AETE
Association Européenne des Technologies de l' Embryon
Association of Embryo Technology in Europe

December 2016 AETE Newsletter Issue 46 Editor: Roger Sturmey

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PRESIDENTS LETTER

Dear Colleagues and friends,

Time flies so fast! Almost 4 months have passed since our 32nd annual meeting held in Barcelona, in the heart of the city in one of the last great works of Catalan Modernism "The Casa de Convalescencia". The meeting was attended by more than 210 delegates, from 16 European countries as well as from North and South America, Asia and Africa, indicating the dynamism of our society. The conference was organised into 5 main talks, 14 short oral presentations selected from the record number of submitted and accepted abstracts (100), including the student competition, 2 workshops and 2 poster sessions during which a record of 100 posters were presented. All invited papers and abstracts published were in July-September 2016 issue of Animal Reproduction (the official journal of the Brazilian College of Animal Reproduction) together with the 30th SBTE's annual meeting, and is available in our web site (www.aete.eu). This year was the first year that we used the FASS system for submission and reviewing – and all section editors and reviewers are very grateful for the ease with which this system worked.

The presentation of **Prof. Henrik Callesen** as the recipient of the AETE Pioneer Award was a special moment of the meeting. Prof. Callesen received the award due to his outstanding work in the field of reproduction, recognized nationally and internationally and his significant influence on the development of the society. **Prof. Torben Greve**, his mentor, delivered the laudatory speech which highlighted the impressive scientific abilities of Prof. Callesen in addition to his great personality as a professor, colleague and friend.

Dr Dimitrios Rizos, Prof Henrik Callesen and Prof Torben Greve

Further invited lectures were given by **Roberto Sartori** from Brazil, representing our sister society SBTE on "Update and overview on assisted reproductive technologies in Brazil"; **Ann Duittoz** from France on "Timing of puberty"; **Trudee Fair** from Ireland on "Embryo maternal immune interactions in cattle"; and **Jane Morrell** from Sweden on "Practical applications of sperm selection techniques for improving reproductive efficiency". Five students were selected, based on their submitted abstracts, to present their work in the student competition. All presentations were scientifically outstanding. However, only one can be the winner and this year **Lies Jordaens** from University of Antwerp, Belgium won by presenting her work on "In vitro monolayer barrier function of bovine oviduct epithelial cells is modified due to high concentration of non-esterified fatty acids". The prize for best oral presentation was won by **Pablo Bermejo**, INIA, Spain for "Optimisation of RNA concentration for genome editing by CRISPR in rabbit zygotes", while the prize for best poster was won by **Svetlana Uzbekova** from INRA, France for "MALDI-TOF mass spectometry analysis of lipids in single bovine oocytes during IVM". We congratulate them all and I am sure these activities will stimulate more and more students to participate and present their results at the AETE annual meeting. Furthermore, a special lunch for early career researchers was organized where they had the chance to speak and discuss with their colleagues and with senior scientists as well as strengthen their own network.

Both workshops were very practical orientated. The first, co-ordinated by Giovanni Gnemmi from Italy and Serge Lacaze from France, was on "CL/P4 and Doppler sonography" and was supported generously by Elexinn. The second, co-ordinated by Patrice Humblot from Sweden and Pascal Salvetti from France was on "Genomics-practical consequences: update-current challenges-future". We thank them all for their excellent contribution to the scientific program.

Two members step down from the board this year – Ian Kippax (United Kingdom) and Peter Vos (The Netherlands) - and I would like to thank them both for all they have done for the society. Ian has served the society as a board member since 2008. He was responsible for European legislation and he was the chair of the local organizing committee for our meeting in Chester in 2011. Peter also joined the board member in 2008 and was the vice president between 2013-2015. His contribution to the AETE web site in the last few years was remarkable.

Of course, two new Board members were elected. Out of the five candidates, it is my pleasure to present **Teresa Mogas** from University Autonoma of Barcelona, Spain and **Hilde Aardema** from University of Utrecht, The Netherlands who received the most votes at the General Assembly meeting in Barcelona. I would like to congratulate and welcome them in the board and I am looking forward to working with them both. Teresa has been appointed as an "secretary-elect" and Hilde has been assigned responsible for the website. I wish you both all the best. At the same time, **Jan Detterer** has been appointed out as our Treasurer-elect.

During the general assembly a decision related with a name change of the society, without changing the acronym (AETE), was made:

(i) Firstly the membership voted to replace the word "Transfer" with "Technology" to capture the majority of the techniques related with embryo handling and

(ii) That the English name of the society would match with the actual acronym "AETE" and henceforth be known as "Association of Embryo Technology in Europe". The translation into French as "Association Eutopéenne des Technologies de l' Embryon".

During the year **Marja Mikkola**, AETE member responsible for the Annual ET statistics in Europe together with George Perry, chair of the IETS Data Retrieval Committee worked together and the IETS Database is running after some major modifications to enable better collection of data. More information will be available in the AETE website or by contacting Marja directly.

As every year, the meeting combined a successful scientific programme with outstanding social events, encouraging exchanges between practitioners, students, scientists and sponsors. Therefore, I would like to acknowledge the excellent organisation of this meeting by the Local Organising Committee chaired by Teresa Mogas from University Autonoma of Barcelona who worked hard to create a memorable event. Their hospitality in Barcelona made us all feel at home. They created a special social atmosphere for all participants at the Gala Dinner and dance party with great views of the city and also in the last evening with the live rumba band at a beach bar. Thank you all for putting together such a great event celebrating the 32nd annual meeting of AETE in Barcelona, Spain.

I also would like to thank all the sponsors and exhibitors for their financial support allowing us to organise a successful meeting like this.

As president of the AETE, I was invited by the Brazilian Embryo Technology Society (SBTE) to the 30th annual meeting at Foz do Iguaçu, August 25 to 27, 2016. The scientific committee, Felipe Perecin, Paula de Carvalho Papa, and José Eduardo Santos, together with the president Jose Buratini Jr. put together an outstanding scientific program on topics related with embryo technology and reproductive biology. I really enjoyed the meeting and the fruitful discussions and of course the Brazilian hospitality.

The preparation of our next AETE meeting in Bath, U.K. on the 8th and 9th of September 2017 is on the way. The Local Organizing Committee, chaired by Brian Graham, EGG Tech and the AETE board is already working hard and I am sure that we will have once again an interesting and enjoyable meeting. More information about the meeting can be found on page 12 of the newsletter.

I wish you all Merry Christmas and a Happy New Year 2017 and looking forward to see you in Bath.

Kind regards,

Dimitrios

Dimitrios Rizos, President, AETE

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From left to right: Jo Leroy, Roger Sturmey, Jan Detterer, Rainer Saner, Teresa Mogas, Hilde Aardema, Marja Mikkola, Urban Besenfelder and Dimitrios Rizos.

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Have an idea for an article for the newsletter?
Have some news to share with the AETE community?
Been to an interesting meeting and wish to share a report?
Keen to get some writing experience and editorial support?
Wish to share details of an upcoming meeting?

Please send your suggestions for articles and contributions, for our next news letter in **July 2017** to the editor.

Deadline for suggestions is **June 1, 2017.**

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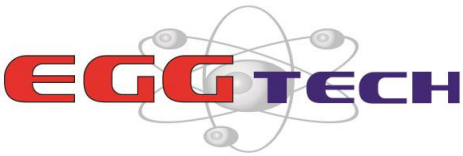

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WORKSHOP REPORT: SONOGRAPHY, DOPPLER, NEW TOOLS FOR DONOR AND RECIPIENT SELECTION?

Workshop at 32nd scientific meeting AETE, Barcelona, Spain, 9th and 10th September 2016. Moderated by Giovanni Gnemmi (BovineVet, Italy) and Serge Lacaza (AURIVA ,France), supported by ELEXIN France.

This workshop was dedicated to memory of Ludovic RICHET, (AURIVA-COOPELSO, France), member of AETE since 1988 and who died in November 2015.

After 3 presentations to introduce the subject, the participants exchanged and discussed in a serious, constructive and important way.

1 – Ultrasonography around luteal period: *Giovanni Gnemmi.*

Ultrasonography is definitely a further examination of extraordinary effectiveness, even for those who deal with bovine reproductive management. The margin of error by palpation is unsustainable: 45-50% in the evaluation of the ovary physio-pathology and 70-80% in the evaluation of the physio-pathology of the uterus. This error margin carries a low yield of hormonal therapy. The ultrasound allows us to increase the results of super-ovulation and increase conception rate of ET, thanks to a better assessment of the recipients. The ultrasound exam may be used in the evaluation of the donor as well as assessment of the recipient. In the donor, it may be possible to check the presence of the corpus luteum, assessing the functionality and the production of progesterone (colour Doppler), occurring, at the same time, the age of CL, indirectly through the evaluation of follicular map. It may be possible to count the number of follicles present in the ovaries (follicular reserve), a parameter which allows with good approximation, an estimation of the outcome of super-ovulation. Before the start of the super-ovulation, we can ablate, under ultrasound guidance, the dominant follicle, eliminating a possible risk factor for the success of the program. However the success of a super-ovulation it is not only bound to the control of the ovaries, but also to the condition of the uterus. Using ultrasonography it is possible to diagnose the presence of a sub-clinical endometritis, capable of compromising the final result of the super-ovulation. At the end of the super-ovulation, with an ultrasound exam, we can see if the cow it is in estrus and to count how many follicles are present: this number is positively correlated with the future number of corpus luteum and embryos. On the basis of the number of Graffian follicles present, it may be possible to decide whether to use or not use of particularly expensive semen doses. Before performing the flushing, it is advisable to carry an ultrasound count of the corpus luteum: this allows to determine how many embryos, approximately, we can expect to collect.

Ultrasonography is also useful in the evaluation of the recipients. First of all introduction of ultrasonography in the evaluation of the recipients, has reduced by 30%, the elimination of the recipients. The ultrasound exam of the recipients, allows us to evaluate the presence of a CL, if the CL can have an age of 6-7-8 days and if that CL has a volume and or a satisfactory size.

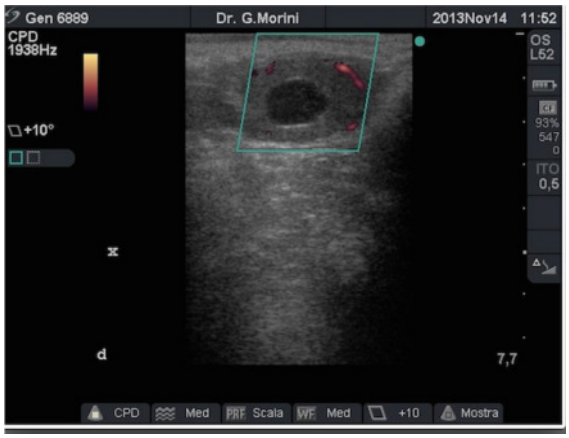

Figure 1: A Functional CL

Ultrasound examination also allows us to realize an early diagnosis pregnancy, also verifying the quality of gestation, identifying with extreme precision poor viability and/or late embryonic death. Between 60-110 days of gestation, it is possible to realize a fetal sexing, test that takes a few seconds, if made by a technician capable, with an accuracy of 99.99%.

2–The interest of ultrasounds for the follicle analysis around follicular period: *DREVILLON Pierrick* – DECHERF Agathe – Elexinn (France)

For the embryo transfer technician, the assessment of number, quality and stage of the corpus luteum (CL) is essential. Precious information may be collected by the analysis of the follicular stage of the recipient which is the purpose of the study that has been made by Pierrick Drevillon and its team (CECNA).

Ultrasounds follow-up has been proceeded with a curvilinear rectal probe, at the frequency of 10 MHz.. A follicular monitoring of 3 cows exposed first to a pre-synchronization treatment has been conducted. Follicular stimulation has been done

with Folltropin, and started on D1 with the injection of 2.8cc in the morning and in the evening.
On D1, a typical corpus luteum of 3 cm diameter was observed on the left ovary: in light yellow, you can see the entire inner portion of the corpus luteum; and in darker yellow, the papilla or champagne cork. One small follicle less than 3 mm was observed on the left ovary and 5 on the right ovary. Two bigger follicles larger than 3 mm were also present (Figure 2).

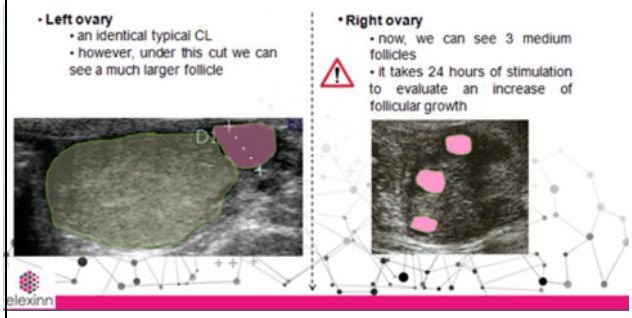

Figure 2

On D2 (Figure 3), the left ovary has not changed except for a larger follicle that was detected the day before. Small follicles are growing on the right ovary and become easier to be counted: 3 medium follicles on the right ovary (size > 3 mm). These follicles start to react to the treatment. Indeed, it is after 24h that the follicular growth begins to be perceptible to the eye.

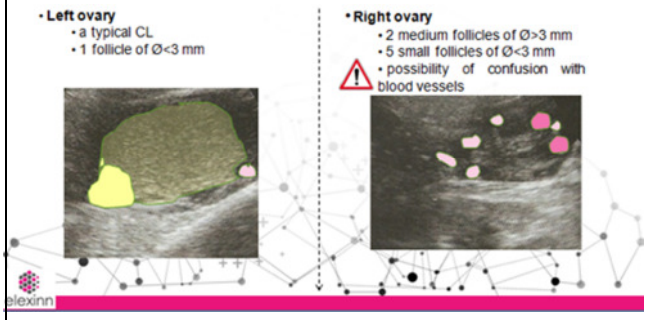

Figure 3

On D4 of the superovulation treatment, the CL found on the left ovary is smaller because of the prostaglandin injections. The 8 mm follicle is still present on that ovary. The right ovary begins to respond to the treatment with the apparition of 4 medium follicles and 5 large follicles. The overall size of the ovary is increasing due to the growth of follicles within the ovary. The heat of the animal is about to start.

Pictures taken on the fifth day of the superovulation treatment (Figure 4) show the leftover of the CL on the left ovary, two times smaller than its original size. It has now 3 large follicles. 7 large follicles can be counted on the right ovary. Attention has to be put on the deformation effect of the ultrasound analysis.

On the ovulation day, the technician can count all of the follicles that have a size of > 10 mm which is the cut-off for follicles to have LH receptors. This counting is important to orientate the insemination strategy: sexed and expensive semen or not. The presence of a small number of follicles with an ovulation size seen by ultrasound is a reliable diagnosis but incomplete.The monitoring of the follicular growth is an interesting tool also to identify post-ovulation situations which would require deep-intra uterine insemination.

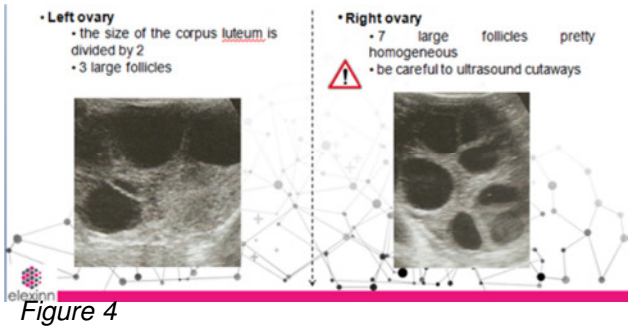

Figure 4

At D+1, young corpora lutea may be identified but this is difficult because the luteal cells are immature and not very echogenic. Non ovulated follicles with their follicular fluid are also identified and finally immature follicles recruited for the next follicular wave. The formation of both corpora lutea is clearly visible on the pictures with the follicular fluid in the middle which will take several days to disappear and around it, the cells of the CL begin developing.

Seven days after ovulation, the presence of CL with cavities and without is observed which doesn't give indications about the result or the quality of the collection to come.

Particular cases of a bad collection due to the presence of a dominant follicle which countered the FSH effect during the stimulation can be observed with the following consequences: no response on the right ovary and a very low response on the left ovary (only 2 CL visible).

Finally, the last case introduced came from the CRRA of Montreal, and related to the collecting day. Non-ovulated follicles appeared to be present which is a misunderstanding of the situation: by ultrasound, it seems like these follicles have not ovulated but by looking closer, the follicular wall is actually thick. The animal has given 15 embryos and 12 with a quality 1. Pictures are actually explained because of the follicular fluid that persisted in the CL but with follicles walls that has thickened enough to give sufficient luteal cells (Figure 5). This syndrome is known in humans and is often associated with hyperstimulation

syndrome.

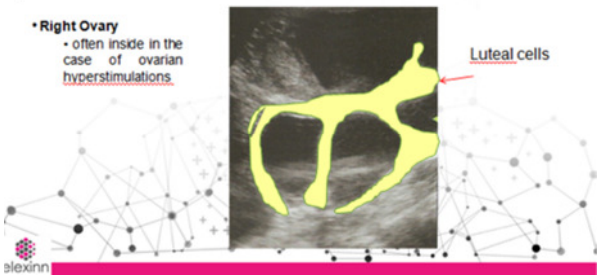

Figure 5

Today the Doppler ultrasound is used in women and helps during the processing to judge whenever to puncture the patient. Doppler ultrasound also allows adapting better the hormonal dosage for a balanced response and quality, which should be investigated for cattle as well.

To conclude, if today the ultrasound for embryo recipients is obvious, the use of ultrasounds on donors seems difficult to implement. The benefits of this use are assured and other ideas could be applied like controlling small follicles on ovulation day to get an idea of the potential of the donor or adapting the treatment and controlling the follicles larger than 12 mm diameter. Ultrasounds will also help to control the number of CL during the day of collect and finally, it could help to count follicles larger than 8 mm the day of AI in order to orientation the choice of the semen used.

3.The selection of recipients: How to determine the quality of the corpus luteum?: *Dr Nicole Hagen*, (Professor of reproduction at the Veterinary School of Toulouse, France)

For the embryo transfer team, the selection of a recipient is a challenge. In addition to the sanitary criteria, the selection of the recipients is based on the quality of the mature corpus luteum (CL) evaluated by rectal palpation or by ultrasonography. Several studies described the occurrence of mature corpora lutea with cavity, with an incidence ranging from 34 to 80 %. However, in the absence of serial ovarian examinations to confirm ovulation, it may be difficult to determine if a luteal structure with a large fluid-filled cavity originated from an ovulated follicle (cavitary corpus luteum) or an anovulatory follicle (luteal cyst).

For the practitioners, an important question is to know if these cavitary CL are able to sustain pregnancy after embryonic transfer. A synthesis of different studies evaluating the function of corpora lutea with a cavity of various sizes was presented during the workshop. The presence and the size of luteal cavity do not affect luteal tissue area, plasma progesterone concentration or ultrasound echotexture. Furthermore, the presence of a cavity does not modify the length of the interovulatory interval. Moreover, pregnancy rates were not different in females that had a mature cavitary CL from those bearing a noncavitary CL. However, in the absence of serial ovarian examinations to confirm ovulation, it may be difficult to determine if a luteal structure with a large fluid-filled cavity originated from an ovulated follicle (cavitary corpus luteum) or an anovulatory follicle (luteal cyst).

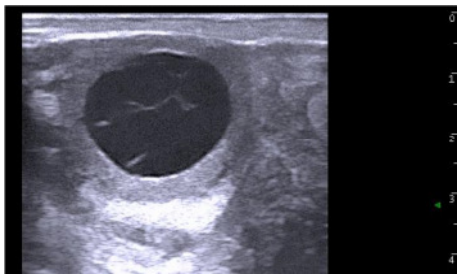

Figure 6 Cavitary CL

In conclusion, ultrasonography of the CL is an accurate method to monitor the quality of the corpus lutea before the embryo transfer. The presence of a fluid-filled cavity is a common characteristic of the bovine CL. And this cavitary CL could be considered as a normal structure that does not reduce the likelihood of pregnancy, provided that the extent of the luteal tissue is sufficient to ensure a normal progesterone production. So as long as recipient is observed in standing estrus 6 to 8 d prior to embryo transfer and has a palpable or ultrasonically evident CL, whether cavitary or noncavitary, at the time of ET, it is eligible to receive an embryo.

Thank for the participants for their exchanges and especially on the subject of the cavitar CL where opinions are very mixed !!!!

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WORKSHOP REPORT: INTEGRATION OF REPRODUCTIVE BIOTECHNOLOGIES IN THE CONTEXT OF GENOMIC SELECTION

Workshop at 32nd scientific meeting AETE, Barcelona, Spain, 9th and 10th September 2016. Moderated by Patrice Humblot and Pascal Salvetti.

After a short introduction, persons representing key actors in the field of animal breeding and genetic selection in EU (Knut Roschlau (Masterrind, Germany), Marja Mikkola (Viking Genetics, Finland), Myriam Reichenbach (Bayern Genetik, Germany), Erik Mullaart (CRV, The Netherlands) and Alexandre Morel (Evolution, France)) presented how reproductive biotechnologies are integrated in selection schemes while considering the present context of genomic selection associated to intensive international competition and societal watching. This abstract underlines the major points raised by the speakers and the audience during the workshop.

For now, the companies do not have the same level of integration of embryo technologies in their genetic schemes. Some companies work mainly with *in vivo* produced embryos whereas others have integrated *in vitro* production embryos ranging for instance from post-mortem IVF without OPU in Bayern Genetik to a routinely used of OPU with about 7000 IVP embryos per year in CRV (see AETE statistics). However, despite these differences, all speakers mentioned that there is a strong need for companies to develop and optimize **the efficiency of embryo technologies** in order to be competitive. Most particularly, the variation in pregnancy rates obtained after the transfer of *in vitro* produced (IVP), biopsied and cryopreserved embryos (the most interesting strategy from a genetic point of view) still represents a major bottleneck in the use of these technologies.

In order to optimize pregnancy rates, several complementary approaches have been cited by the speakers while focusing on the quality of IVP embryos which is a large field covering from the quality of punctured oocytes to the formulation of culture media (K.R.) or on the cryopreservation protocols (E.M. and M.R.). Vitrification is still promising but not really user friendly because direct transfer after thawing is not possible yet. The quality of the recipient is also an interesting research topic but not so much approached yet (M.M). Finally, the question about the interest of embryo splitting to increase the pregnancy rates/ chances to get a calf of a high genetic merit was raised (E.M.) and the cost-benefits for the industry of developing their own recipient's herds discussed in relation with the need of high numbers of recipients and relatively low pregnancy rates (A.M.).

Limitations in embryo production are encountered also due to use of young animals to reduce the generation interval. Genomic allows a very early identification of candidates for the selection scheme but the challenge is now to produce enough good quality gametes and embryos from young animals. Thus, a very important research area is opened to satisfy gametes production from pre-pubertal animals and/or to hasten puberty. In parallel, to decrease the costs of selection schemes, it is critical to develop tools to predict the donor's superovulation responses to avoid the inefficient treatment of poor responders.

A major challenge for the efficiency of the selection schemes remains the development of a rapid, easy and reliable embryo pre-implantory diagnosis allowing sex determination, the estimation of breeding values and possible undesired genetic status (hereditary defects, horn status…). Different levels of integration have been reached by the operators. However, today, the technique is still time consuming on field and there is limitations due mainly to logistic problems (long time for the genome analysis, particularly when it's not integrated is the routine breeding value estimation (M. M.)).

The question of the management of the genetic variability was raised in the audience due to possible unfavorable impacts of genetic selection on biodiversity especially in highly selected dairy breeds. This remark illustrates well also the present concerns from the society. The discussion showed that the breeding industry is aware of problems raised by society about the long term consequences and safety of their selection schemes and possible impacts on the evolution of the associated legal framework in EU (comments from E.M. and A.M.). Being prepared is an important issue in a societal context where animal productions are rejected by part of the population. The place of embryo technologies in this context is central. The perception of the use of embryo technologies can be unfavorable while being associated with the reduction of genetic variability, use of hormones in the protocols and possible induction of undesired epigenetic effects. On the other hand, their use can help to answer some of the major issues raised by the society. A balanced use together with genomic selection allows the selection for new traits (like diseases resistance, feeding efficiency, reduction of methane emission, quality of products…) which strongly meet wishes from the society.

The development of genomic selection was associated to major changes in the objectives of breeding companies inducing a regain of interest for embryo technologies which occupy a central place in the context of high worldwide competition for genetic gain. We are very grateful to all speakers and participants for highlighting some important bottlenecks that need further research to optimize genetic scheme for the sake of their sustainability and social acceptance.

2016 PRIZE WINNERS

Student Prize

Chosen by Members of the AETE Board

Winner: Lies Jordaens

University of Antwerp

Email: Lies.jordaens@uantwerpen.be

In vitro monolayer barrier function of bovine oviduct epithelial cells is modified due to high concentrations of non-esterified fatty acids

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[b] *Laboratory for Animal Nutrition and Animal Product Quality, Ghent University, Belgium*

[c]*Core Facility for Biomedical Microscopic Imaging, University of Antwerp, Antwerp, Belgium*

Early post-partum negative energy balance in high yielding dairy cows has considerable repercussions on reproductive ability and economic merit of these animals. Typically, lipolysis is upregulated and the associated rise in non-esterified fatty acids (NEFAs) in both plasma, and follicular fluid has been proposed as a key factor in the decline of oocyte and embryo quality. The effects of elevated NEFAs on the oviductal micro-environment, however, remain largely unknown despite the significance of the oviduct in female reproduction. In this study, we focused on the potential effects of maternal metabolism on the oviduct and hypothesized that elevated NEFAs modify *in vitro* bovine oviduct epithelial cell (BOEC) physiology by altering the BOEC barrier function, and thus potentially affect overall fertility. Hereto, we aimed to characterize the effects of elevated NEFA levels on fatty acid (FA)-transfer across the BOEC-monolayers, monolayer permeability linked to transepithelial electric resistance (TER), BOEC tight junction protein 1 (*TJP1)* expression and intracellular lipid accumulation, by using a polarized cell culture system.

In 4 repeats, early luteal BOECs from 4 oviducts per repeat were freshly seeded in a polarized cell culture (PCC)-system with hanging inserts. After reaching 100% confluency (Day 9), two distinct compartments were created: an apical and a basal compartment, respectively representing the oviduct lumen and its blood supply. Monolayers were subsequently NEFA-exposed to 230µM palmitic acid, 280µM stearic acid and 210µM oleic acid, mimicking the average serum NEFA-concentrations during lipolytic metabolic disorders, and the following 4 treatment groups were implemented: 1) CONTROL (0µM NEFA + 0%EtOH), 2) SOLVENT CONTROL (0µM NEFA + 0.45%EtOH), 3) BASAL NEFA (720µM NEFA + 0.45%EtOH in the basal compartment), 4) APICAL NEFA (720µM NEFA + 0.45%EtOH in the apical compartment). After a 24h NEFA-exposure period, spent medium was photometrically evaluated for total FA-concentration and subjected to gas chromatography for FA-profiling. Also, a 3h permeability assay using FITC-albumin was performed, and related to pre- and post-exposure TER-measurements. BOEC-mRNA was retrieved to assess expression levels of tight junction protein 1 (*TJP1*) using qRT-PCR. Intracellular lipid accumulation was studied using confocal imaging after Bodipy® 493/503 and DAPI staining. All data were analyzed with one way ANOVA.

Spent medium analyses showed a 19.5% (122.5 ± 4.3 µM) NEFA-decrease in the supplemented compartment of BASAL NEFA, with limited passage to the non-supplemented, apical compartment of PA (56.0%↑), SA (60.0%↑), OA (33.5%↑) as free FAs. However, in APICAL NEFA 53.4% (334.2 ± 28.2µM) of FA-decrease was observed in the supplemented compartment, while no FA-increase was apparent at the non-supplemented side. This suggests intracellular FA-uptake in APICAL NEFA and was supported by an abundant presence of intracellular lipid droplets, which was limited to absent in the other treatments. FITC-albumin flux increased significantly (27.59%) in APICAL NEFA compared to the controls, and was associated with a reduced relative TER-increase (46.85%) during the NEFA-exposure. *TJP1*-expression was not affected by the treatments.

In conclusion, the *in vitro* oviduct culture system allows to observe FA-transfer across BOEC-monolayers and the resulting response strongly depends on cell polarity. In this context, elevated NEFAs in the apical 'oviductal lumen' compartment decreased the tightness of cell-cell interactions. BOEC barrier function was thereby compromised, specifically when NEFAs were supplied to the apical cell side in the oviduct lumen. These data substantiate the concept of the oviduct as a possible gatekeeper that shields its micro-environment from detrimental metabolites, such as high NEFAs, to create optimal developmental conditions for the pre-implantation embryo. The latter theory, however, needs to be confirmed in future experiments.

The data presented in this report are part of a PhD thesis in which the effects of maternal metabolic conditions on the oviduct micro-environment are characterized, in order to further elucidate the complexity of metabolically induced female infertility. For updates in this research field, please visit https://www.uantwerpen.be/en/rg/vpb/.

Best Oral Presentation

Winner: Pablo Bermejo-Álvarez

INIA Madrid

Email: borrillobermejo@hotmail.com

Optimization of RNA concentration for genome editing by CRISPR in rabbit zygotes

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Site-specific genetic modification aiming to delete (knock-out) a gene provides an unequivocal answer to elucidate the function of such particular gene in the whole organism. Site-specific genetic modification has been achieved by homologous recombination, generally in embryonic stem cells, which has made the mouse the most widely used mammalian model. However, the mouse model is not appropriate to study some biological functions or to recapitulate some human diseases. As an example, ZP4, one of the four proteins of the zona pellucida in humans and rabbits, is not present in mice, so its function remains elusive due to the lack of a knock-out model. The use of the newly developed site-specific endonucleases, such as CRISPR, allows site-specific genetic modification in zygotes, being a suitable technique for genetic modification in domestic mammalian species. The aim of this experiment has been to determine the optimal concentration of the two components of the CRISPR system (Cas9 mRNA and gdRNA) for genome editing following microinjection of rabbit zygotes. Capped polyadenylated Cas9 mRNA was produced by *in vitro* transcription from BstBI digested pMJ920 plasmid. A gdRNA was designed against the first exon of rabbit ZP4 gene, cloned into the plasmid px330, amplified by PCR adding T7 promoter and *in vitro* transcribed. Rabbit zygotes were obtained from the oviduct 14 hours after mating. Immediately after collection, zygotes were microinjected into the ooplasm with approximately 10 picoliters of three different combinations of Cas9 capped polyadenylated mRNA and gdRNA: 1) 300 ng/µl Cas9 and 150 ng/µl of gdRNA (300:150), 2) 150 ng/µl Cas9 and 50 ng/µl gdRNA (150:50) and 3) 100 ng/µl Cas9 and 25 ng/µl gdRNA (100:25). Following microinjection, embryos were cultured in TCM199 supplemented with 5 % FCS at 38.5 ºC in a 5 % CO2, 5 % O2 and 90 % N2 water saturated atmosphere. CRISPR components did not affect preimplantation embryo development, as all embryos surviving microinjection (~90 %) developed to the blastocyst stage. At the blastocyst stage, the zona pellucida was removed and blastocysts were individually stored at -20 ºC. Blastocysts were digested in 8 µl of a 100 µg/ml proteinase K buffered solution and 2 µl of the lysate were used to amplify the genomic sequence including the CRISPR target site. PCR products were purified and sequenced to determine genome edition around the target site. All combinations were similarly effective in generating insertion/deletions around the target site: in the groups 300:150 and 150:50 all blastocysts analysed (6/6 in both groups) were edited, whereas in the group 100:25 only one blastocyst out of six was not edited. To determine the number of alleles generated by CRISPR on each individual, PCR products were cloned into pMD20 plasmid and 10 clones were sequenced. Clonal sequencing revealed that CRISPR generated chimeras composed by 4 alleles, suggesting that the double-strand break generated by CRISPR and repaired by Non-Homologous End Joining (NHEJ) occurred after parental DNA replication. In conclusion, CRISPR system constitutes an effective means for genome editing in rabbit zygotes and the ooplasm microinjection of 100 ng/µl capped polyadenylated Cas9 mRNA and 25 ng/µl gdRNA achieves high genome editing efficiencies.

This study is supported by the projects AGL2014-58739-R and RYC-2012-10193 (to PBA), AGL2012-40180-C03-02 (to MA), AGL2011-23822 (to PGR and PL) and AGL2012-39652-C02-01 (to AGA). NFB is supported by a FPI grant.

Best Poster Presentation

Winner: Svetlana Uzbekova

INRA, Tours

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MALDI-TOF mass spectrometry analysis of lipids in single bovine oocytes during IVM

Priscila Bertevello[1], Olfa Ghazouani[1], Sebastien Elis[1], Charles Banliat[1], Ana-Paula Teixeira-Gomes[1], Virginie Maillard[1], Valerie Labas[1] and Svetlana Uzbekova[1#].

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Bovine oocyte is rich in intracellular lipids which are involved in membrane composition, intracellular signaling and energy storage. We have recently showed that level of neutral lipids containing in lipid droplets was diminished during oocyte *in vitro* maturation, IVM (Auclair et al. 2013). We also reported that that Intact Cell Matrix-assisted laser desorption/ionization time of flight Mass Spectrometry (ICM-MS) analysis of lipid profiles of cumulus cells was able to discriminate immature and mature oocytes (*Sanchez-Lazo et al. 2014*).

The objectives of this work were to adapt ICM-MS technology to single bovine oocytes and to compare lipid contents in the oocytes before and after IVM.

IVM was performed on bovine oocyte-cumulus complexes from 4-6 mm ovarian follicles in culture medium containing 10% of fetal bovine serum (MP Biomedicals, Illkirch, France), growth factors and gonadotropins. ICM-MS was performed on individual immature (n=12) and mature (n=12) oocytes, completely denuded from CC as shown in workflow (**Figure 1)**.

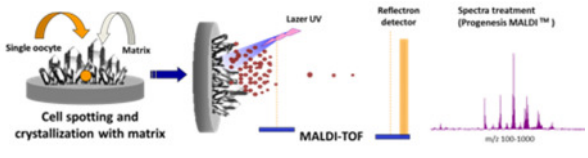

Figure 1. ICM-MS lipid profiling workflow.

Oocytes were stripped from surrounded cells, washed in Tris-sucrose buffer and individually deposited on MALDI plate with 20 mg/mL DHAP / 90% methanol / 2% TFA / 8% H20. Lipid spectral profiles (3000 shots per spectra) were acquired from each oocyte, cocrystallized with 2,5-dihydroxyacetophenone (DHAP) matrix, using an UltrafleXtreme MALDI-TOF/TOF instrument (Bruker) in positive reflector mode. M/z peaks were detected in the range of 160 to 1000 m/z and values of the normalized peak heights (NPH) were quantified using Progenesis MALDI™ (Nonlinear Dynamics).

Coefficient of variation (CV %) was calculated for each m/z peak from 3 technical replicates using 20 immature oocytes. Multivariate Principal Component Analysis (PCA) and Student test were applied to NPH values for hunting lipid content variations between immature and mature oocytes. Lipids were extracted from follicles; several peaks were fragmented by high resolution MSMS top-down analysis using LTQ Velos Orbitrap operating in positive mode and annotated using LipidMaps.

A total of 266 distinct peaks ranging from m/z 163.27 to 951.62 were detected. Mean CV% of all the peaks was 32%. 72 peaks were differential between immature and mature oocytes (38 up- and 34 down-regulated during IVM, $p<0.01$, fold change >2.0), as shown in **Figure 2**.

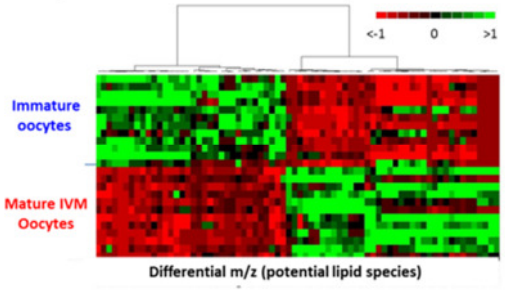

Figure 2. *Heat map representation of differently abundant lipids in the oocytes before (immature, n=12) and after 22h IVM (n=12).*

Groups of immature and mature oocytes therefore could be clearly discriminated by PCA (Figure 3)

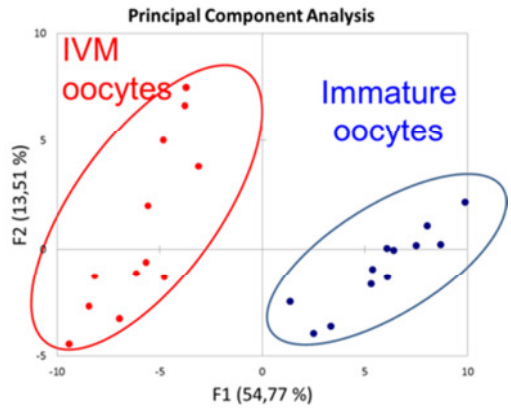

Figure 3. PCA of differently abundant lipids in immature and mature (22h IVM) bovine oocytes

Among differential m/z, several up-regulated peaks (2-68 fold increase during IVM) ranging from m/z 700 to m/z 815 were identified as phosphatidylcholines (32:0, 32:1, 33:1, 34:2, 36:2, etc) and sphingomyelins (36:1, 42:2). Among the down-regulated peaks, fatty acids C14:0 (16-fold decrease during IVM) and C17:0 (2-fold decrease) were annotated.

In conclusion, ICM-MS lipid profiling approach on single bovine oocyte allowed discrimination of the oocytes in relation to their maturation state. A number of lipid markers were revealed as m/z peaks which can be quantified in individual oocytes. Lipid content significantly varied in the oocytes before or after IVM may be due to both changes of oocyte follicular environment in vitro and to proper intracellular fatty metabolism (lipogenesis, lipolysis…) leading to structural modifications in the oocyte.

Financial support: Bovomega3 project funded by Val de Loire Region, France; P. Bertevello is funded by University do Estado do Rio Grande do Norte (Brazil).

References.

1. Auclair, S, Uzbekov, R., Elis, S., Sanchez, L., Lardic, L., Dalbies-Tran, R. and **Uzbekova, S.** Absence of cumulus cells during in vitro maturation affected lipid metabolism in bovine oocyte. *Am J of Physiology Endocrinology and Metabolism* **2013** Mar 15;304(6):E599-613
2. Sanchez-Lazo L, Brisard D, Elis S, Maillard V, Uzbekov R, Labas V, Desmarchais A, Papillier P, Monget P, **Uzbekova S**. Fatty acid synthesis and oxidation in cumulus cells support oocyte maturation in bovine. Mol Endocrinol. **2014** Sep;28(9):1502-21.

EUROPEAN STATISTICAL DATA ON EMBRYO TRANSFER ACTIVITY 2015

In a departure from the norm, we have decided to not include the full report on the ET activity for 2015 in the newsletter. This may come as an unexpected shock to readers, but fear not; the data are still available, having been compiled with great accuracy by Marja Mikkola, and available by clicking here.

The data collection form was sent to representatives from 38 countries, of which 31 replied. The data that form the report are based on embryo transfer activities for breeding and commercial embryo production reported by these European countries (countries that have at least part of their country in Europe) in 2015. The presented data include numbers on embryo production (MOET and OPU-IVP) and transfers for bovine and other species (sheep, goat and horse). These data are included in the report of the International Embryo Technology Society (IETS Data Retrieval Committee) on embryo transfer activities worldwide. There were 180 approved embryo collection teams, out of which 124 (69%) provided data.

In **summary** the number of reported embryo collections and transfers in Europe decreased in 2015 compared to two previous years for in vivo embryos. For *in vitro* embryos, the number of OPU-sessions decreased slightly (6%), but there was a moderate increase on the number of IVP embryo transfers. The proportion of *in vitro* embryos of all transferred embryos has been increasing by approximately one percentage points during the last years.

INVITATION TO THE 33RD ANNUAL MEETING OF THE AETE –SEPT 8-9, 2017

INVITATION TO BATH - UK!

On behalf of the European Embryo Transfer Association, the local organizing committee cordially invites you to the 33rd scientific meeting in the city of Bath, south west England, from the 8th to the 9th of September 2017.

Panoramic view of Bath including Royal Crescent
Image sourced from Wikimedia Commons and used according to creative commons license

The Local Organizing Committee will be chaired by **Mr. Brian Graham**, EGG Tech, UK

The Conference Location

In 2017, the meeting will take place in Bath, at the "Assembly Rooms" in the World Heritage City of Bath, England. Designed by John Wood the Younger in 1769 this fine set of public rooms was purpose built for a particular eighteenth century form of entertainment: the assembly. When they were completed in 1771, they were described as ' the most noble and elegant of any in the kingdom'. Built of Bath Stone the building has rooms arranged in a U shape. There are four main function rooms in the complex: the 100-foot-long (30 m) ballroom; the tea room; the card room; and the octagon.

Welcome to Bath:
Some reasons to come to Bath.

500 BC (some say much earlier), legend has it that Bladud, father of Shakespeare's King Lear, discovered the thermal springs and the locally living Celts began to worship here, dedicating the springs to their God, Sul.

From **AD 43** the Romans started the development of Bath as a city of recreation, rather than a garrison, and built around the hot springs a sophisticated series of baths used for bathing and curative purposes. A temple, dedicated to the goddess Minerva, was built alongside the baths and this area formed the centre of Aquae Sulis.

Bath is the only destination in the UK to have the whole city designated a World Heritage site by UNESCO. Since 1987 Bath has been listed as a 'cultural site' with outstanding universal value and cultural significance.

During the 18th century, three ambitious local entrepreneurs set out to make Bath one of the most beautiful cities in Europe. A former mayor of Bath, Ralph Allen, created the beautiful and intimate Prior Park Landscape Garden, Richard 'Beau' Nash played a leading role in making Bath the most fashionable resort in 18th century England and John Wood the Elder designed many streets and iconic buildings, such as the Circus and Queen Square. His son, John Wood the Younger, followed in his footsteps and created the Assembly Rooms and The Royal Crescent.

Today Bath has around 5,000 listed buildings. The most famous is the Royal Crescent, comprising of 30 houses laid out in a crescent shape. Built between 1767 and 1774, it is among the greatest examples of Georgian architecture in the world.

To experience Royal Crescent life in its original style, No. 1 Royal Crescent, the first house to be built on the crescent, is open to the public as a museum maintained by the Bath Preservation Trust. The house illustrates how wealthy property owners of the 18th century might have furnished such a wonderful home. Prepare to encounter many surprises as friendly, knowledgeable guides positioned in each room of the house reveal the secret history of the house and its former residents and guests. You can also find out how the city was transformed in the 18th century and how Georgian Bath was built by visiting the Museum of Bath Architecture.

If you find additional time during your trip why not consider visiting some other attractions not too far from bath.
Stonehenge
Cheddar Gorge
Bristol Zoo
Longleat Estate and safari park
Salisbury Cathedral
Or take a trip to London.

Where to stay in Bath?

Plenty of accommodation options in Bath:
Try exploring the Official Tourist Bureau
Some specific suggestions:
Accor Francis
Hilton Bath City
Abbey Hotel Bath
Villamagdala
Holiday Inn Express Bath
Premier inn Bath
Apex Hotel Bath – OPENING SUMMER 2017

How to travel to Bath?
By Air

Bristol Airport:
Bristol international Airport is the closest and easiest airport for travelling to bath with over 60 European cities able to access the airport. The airport is approximately 19 miles from bath and around 8 miles from the centre of Bristol. Please click here below to view a list of cities connected to Bristol Airport.

Bristol Airport to Bath by train & bus:
Board the A1 Bristol flyer bus from the west airport terminal heading for Bristol Temple meads. Two busses depart every hour. It is a two minute walk from the bus stop to Bristol temple meads train station. Bath is a 11-14 minute train journey from Bristol temple meads with trains departing every 15-20 minutes please use click here to view train times.

Taxi:
There are over 400 taxi companies serving Bristol airport and the surrounding areas. Bath is approximately 45 minutes from Bristol Airport via taxi, but can be considerably longer when congestion is high. Pre-book airport taxis by checking out Bristol airport Taxi Listings. The listings include contact details and a description of each company's services to help you book a taxi to suit your needs.

Heathrow Airport

Heathrow Airport:
Heathrow airport has good connections to Bath via the M4 and the railway system. Heathrow is accessible from many airports around the world and is approximately 100 miles from bath.

Train:
The train station at Heathrow airport is based at Terminals 1-3. Board the Heathrow express to Paddington station from platform 2. This is a nonstop service that will take around 16 minutes. Once at Paddington station you will need to board the train heading for Taunton. Please click here for train time from Paddington. The journey from Paddington to Bath should take around 2 hours.

Bus:
The National express coach service runs between Heathrow and Bristol. Once in Bristol there are trains, buses and taxis available for Bath. The journey time is approximately 3 hours and 15 minutes. Please click here to view National express time tables and fares:

Taxi/Car
Both taxis and hire cars will be available from Heathrow airport. The journey to Bath is fairly direct using the M4.

Other UK Airports in the south of England:
Gatwick, London City Airport, Southampton and Cardiff (wales).

By Sea
If you wish to drive to bath and take in some of the sights that the south of England has to offer, there are multiple sea ports along the south coast.

Please see some examples of travels times to bath from some of the ports:

Portsmouth – Bath:	2 hours 10 minutes
Southampton – Bath:	2 hours
Poole – Bath:	1 hour 55 minutes
Dover – Bath:	4 hours

By Eurostar
Having the option of either driving or being a foot passenger, the Eurostar offers easy access to London from the continent. On arrival in St Pancras you will need to make your way to Paddington station using either a taxi or the underground service. Please click here for tube map of London:

Please click here for more Eurostar travel information including stations and time tables:

LOC representatives (in alphabetical order)
Brian Graham (Chair)
Jake Oliver
Dr. John Dawson
Mr. Mark Nutsford
Dr. Peter May
Dr. Roger Sturmey
Sharon Graham
Sue Williams

Should you require any assistance from the LOC please email 0ffice@eggtech.co.uk

We look forward to seeing you in 2017 in Bath
Local Organizing Committee

INIA
Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria

UPCOMING EVENTS

IETS
January 14-17, 2017
Renaissance Austin Hotel, Texas, USA
Meeting homepage
Programme

50th Annual Meeting of the Society for the Study of Reproduction
13–16 July 2017
Marriott Wardman Park—Washington D.C., USA
Meeting homepage

31st Annual Meeting of the Brazilian Embryo Technology Society (SBTE)
17-19 August 2017
Cabo de Santo Agostinho, Brazil

21st Annual ESDAR Conference 2017
24-26 August 2017
VetSuisse University of Bern, Switzerland
Meeting details

33rd Annual Meeting of the AETE
8-9 September 2017
Bath, UK
Early Bird Registration until July 15
Meeting homepage

AETA & CETE/ACTE JOINT CONVENTION
26-28 October 2017
Caribe Royale, Orlando, Florida, USA
Meeting details **Keep checking for details**

Looking ahead…
11th Bienniel Meeting of the Association for Applied Animal Andrology
14-16 July 2018
New Orleans, USA
Meeting details

International Ruminant Reproduction Symposium
16-20 September 2018
Foz Do Iguacu, Brazil
Details

19th International Congress on Animal Reproduction
28 June-02 July 2020
Bologna, Italy
Meeting details