HEALTH AND SAFETY ADVISORY COMMITTEE REPORT

Dr Claire PONSART

Chair of the committee

Paris, 3/25/2014

REPORT TO THE IETS BOARD OF GOVERNORS

I. Research subcommittee

Ann Van Soom confirmed her stepping down after this meeting and Francis Fieni volunteered to become the chair starting from 2015.
New members are George Perry and Patrice Humblot.

1. Review minutes of Hannover 2013

Much of focus of the meeting was on scrapie, both on classical and atypical scrapie. Selecting animals for markers of disease resistance was also discussed as it is an important topic of current research. The electronic working groups that were started last year on the topic of scrapie were very productive and should remain active so that the Research subcommittee can discuss important issues between meetings. Francis will take care of this as the new chair.

It is proposed to discuss important matters also in the general HASAC committee rather than just the HASAC Research subcommittee. Last year an observer from one country expressed some reservations with our conclusions as regards scrapie. However, the conclusions are not for transmission to the authorities of the different countries, but to the IETS Board of Governors. Originally, the Research subcommittee was only scientists and did not include veterinarians from veterinary authorities. Now they are more prominently present as their support is important for funding on future research.
The Research subcommittee provides scientific evaluation of recent research and the policy makers use this to assist with formulating official regulation and policies.

Working with IVF embryos is becoming very important : since the data we have on embryo-pathogen interaction show that IVF embryos do not react in the same way to decontamination procedures, we cannot extrapolate data gained from in vivo embryos, and this is obstructing the international trade. The Board of Governors will officially ask for a review on the problems associated with IVF embryos and will send a request for this review to Claire Ponsart, the new head of HASAC since Pascale is also stepping down after this meeting.

More money is desperately needed for research on embryo-pathogen interaction, especially IVF embryos – the situation for funding is very bad. The Board sent out a letter last year without result. Next year there will be a presymposium on HASAC issues organized by Claire and **hopefully under the auspice of OIE** at the IETS.

In the minutes of last year there was an unpublished paper on BTV which is still unpublished because other priorities occurred. Similar problems occurred with new data on BTV-8 (two abstracts).
The committee would like to see the data published.

P Blondin asked more information about semen decontamination. The EWG has not been started yet but it will soon be online.
A motion to approve the minutes was put forward and **approved.**

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2. Review 2013 Research update

PART A : New Research

1. **Bielanski A, Algire J, Lalonde A, Garceac A**

Prevention of bovine herpesvirus-1 transmission by the transfer of embryos disinfected with recombinant bovine trypsin.

Theriogenology. 2013 Dec;80(9):1104-8.

In this study it was concluded that the use of RBTr could be considered as an alternative method of rendering embryos free of BHV-1 and thus reduce the potential risk of disease transmission to embryo recipients and offspring.

The committee agreed to the significance of this paper on the treatment of bovine in-vivo embryos
The following two recommendations were proposed

1. To approve the use of recombinant trypsin for cattle as an alternative to porcine trypsin
2. To approve trypsinisation for 120 s instead of 60-90s

MOTION
The committee recommended the recombinant bovine trypsin (RBTr) for in vivo derived cattle embryos be used in accordance with the procedures described in Andrej Bielanski's paper:
" The disinfection protocol using RBTr involved three washes in PBS culture medium supplemented with 0.4% BSA, followed by two treatments with RBTr for 60 s each, one wash in soybean trypsin inhibitor at 80 mg/mL for 60 s and four further washes in PBS medium containing 0.4% BSA".

Motion approved.

2. **Bielanski A, Algire J, Lalonde A, Garceac A.**
Embryos produced from fertilization with bovine viral diarrhea virus (BVDV)-infected semen and the risk of disease transmission to embryo transfer (ET) recipients and offspring.

Theriogenology. 2013 Sep 15;80(5):451-5

In conclusion, results herein suggest that BVDV can be transmitted by AI resulting in the production of some proportion of contaminated embryos. However, it appears that such embryos, when washed according to International Embryo Transfer Society and the World Organization for Animal Health guidelines do not cause BVDV transmission to recipients or their offspring.

The committee agreed the results are dependent on the strain – some remain attached to the ZP. However BVDV can cause death of the embryo.
The IETS/HASAC can never approve or recommend ET from a PI infected bull/cow.

This paper, although interesting, does not provide new evidence to change the IETS- recommended policy.

3. **Foster JD, Goldmann W, Hunter N.**

Evidence in sheep for pre-natal transmission of scrapie to lambs from infected mothers.

PLoS One. 2013 Nov 18;8(11):e79433.

Scrapie clinical disease, caused by both natural scrapie and SSBP/1, occurred in the progeny but evidence (including mouse strain typing) of SSBP/1 infection was found only in lambs born to fully susceptible recipient mothers. Progeny were not protected from transmission of natural scrapie or SSBP/1 by washing of embryos to International Embryo Transfer Society standards or by caesarean derivation and complete separation from their birth mothers. Our results strongly suggest that pre-natal (in utero) transmission of scrapie may have occurred in these sheep.

These data are not relevant for the IETS washing procedure since the embryo is not determining the offspring health but it is the recipient mother which is susceptible and will confer the scrapie to the offspring whether or not the embryos was washed.

They did not mention whether the instruments were properly sterilized. This is probably not an issue because they have a lot of experience with this type of study. They only discussed ewes where they got lambs from but they never discussed ewes that received an embryo but were not pregnant.

4. Bielanski A, Algire J, Lalonde A, Garceac A, Pollard JW, Plante C.

Nontransmission of porcine circovirus 2 (PCV2) by embryo transfer.

Theriogenology. 2013 Jul 15;80(2):77-83

The results obtained in this study indicate that the transmission of PCV2 from infected donors by embryo transfer is unlikely if the sanitary recommendations of the International Embryo Transfer Society are followed. In practical terms, this means that embryo transfer can be successfully used for the intentional elimination of PCV2 and to create virus-free offspring for the safe exchange of swine genetic materials.

PCV2 is at present listed by OIE in category 4.

We suggest to the regulatory subcommittee to move PCV2 to category 3, for the following reasons:

It was demonstrated that the risk of disease transmission is negligible after flushing pig embryos from infected donors provided that the IETS guidelines for washing are followed. This was based on the finding that 1019 embryos were collected and 364 embryos were transferred from 59 PCV2 infected donors into 24 seronegative recipients which did not seroconvert and all piglets (N= 76) were PCV2- free (Bielanski et al. 2013, Theriogenology 80, 77-83).

5. **Alsaleh A, Fieni F, Rodolakis A, Bruyas JF, Roux C, Larrat M, Chatagnon G, Pellerin JL.**
Can Coxiella burnetii be transmitted by embryo transfer in goats?
Theriogenology. **2013 Oct 1;80(6):571-5.**
The bacterium shows a strong tendency to adhere to the ZP after in vitro infection, and the washing procedure recommended by the IETS for bovine embryos failed to remove it. The persistence of these bacteria makes the embryo a potential means of transmission to recipient goats. Further studies are needed to investigate whether the enzymatic treatment of caprine embryos infected by C. burnetii would eliminate the bacteria from the ZP.

MOTION

We suggest to the regulatory subcommittee to add Coxiella burnetii to category 4, following Fieni's paper it has been shown that in a worst case scenario (in vitro-in vitro study : very high dose of experimental infection) Coxiella can adhere to the ZP of in vivo derived caprine embryos and not be removed by appropriate washing procedures.

Category 4 *diseases* or pathogenic agents are those for which studies have been done, or are in progress, that indicate:

i)that no conclusions are yet possible with regard to the level of transmission risk; or
ii) the risk of transmission via embryo transfer might not be negligible even if the embryos are properly handled according to the IETS Manual between collection and transfer.

Motion approved.

6. **Lamara A, Fieni F, Chatagnon G, Larrat M, Dubreil L, Chebloune Y.**
Caprine arthritis encephalitis virus (CAEV) replicates productively in cultured epididymal cells from goats.
Comp Immunol Microbiol Infect Dis. **2013 Jul;36(4):397-404**
Our findings clearly demonstrate that cells of the buck genital tract are targets of CAEV and are thus a potential reservoir that sheds infectious CAEV into the semen of infected animals. These data suggest the use of sperm from CAEV-free goat males for artificial insemination in genetic selection programs to minimize CAEV dissemination.

This paper provides for the first time interesting data on target cells in the male goat that are not from the hemo-endothelial line and in which CAEV can replicate.

7. Alsaleh A, , Fieni F*, Moreno D, Rousset E, Tainturier D, Bruyas JF, Pellerin JL
Risk of *Coxiella burnetii* transmission via embryo transfer using *in vitro* early bovine embryos
Theriogenology accepted
These results demonstrate that *C. burnetii* adheres to and/or penetrates the early embryonic cells as well as the ZP of *in vitro* bovine embryos after *in vitro* infection, and that the standard washing protocol recommended by the IETS for bovine embryos, failed to remove it. The persistence of these bacteria after washing makes the embryo a potential means of transmission of the bacterium during embryo transfer from infected donor cows to healthy recipients and /or their offspring. Further studies are required to investigate whether enzymatic and/or antibiotic treatment of bovine embryos infected by *C. burnetii* would eliminate the bacteria from the ZP and to verify if similarly results are obtain with *in vivo*-derived embryos.

For animal health we need more research before reaching any conclusion.
For human health we refer to medical authorities, but we recommend that when working in close contact with positive animals at least wear a mask and glasses/goggles, especially for women (pregnancy risk).

8. **González Altamiranda EA, Kaiser GG, Mucci NC, Verna AE, Campero CM, Odeón AC.**
Effect of Bovine Viral Diarrhea Virus on the ovarian functionality and in vitro reproductive performance of persistently infected heifers.
Vet Microbiol. 2013 Aug 30;165(3-4):326-32
The results of this study demonstrate that BVDV PI heifers exhibit alterations in follicular population through of the early interaction between the virus and germ cell line affecting directly the mechanisms involved in the ontogenesis of the ovary.

This paper is a reiteration of previous studies (Fray) involving production of oocytes from PI BVD animals : it is a confirmative study. There are some reservations about this paper. The authors claim they have 20 % of embryos from PI animals but they did not define the stage so if it were morulae it could have been degenerated embryos as well . They also do not mention the culture medium additions : there should be some protein in it and if they used FCS it could be a source of BVD contamination.

9. Galuppo AG, Junior NB, Arruda NS, Corbellini AO, Chiappetta CM, Pavão DL, D'Angelo M, Canal CW, Rodrigues JL.
Evaluation of the effectiveness of semen processing techniques to remove bovine viral diarrhea virus from experimentally contaminated semen samples.
J Virol Methods. 2013 Feb;187(2):443-8
The data showed that the combination of Swim up/Percoll gradient centrifugation promoted the elimination of BVDV from the semen samples without damaging spermatozoa cells and also allowed successful in vitro embryo production free of BVDV. Hence, the risk of BVDV contamination is negligible for the embryo

The semen was spiked so this may be the reason why it was more easy to remove it. In naturally infected semen BVD might be located in somatic cells. It is very difficult to detect virus in natural semen without processing the semen. However this paper provides interesting information for safer in vitro embryo production in cattle.

PART B UPDATE : Unpublished data of 2013

No unpublished data were provided

PART C UPDATE : Reviews of 2013

1. **Cortez-Romero C, Pellerin JL, Ali-Al-Ahmad MZ, Chebloune Y, Gallegos-Sánchez J, Lamara A, Pépin M, Fieni F.**
The risk of small ruminant lentivirus (SRLV) transmission with reproductive biotechnologies: state-of-the-art review.
Theriogenology. 2013 Jan 1;79(1):1-9.
This review was discussed last year

Ponsart C., N. Pozzi
Sanitary requirements for bovine gametes and embryos in international trade
Anim. Reprod., v.10, n.3, p.283-296, Jul./Sept. 2013 _____

3. List business arising from email communications with HASAC research members :

4.1 Sterilization of ET instruments

Some ET practitioners work in different countries. There are differences in recommendations by the IETS, OIE and WHO.

Recommendations proposed by George

The IETS Manual Chapter 8 should update the section on equipment sterilisation and include the OIE recommendation that "*used equipment should not be transferred between countries for re-use by the embryo collection team*" is adequate to manage the risk of international transmission of prion infectious material.

The OIE be advised the IETS advises the instrument/equipment recommendations in the current edition of the IETS Manual is not adequate to manage the risks of scrapie transmission during embryo/oocyte collection and embryo transfer.

An EWG will be started on this topic with the aim that the next edition of the IETS manual properly addresses this particular issue. George Perry will lead the EWG.

1.2. EU 2013 review on scrapie transmission

Last year the committee decided not to make any recommendation based on genotype as it was agreed the genotype is not relevant in ET industry in the sheep.

1.3. IVF embryo transport

The current OIE guidelines for international trade in IVF embryos refer only to frozen embryos:

4.8.7 Conditions applicable to the storage and transport of embryos

1. Only embryos from the same individual donor or from the same batch collection should be stored together in the same ampoule, vial or straw.
2. The embryos should if possible, depending on the species, be frozen in fresh liquid nitrogen or other cryoprotectant and then stored in fresh cryoprotectant in cleaned and sterilised tanks or containers under strict hygienic conditions at a storage place.
3. Ampoules, vials or straws should be sealed at the time of freezing and should be labelled according to the IETS Manual[1].
4. Liquid nitrogen containers should be sealed prior to shipment from the *exporting country*.
5. Embryos should not be exported until the appropriate veterinary certificates are completed.

It was proposed the committee should think about international transport of fresh bovine/equine IVP embryos. From a scientific point of view the conditions required for frozen should also be required for fresh embryos. Post-collection tests however cannot be performed. It will be necessary to list diseases that can create problems. It was suggested the collection of oocytes be considered equal to the export of a live animal and donors be tested and prepared as if they were going to be exported.

The group recognizes the concerns about this issue and proposes that an EWG works on this topic during the next year led by George. We should find some practitioners that are involved in this practice.

1.4. Quality control with in vitro embryo production

One member thought The Manual is inadequate on air quality control. Sybrand Merton published a paper on this topic : by using a CODA filter during IVP, there was no effect on embryo stage or quality. However, the pregnancy rate was improved (P=0.043) for both fresh (46.3 vs. 41.0%) and frozen/thawed embryos (40.8 vs. 35.6%). These results show that atmospheric purification by the CODA intra-incubator air purification unit significantly increased pregnancy rate following transfer of in vitro-produced bovine embryos.

It was pointed out equipment is very expensive and such a filter can be used if so desired. The manual adequately addresses important quality control issues to consider.

5. Requests from OIE, any update at this stage?

This year the OIE set up a working group for Infectious Epizootic Hemorrhagic Disease virus (EHD) draft w/o consideration for embryos. We have very few publications on this topic. There was one paper on bovine embryo interaction (Dinkins et al., 2001). Trypsin seemed not effective to remove the virus.

The group recommends that new research will be performed on EHD.

The first priority is to see if washing procedures are adequate to remove virus from in vivo derived embryos with an in vitro – in vitro approach.

6. Regulatory subcommittee requests?

No requests were made by the regulatory subcommittee. If we follow the agenda of the Regulatory subcommittee the following points are addressed

1.1. Trypsin temperature

Ann Van Sooms group reports on the trypsin treatment combined with temperature (37°C vs RT) in the abstract presented at this conference (**Failure to remove BlueTongue serotype 8 virus (BTV-8) from in vitro produced bovine embryos. Oliveira et al. Abstract # 104)**

These results show that the wash procedure is efficient to remove the virus from the wash media, but it failed to remove the virus from bovine embryos produced in vitro. The temperature (37 °C or RT) did not influence the efficiency of the trypsin treatment.

1.2. BTV-8

We conclude the following: due to the lack of substantial embryo transfer data (only 3 ET), the research subcommittee is not in a position to make a recommendation regarding BTV-8 to the regulatory subcommittee. Jennifer Koziol and Julie Gard will collaborate with Ann van Soom to write up a full paper on these BTV-8 data (6.1 and 6.2).

1.3 Other updates

-Update on Schmallenberg will be given by Claire – next year it will be on the agenda of the Research subcommittee since the experiments are still going on.

-Update on Chlamydia will be given by Francis Fieni next year. Dr Kaltenbach (USA) does a lot of research on Chlamydia, also Daisy Van Rompay (BE).

-Update on Sterilization of laparoscopy equipment : this will be covered by the EWG to be led by George

7. AOB :

Peter Hansen spoke on behalf of the Board. They value very much the activities of the Research subcommittee and is open to any suggestions. Pascale says that one night of hotel stay can be claimed from the IETS for participating members of the Research Subcommittee.

8. **Date for next meeting.** Friday 9th of January 2015 in Versailles, France.

II. Regulatory subcommittee

1. Review of the minutes of the previous meeting in Hannover, Germany

Motion approved.

-Laboratory capacity/activity in the field of embryo transfer epidemiology worldwide

More research is needed on emerging diseases and also those diseases requiring vector proofing. Issue with vector borne diseases is ideally they should be conducted in vector proof facilities as else this may invalidate the findings of the study. Option of vector free zones was discussed.

MOTIONS
1) The Regulatory Subcommittee recommends to the board to continue to lobby internationally for further research in this area. 2) **The Research Subcommittee should develop a priority list of disease where research is needed.**
Motions approved.

-Trypsin wash update

For the Research Subcommittee Bielanski et al.'s paper was quoted "**Prevention of bovine herpesvirus-1 transmission by the transfer of embryos disinfected with recombinant bovine trypsin." In Theriogenology. 2013 Dec;80(9):1104-8.**

This study deals with the potential for the introduction of infectious agents through the use of animal-derived products. The efficacy of a recombinant bovine trypsin (RBTr) as a replacement for porcine pancreatic trypsin and a disinfectant for bovine herpesvirus-1 (BHV-1)-infected embryos was investigated according to the sanitary guidelines of the International Embryo Transfer Society. Treatment of in vivo and in vitro fertilized embryos contaminated with BHV-1 (10(5) TCID50/mL) in the presence of RBTr (525 U/mL) for 120 s, effectively removed the infectious virus compared with untreated and washed embryos (P < 0.05). Transfer of in vivo fertilized and disinfected embryos to BHV-1 seronegative recipients (n = 24) resulted in 14 pregnancies and 11 calves born free of BHV-1. In contrast, transfer of

unwashed or undisinfected embryos to four recipients resulted in seroconversion and no pregnancies at term. It was concluded that the use of RBTr could be considered as an alternative method of rendering embryos free of BHV-1 and thus reduce the potential risk of disease transmission to embryo recipients and offspring.

There are 4 areas for trypsin, temperature, duration, source of trypsin and storage/use of batches. It was raised that research is only covering one pathogen so it is difficult to extrapolate these results to another. The Research Subcommittee is to look into the trypsin issue again.

a) Bluetongue virus 8 update

Disappeared from Europe, some further papers are work in progress.

b) Atypical scrapie

i) Is there a syndrome associated? Need we be concerned?
ii) Ovine derived FSH from atypical scrapie designated donors

Nothing further to discuss.

c) Recommendations re LN2 and vacuum tank sterilization

A letter from the OIE has addressed this, all covered by OIE Code.

d) Update on Schmallenberg virus

Comment that SBV has very short virameic phase.

MOTION
There is not enough data to available to make further recommendations for SBV so should follow OIE guidelines. Majority agreed.

e) Update on Chlamydia

Chlamydia in sheep and goats – proposed article sent to OIE has been accepted.

f) Sterilization of laparoscopy equipment (Scrapie)

George to lead an electronic working group on this issue, this will fall under Research Subcommittee.

g) Update on equine viral arteritis

Proposed article sent to OIE has been accepted.

7. New business

Discussed the issue of movement of semen under official control that would be used for fertilising oocytes as well as storage of semen/embryos and agents being transmitted between stored embryos/semen.
An electronic working group will be established to discuss this further.

Recommendation for USDA and DG SANCO (EU) to join electronic working group and attend future IETS Conferences.

a) Embryo storage – are separate tanks needed for different destinations?

Discussed that it is up to the importing country to set these requirements. In general embryos all stored together.

b) International movement of in vitro produced embryos, fresh and frozen

An Electronic Working Group has been established in the Research Subcommittee to look into this. George suggested that he do a risk assessment to look at the disease risks.

MOTION
The **recommendation that for the trade in vitro embryos the oocyte donors meet the requirements for importing live cattle into that country, the semen used should also meet the country's import requirements and that the processing of the embryos follows IETS requirements. Majority agreed.**

c) Report from the OIE and discussion (Michel Thibier)

Discussion that at the Research Committee they will recommend porcine circovirus be moved from category 3 from 4 and move Coxiella burnetii to category 4.

Discussion on Epizootic Hemorrhagic Disease OIE Code chapter due 24th February for the OIE. This will be circulated to the group to discuss electronically.

d) Report from the Rep Vet Committee of the European AI Vets.

Discussion on new vet law that will simplify EU legislation.

III. Forms and certificates subcommittee

Temporary chair for the meeting : Reuben Mapletoft.

No agenda provided prior to meeting.
Went through last year's meeting minutes.

1) Form A1 from the IETS Manual : how this can be used to accommodate embryos produced from multiple donors in the same culture system ?

According to the IETS Manual, embryos from multiple donors should not be washed together. However, it is becoming normal practice fertilize oocytes from multiple oocyte donors with the same sire and culture zygotes together. Parentage of offspring will be determined once calf is born by genetic testing.
Form A1 is mainly needed for the breed associations for tracking parentage.

Motion
To allow for the existing A1 form to be used and modified by practitioners to identify multiple female donors along with a note that the embryos from these donors have been cultured and washed together. Majority in favour.

Motion: To create a second form (e.g. A1a form) that will accommodate multiple female donors and include in IETS Manual. Majority in favour

2) Discussion on how to communicate this to practitioners, suggestion that the IETS newsletter can be used.

3) "update of the IETS Manual"

Motions
1)Present to the Board the urgent need to make the manual easily updatable and downloadable. All in favour.

2) information on embryos from porcine, equine and small ruminants is needed. These should be a priority for the updated manual. All in favour.

Francis FIENI is leading this work and Canadian practitioners are already undertaking this work

Discussion on the web access to the IETS manual.

Motion
To propose to the board that the IETS Manual be open access. Majority in favour

4) Discussions about the need for teleconferences through the year from Chairs from the different committees to keep momentum of issues arising from these meetings.

The subcommittee wish to consider the Chair for this subcommittee : Richard REMILLARD (TRANSOVA) volunteered, accepted.

5) "Problem with identification of straws when labelled by hand."

Motion
For all embryos for international trade to be machine labelled (not hand labelled) by 1 January 2015. Majority in favour.

Motion
In the revision of the IETS manual to reduce the amount of duplicate information on the straw label. [Motion did not pass]. Continue with normal practice.

6) **Representatives from South America, Asia and Africa. This will be a job for the new chair to get this representation to this committee and meeting.**

Comment that the IETS Forms are only used by North America and no other countries, a cause for concern. How do we get countries around the world to use IETS developed forms? This should be pursued at future meetings.

IV. Emerging technologies

1. Approval of the last minutes.

The motion has been reviewed by the group.

This subcommittee should serve as the nucleus of presently used, new and developing technologies
To identify and describe the new technologies, and raise questions related to the technologies, including the hazards and potential benefits
To identify the experts that can speak for these technologies and develop language for messaging
We need to provide substantive commentaries based on scientific data on requests of regulatory authorities developing regulations, …
The subcommittee does not wish to be seen as endorsing any particular commercial product
The subcommittee should provide factual information and issue consensus opinions on broad topics dealing with scientific process and integrity related to technologies reviewed by the subcommittee.

2) Discussions about new issues / organization of the group

New issue : the group is raising the question of meganucleases.
The objective is not to regulate this new technologies. However, one group (Carlson) is working on new tools to develop embryos using meganucleases. One application goal is to produce "polled embryos". It could be commercialized soon.

Should the “meganucleases” issued animal considered as “transgenic” animals ?
Some candidate genes already exist for such genetic engeneering techniques: horn, myostatin genes.
Some applications are also being developed in pigs.

One EWG could be started to produce an opinion about “meganucleases”.
This group is an group with experts in new technologies.
To discuss properly the emerging technologies issues, this subgroup would need a 3 hour meeting / half a day.