Murcia (rromar@um.es).

Welcome Reception and Congress Venue

For those of you who will be arriving on the12 the welcome reception will take place at the Hotel Nelva where registration and the AETE scientific program will be held. We will all meet and enjoy a fantastic cocktail of "tapas" in the outside gardens of this comfortable and elegant hotel.



Hotel Neva – Conference Venue

Gala Dinner

After a first day of science on Friday 13th, we will walk from congress venue to Royal Casino (http://realcasinomurcia.com/), an architecturally unique building built in the late 19th century. This iconic spot in Murcia contains many different styles including a classical and modernist facade and Moorish and Pompeian patios. After visiting this emblematic building we will be served a tasteful dinner based on local Murcian gastronomy and we will enjoy live music and dancing. The Casino is located 1 minute walking from Cathedral's square so do not miss to visit that!

Casino Fascade

Casino's indoor area

Farewell party

On Saturday 14, we will take the bus to Campoamor beach (45 minutes) where we will be meeting at Montepiedra Playa Restaurant, located right by the beach sand to enjoy typical dinner and a get-together party with live music. Buses will take us back to Murcia but for those of you who prefer to spend the night in Campoamor and enjoy the beach please visit the option at the hotels page.

Montepiedra Playa Restaurant (Farewell Party)

How to get Murcia

The city of Murcia, the sixth most populated city in Spain, is located to the southeast of the Iberian Peninsula. It is the capital of the Autonomous Community of the same name with an area of 11,317 km2.

By plane

Alicante-El Altet International airport (70 km from Murcia downtown). www.aena.es/en/alicante-airport/index.html

Once landed you can reach Murcia by bus (www.alsa.com/en; Single ticket for 5.19€ and Roundtrip ticket for 9.34€), by taxi (around 50€) or by transfer.

San Javier (Murcia) airport is another option with connections to the city by taxi (around 40€) or transfer. The airport is located at 50 km from Murcia downtown. www.aena.es/en/murcia-san-javier- airport/index.html
For special deals on transfers from airports to Murcia you can contact our partner congresosmurcia@viajeseci.es.

By car

Spain has a modern road system so if you prefer to take the car here you have some distances and connections to Murcia:
- From Barcelona the trip takes around 5h 45min (578 km) by AP-7 highway.
- From Madrid the trip takes around 3h 45min (400 km) by A-3 and A-30 highways.
- From Valencia the trip takes around 2h 20min (218 km) by A-33 highway.
- From Alicante the trip takes around 1h (60 km) by A-7 highway.

Accommodation

You can find many options for accommodation in Murcia and some nice hotels 5-10 minutes walking from the venue. Our partner offers special prices for all AETE attendants. Please log in http://viajeselcorteingles.sym.posium.com/24198/section/14197/35th-scientific-meeting-of-the-aete-2019.html to make your choice or to ask for more options.

Hotel Nelva****sup (Congress Venue)
www.hotelmurcianelva.com/en/

Hotel Agalia ****
www.agaliahotel.es/en/

Hotel Azarbe ****
www.hotelazarbe.com/en/

Hotel El Churra ***
www.elchurrahotelmurcia.com/

More information at
hwww.turismodemurcia.es/en/dormir.

You can find more detailed information about the scientific and social program of the AETE meeting on the AETE website. The Local Organizing Committee encourages you to join us in this meeting that we are sure will be worth it both scientifically and professionally speaking. In addition, the organized social events will allow us to meet and enjoy good moments together. We will do our best to make your stay in Murcia as pleasant as possible.

We all look forward to seeing you in September 2019 in Murcia.

The LOC (in alphabetical order)

Estefanía ALCAZAR TRIVIÑO
Sebastián CÁNOVAS BERNABÉ (Co-chair)
Pilar COY FUSTER
Joaquín GADEA MATEOS
Josep GINER TORRES
María JIMÉNEZ MOVILLA
Pablo LARROSA MORALES (El Barranquillo SL)
Carmen MATÁS PARRA
Daniel MARTÍNEZ BELLO
Juan Eladio OLIVA TRISTANTE
Raquel ROMAR ANDRÉS (Chair)
Salvador RUIZ LÓPEZ

Some reasons to come to Murcia

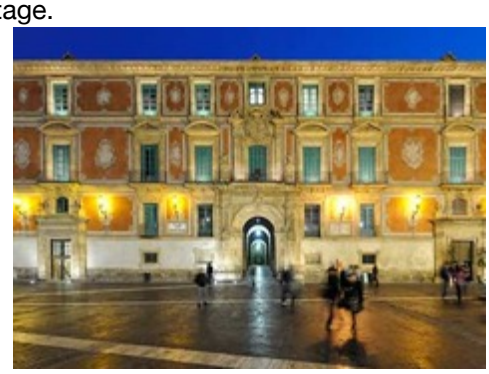
Region of Murcia is located on the Mediterrean arc in the area known as *Costa Calida,* one of the lesser known tourist spots in Spain what makes it the ideal place to travel to get an authentic Spanish experience without the usual crowds. Murcia is a city made to measure by its inhabitants, perfect for long walks to allow visitors to uncover its great exuberant personality and Islamic soul. Come and explore the *Murcia* region, known as the garden of Spain and the orchard of Europe.

Some of the areas in the city that deserve a visit are:

The Cathedral and its tower

It was built over the course of five centuries (14th-18th), and its various styles (Gothic, Renaissance and Baroque) are an impressive testimony to the history of Murcia. Construction began on the building in 14th century, on the ground that formerly housed the largest mosque in the city.

The Bishop´s Palace
In the same Cathedral Square, next to the magnificent façade of the Cathedral, stands the Bishop's Palace. The official seat of the Diocese of Cartagena, it was constructed in the 18th century, and today is one of the most important buildings of the city of Murcia's monumental heritage.



The Malecon esplanade
"El Malecón" is an embankment wall to protect against the flow of the river Segura. It dates back to 1420 when, following two major floods, the City Council decided to demolish the old and damaged houses in order to build a wall, which was built on the same boundaries of the current esplanade.

Flowers Square
This beautiful and vivacious square, in the center of the city is surrounded by nineteenth-century buildings and is the place of "tapas" par excellence.

You cannot leave "Plaza de las Flores" without a stop at the pastry shop Bonache, one of the most famous in the city, and try a unique Murcian speciality: the meat pie.

Gastronomy
Murcia is known as the Orchard of Europe. If look at the label of the fruit and vegetables that you find in your supermarket you will probably find it comes from Murcia region. Tasting fruits and vegetables from Murcia and its traditional recipes of rice dishes, grilled meats and fish, is a pleasure.

Some of our traditional dishes from "la huerta" (orchard) include Paparajotes, a typical Murcian sweet treat, made with lemon leaves.

"Paparajotes", traditional Murcian sweet.

Weather
Finally, our prevailing good weather is yet another reason to visit us any time of year. One of the most characteristic aspects of Murcia is its constant blue skies, and the bright sunlight that permeates the city.

In September temperatures typically ranges from 18º to 31ºC. Bring light clothes and swimming dress!

With two seas on one coastline, the Mar Menor and the Mediterranean sea, beaches of endless white sand and wild coves with crystal-clear water, the Costa Calida has the beaches you have to visit no matter which (Calbanche, La Manga, San Pedro, Mazarrón, Aguilas, …).

Outside Murcia city
Murcia Region retains innumerable vestiges of the past in different places such as **Cartagena**, a port fronting on the Mediterranean Sea, **Lorca**, the town of a Hundred Coats of Arms, or **Caravaca**, the Holy Town. The abundant remains and archaeological sites include rock-paintings in cave-shelters dating back to the Iberian period, the splendour of Roman antiquity, Visigothic cities, Arab medinas, Christian castles, watch-towers or churches and temples. Just 45 minutes by car from Murcia, in Cartagena, a city with more than 3,000 years of history, you can visit the Roman Theatre, built in the 1st century before Christ by Emperor Augustus.

For special deals on trips and visits to touristic spots please contact our partner congresosmurcia@viajeseci.es

UPCOMING EVENTS

FERTILITY 2019
3-5 January 2019
Birmingham, UK
www.fertilityconference.org
Programme: https://bit.ly/2USSoRE

IETS
20-23 January 2019
New Orleans, Louisiana, USA
www.iets.org/2019
Programme: https://bit.ly/2SQzngK

AET-D
4-5 July 2019
Eschikon, Lindau , Switzerland
https://www.aet-d.de/aktuelle-tagung/

SSR
17-20 July 2019
San Jose, California, USA
http://www.ssr.org/ssr-2019-annual-meeting

AETE
13-14 September 2019
Murcia, Spain
http://www.aete.eu/images/PDF/Invitation35thAETEMeeting.pdf

ESDAR
19-22 September 2019
St. Petersburg-Pushkin, Russia
http://www.esdar.org/esdar-conference-2019/announcement-2019-gb-1.html

SBTE
Details announced soon – keep checking the website
http://www.sbte.org.br/index

AETA & CETA/ACTE JOINT ANNUAL CONVENTION
24-26 October 2019
Colorado Springs, Colorado, USA
https://www.aeta.org/mtg_future.asp?autotry=true&ULnotkn=true

ICAR
28 June – 2 July 2020
Bologna, Italy
http://animalreproduction.org/current/

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PRELIMINARY PROGRAMME – AETE 2019

AETE

Association Européenne des Technologies de l' Embryon

Association of Embryo Technology in Europe

35th SCIENTIFIC MEETING

HOTEL NELVA

Murcia, Spain

P R O G R A M M E

13th and 14th September, 2019

THURSDAY, September 12th 2019

18.30-20.00: Registration
20.00-22.00: Welcome Reception

FRIDAY, September 13th 2019

08.00-17.00: Registration

08.45-18.00:

Opening meeting

SESSION 1: *First invited lecture:*

Felipe Perecin, Faculty of Animal Sciences and Food Engineering, Veterinary Medicine Department, University of Sao Paulo, Brazil:

Cellular interactions during oocyte development and maturation

Second invited lecture:

Olli Peltoniemi, Faculty of Veterinary Medicine,University of Helsinki, Finland:

Developments of reproductive management and biotechnology in the pig

POSTER SESSION 1 and coffee break

Short oral communications: *Student Competition*

Lunch

SESSION 2: *Third invited lecture:*

Sylvie Chastant-Maillard, National Veterinary School of Toulouse, France

Inflammation: friend or foe of cow reproduction?

Short oral communications

POSTER SESSION 2 and coffee break

Workshop I: Social acceptance of reproductive technologies in livestock

chaired by Hélène Quinton, Evolution, France

20.00-02:00: Gala Dinner. Royal Casino

SATURDAY, September 14th 2019

08:00-18.00:

SESSION 3: *Fourth invited lecture:*

Peter J Hansen, Department of Animal Sciences, University of Florida: *Reproductive physiology of the heat-stressed cow: implications for fertility and assisted reproduction*.

Short oral communications

Sponsor presentation

General Assembly

POSTER SESSION 3 and coffee break

Lunch/Student Lunch

SESSION 4: *Fifth invited lecture*

Poul Hyttel, Department of Veterinary and Animal Sciences,Faculty of Health and Medical Sciences, University of Copenhagen, Denmark

Oocytes, embryos and stem cells in a one health perspective

Pioneer award 2018 – Poul Hyttel AETE Medallist Presentation introduced by Trudee Fair (Ireland)

Short oral communications

POSTER SESSION 4 and coffee break

Workshop II: The impact of heat stress on dairy cows fertility-The management point of view.

chaired by Zvi Roth, The Hebrew University of Jerusalem, Israel

Closing session:

Student Competition results and invitation to the AETE Conference 2020

20.00-24:00: Farewell party. Campoamor beach

SPEAKER BIOGRAPHIES – AETE 2019

Dr. Sylvie Chastant-Maillard

Dr. Sylvie Chastant-Maillard is a French veterinarian, graduated from Alfort National Veterinary School (near Paris, France) and Diplomate of the European College of Animal Reproduction. She spent 15 years teaching reproduction in Alfort National Veterinary School and since 2010, in Toulouse National Vet School, in the South of France. Her real passion is teaching, especially to share knowledge on reproduction. Her second professional interest is applied research (in the field of reproduction, of course), trying to answer field questions. I have a special interest in bovine endometritis, ovarian resumption of cyclicity and precision livestock farming.

Dr. Peter J. Hansen

Dr. Peter J. Hansen is a Distinguished Professor and L.E. “Red” Larson Professor of Animal Sciences at the University of Florida. His research focuses on the biology of pregnancy and embryonic survival and development of methods to improve fertility and assisted reproductive technologies in livestock (particularly dairy cattle). Particular emphasis is placed on elucidating effects of elevated temperature on pregnancy, characterizing the nature of maternal control of early embryonic development and identifying genes controlling embryonic survival and fertility. In addition, work is underway to develop methods to improve dairy cow fertility during heat stress and to increase profitable uses of embryo transfer. Hansen received the B.S. in Agricultural Sciences from the University of Illinois in 1978 and the MS and PhD degrees from University of Wisconsin (M.S., 1980; Ph.D., 1983). Hansen served as President of the International Embryo Technology Society (2013-2014) and other organizations.

Dr. Poul Hyttel

Dr. Poul Hyttel graduated as Doctor of Veterinary Medicine (DVM) in 1979 from the Royal Veterinary and Agricultural University in Copenhagen. He received his PhD degree in 1982 and his degree as Doctor of Veterinary Sciences in 1988 from the same university, where he also lectured as an Assistant and later Associate Professor until 1990. Since 1990, he has been the Professor of Anatomy at the Department of Anatomy and Physiology at the Royal Veterinary and Agricultural University; now Department of

Veterinary and Animal Sciences (DVAS), Faculty of Health and Medical Sciences at University of Copenhagen.

Poul Hyttel's current research activities are directed towards pluripotent animal and human stem cells and in vitro embryo production. A fully equipped stem cell laboratory approved for genetically modified cells has been established at DVAS. The major contemporary projects focus on (1) establishment of human patient-specific neural in vitro cell models ("microbrains") for modeling of neurodegenerative disorders by the use of induced pluripotent stem cells (iPSC); (2) establishment of animal iPSCs for the development of the domestic animals as a translational model for iPSC-based cell therapy; and (3) in vitro production of bovine embryos, where the technologies for oocyte maturation, fertilization and embryo culture in vitro are optimized and genomics is applied for precise selection of high quality embryos.

In 1997-1998 Prof. Hyttel was President of the International Embryo Transfer Society, in 2011 he was awarded Doctor Honoris Causa at University of Antwerp, Belgium, in 2015 he was knighted 1st Class Order for his services to Denmark, and in 2018 he was awarded Doctor Honoris Causa at Estonian University of Life Sciences, Tartu, Estonia.

Dr, Olli Peltoniemi

Dr. Olli Peltoniemi graduated as a veterinarian from the Faculty of Veterinary Medicine at the University of Helsinki in 1992. He then studied reproduction in domestic animals at the University of Sydney, Australia (1993-1994) leading to a Master of Veterinary Sciences degree in 1995. He completed the requirements as a National specialist in Production Animal Medicine in 1997 and was awarded a PhD in domestic animal reproduction by the University of Helsinki in 1999. Olli became a Diplomate of the European College of Animal Reproduction in 2001 and the European College of Porcine Health Management in 2005.

Olli joined the staff of the Faculty of Veterinary medicine in Helsinki in 1995 and has held various roles, mainly in clinical sciences and domestic animal reproduction, culminating in a Full Professorship in domestic animal reproduction in 2010. Regarding leadership, in addition to leading his own pig research group for 20 years, he has had a number of leading roles at the Faculty including the deputy head of the Production Animal medicine Department from 2007-2009, chairing the research community from 2010-2012, and has been the Vice-dean for research since 2010. He was appointed as a board member of TINE, the Research Council of the University of Helsinki in 2014. Olli has served on the ECAR examination committee from 2003-2005, chairing the committee in 2005, served as secretary from 2009-13 and is currently the President of ECAR as well as being a member of the EBVS Board. The most recent challenge is that of a director of Helsinki One Health at the University of Helsinki.

Further details:

https://tuhat.helsinki.fi/portal/en/persons/olli-peltoniemi(8545e559-66fb-4b1f-b47b-accd36e64f3b).html

Dr. Felipe Perecin

Dr. Felipe Perecin received a bachelor´s Degree in Veterinary Medicine in 2002 and a PhD in Animal Reproduction- Veterinary Medicine in 2007, both from the Sao Paulo State University in Brazil. He completed his post-doctoral studies (2007-2008) at the University of Sao Paulo at Pirassununga (Brazil).

Since 2016, Dr. Perecin is an Associate Professor at the Veterinary Medicine Department at the Faculty of Animal Sciences and Food Engineering of the University of Sao Paulo although he started lecturing in the same department as an Assistant Professor in 2009. Since 2017, he is the Head of the Graduate Program in Animal Bioscience at the University of Sao Paulo. He was member of the board (Scientific director- 2016-2017) of the Brazilian Embryo Technology Society (SBTE) and since 2015 he is the Academic Editor of the Animal Reproduction journal.
His research group is dedicated to understanding cellular and molecular mechanisms underlying oocyte and embryo development. They investigate metabolic and signaling pathways leading to the acquisition of oocyte competence as well as the disturbances caused by in vitro culture conditions on oocytes and embryos. In the last years, they increased their interest in mechanisms that allows intercellular communication within the ovarian follicle and between the embryo and maternal tissues (i.e. transzonal projections and extracellular vesicles). The group goals include the development of
strategies aiming improvements on assisted reproduction technologies and fertility in
domestic animals.