From the President

Dear IETS Members,

Time is flying by! It’s hard to believe we are already midway between two annual conferences.

Plans for the 2010 meeting in Cordoba, Argentina are forging ahead thanks to the hard work of the local organizing committee spear-headed by Gabriel Bo. Remember that this meeting is somewhat of a new venture for IETS as we embark on our first truly joint meeting with another society – the Brazilian Embryo Transfer Society (SBTE). Given the large numbers of embryos produced and transferred in South America each year the Program Chairs, Matt Wheeler and Gabriel Bo, have put together an exciting program for the main conference entitled ‘Back to basics: from the bench to the field’. There will be sessions dealing with aspects of the entire developmental axis from oocyte to offspring including (i) follicle and oocyte development, (ii) environmental effects on success in the field and the lab, (iii) embryo manipulation, (iv) pregnancy establishment and (v) live offspring delivery. The Practitioner’s Forum will deal with the issue of commercial in vitro embryo production and will be organized by our colleagues in SBTE. Given the range of invited speakers the program of the main conference promises to have something for the scientist and practitioner alike. In addition, a pre-conference symposium organized by Reuben Mapletoft and our partners Bioniche will deal with new developments in the practice of embryo transfer while Fulvio Gandolfi will organize a half day workshop on current progress in embryonic stem cell research in domestic animals.

This year there will not be any post-conference symposia. On Tuesday night, the last night of the conference, we will have our gala dinner and traditional party. While plans are still being finalized, currently this event is timetabled to run from 8pm to 8am! Apparently this is not unusual in South America…….

You should have received an e-mail message containing the first call for abstracts for the 2010 meeting within the last two weeks. All the information you need to submit an abstract is posted at http://www.iets.org/2010/. Please ensure to prepare your abstracts for the deadline of July 30th.

In addition, as an IETS member, you should have received an e-mail message within the last week about an exciting project that has been undertaken by the IETS Foundation, the educational arm of IETS. The new ‘Educational Support Grant Program’ is intended to develop educational materials to 1) stimulate development of educational materials to meet the educational mission of the IETS Foundation; and 2) encourage use of electronic technologies to deliver educational information regarding the development, application and research in embryo related technologies. If any of you have burning ideas that might be useful to educate the public and/or students and scientists on some aspect of embryo technology now is your chance to get a little funding to bring it to fruition. Full details are on the website at http://www.iets.org/edgrants.htm.

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*June 2009*
Letter of Invitation

To: IETS and SBTE Members
From: Matthew B. Wheeler and Gabriel Bo, 2010 Program Co-Chairs
Subject: Call for Abstracts

On behalf of the IETS, the SBTE and the Co-Chairs of the Local Organizing Committee, Lucas Cutaia and Humberto Tribulo, we would like to extend a warm invitation to all IETS and SBTE members to join us at the joint annual conference of the IETS and SBTE, scheduled for 9-12 January 2010 in Cordoba, Argentina.

The theme of the 2010 Annual Meeting program is:

“Back to the Basics, From the Bench to the Field”

The program topics include:

• The Donor Animal
• Control of Environmental Conditions in the Lab and Field
• Embryo Manipulation
• Production of Pregnancies
• Production of Live Offspring

There also will be a Practitioner’s Forum on Commercial IVF and the program will conclude with a keynote address by Dr. Nathan Price, titled “Early Embryo Development a Systems Biology Approach.”

The Local Organizing Committee is planning a variety of social events. That will allow you to have a good taste of the history, culture, dances and food of Argentina during the Sunday opening reception, Monday evening cocktail and Tuesday Evening closing banquet and dance. Details about the program, including the invited speakers and titles of their presentations, are currently available on the IETS website at:

http://www.iets.org/2010

In addition to the main program, there are plans to hold a Pre-Conference Symposia on Saturday (9 January 2010) “New Developments in the Practice of Embryo Transfer” organized by Dr. Reuben Mapletoft and a Pre-Conference Workshop on Saturday (9 January 2010) “Current progress in domestic animals embryonic stem cell research” organized by Dr. Fulvio Gandolfi and the DABE committee. All of these events also will be held at the Sheraton Cordoba Hotel in Cordoba, Argentina. Registration information will be posted on the website as soon as it is available.

We once again urge all members to utilize the electronic submission format in order to help keep costs to a minimum.

We sincerely hope that you will all be able to join us in Cordoba to participate in IETS 2010!
A decade of progress since the birth of Dolly

Ian Wilmut\textsuperscript{A,B}, Gareth Sullivan\textsuperscript{A} and Jane Taylor\textsuperscript{A}

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Abstract. The greatest effect of the birth Dolly, the first cloned animal derived from an adult, has been in prompting biologists to consider ways of reprogramming adult nuclei to a pluripotent state directly. The first procedure depends upon use of viral vectors to introduce selected transcription factors, but this procedure is slow and very inefficient. Research in our laboratory has demonstrated that exposure of differentiated nuclei to an extract of embryo stem cells induces expression of key pluripotency genes within 8 h, suggesting that it may be possible to identify and use other factors to enhance direct reprogramming. A study of mechanisms that bring about changes in DNA methylation in early sheep embryos identified a developmental isofrom of Dnmt1, the expression of which was limited to early stages of pregnancy. Reduction in the level of transcript of this isofrom at the time of fertilisation caused sheep embryo development to cease at the early morula stage, revealing a key role for the isofrom that remains to be characterised. The ability to obtain pluripotent cells from specific patients is providing important new opportunities to study inherited diseases when the causative mutation is not known. The initial objective of this research is not cell therapy, but to use cells with the characteristics of those in a patient who has inherited the disease to establish a high-throughput screen to identify drugs that are able to prevent progression of the symptoms of the disease. Research is in progress with cells from patients with amyotrophic lateral sclerosis.

Introduction

During the period since 1986, a significant amount of ground-breaking research has been performed to develop methods for nuclear transfer (NT) in livestock. This era was marked by the birth of the first offspring following NT from blastomeres (Willadsen 1986), while 20 years later offspring were obtained following NT using cells from adult animals (Wilmut et al. 1997). Those involved in the research had several objectives in mind. There was the appeal of being able to produce groups of genetically identical calves from selected animals either for research or commercial reasons. Second, there was the thought that it may be possible to genetically modify livestock precisely if offspring could be obtained from cells in which genetic change had been engineered. Certainly the primary objective of the group at Roslin Institute was in the modification of the genes of livestock. Finally, this research addresses fundamental questions as to whether nuclei from differentiated cells retain a complete copy of the genome or whether differentiation involves loss of specific gene sequences.

Looking back over that period and the 10 years since the birth of Dolly, it seems that the use of the technique in livestock production has been very limited because only small numbers of cloned animals are produced commercially. In contrast, considerable progress has been made in the genetic modification of animals for biomedical purposes. Pigs have been modified with a view to the transfer of their organs into human patients (Colman and Morser 1979; Lane et al. 1980; Colman et al. 1981; Colman 1996, 1998; McWhir et al. 1996; Kind and Colman 1999; Polejaeva et al. 2000; Dai et al. 2002; Lai et al. 2002). Although modified tissues survive in non-human primates for much longer than those from unmodified pigs, they are still not an effective source of organs for transplantation (Kuwaki et al. 2005). The most striking use of genetic modification has been to breed cattle that only produce human antibodies (Kuroiwa et al. 2002, 2004).

This technical tour de force is dependent upon being able to obtain live offspring following the transfer of nuclei from cells that had been cultured for the prolonged periods required for genetic modification. Previous experience showed that embryos produced with such cultured cells frequently died late in pregnancy or had developmental abnormalities (e.g. Denning et al. 2001). This limitation was overcome by recovering cells from cloned fetuses and carrying out a second NT, a step that also allowed the group to confirm that the transgene was present (Kuroiwa et al. 2002). The human immunoglobulin loci are a far larger DNA fragment than had previously been transferred. Successful transfer was achieved by incorporating the entire, unarranged heavy and light chain sequences into a human artificial chromosome (approximately 10 Mb). The authors confirmed that the human genes were expressed in an appropriate tissue-specific manner in the cloned calves (Kuroiwa et al. 2002), but the animals also produced bovine proteins from their own genes. In a second phase, the bovine genes were inactivated before two further rounds of NT (Kuroiwa et al. 2004). In this way, animals were produced that only secrete human antibodies. Antibodies raised in these animals in response to viral proteins or proteins isolated from tumours may offer new opportunities to address human diseases. Because the bovine immunoglobulin
genes have been inactivated and replaced with their humanised counterparts, the antibodies (humanised) produced should not promote an immune response in patients.

In addition to these biomedical applications, there was another far-reaching effect of the birth of Dolly in that it enabled biologists to think differently. The birth of Dolly and subsequent animals following the transfer of nuclei from adult cells has generated considerable interest in the possibility of being able to reverse the differentiation of such cells by direct treatment. Before Dolly’s birth, it was generally considered that the mechanisms that bring about differentiation are so complex and so rigidly fixed that it would not be possible to reverse them. Dolly’s birth established that at least in some cases this is not true. This has led to research to identify factors that have this ‘reprogramming’ ability. The first group to unlock these mechanisms was led by Shinya Yamanaka at the University of Kyoto (Takahashi and Yamanaka 2006). He demonstrated that by introducing four key transcription factors it is possible to establish a pattern of gene expression that is very similar to that of embryo stem cells. Yamanaka termed these cells ‘induced pluripotent stem cells’ (iPS cells). He and others have since demonstrated that similar approaches are effective with human cells (Takahashi et al. 2007; Park et al. 2008b).

The birth of Dolly was followed rapidly by the birth of the first clones from adults of a variety of species (for a review, see Wilmut et al. 2002). It was also shown that cloned offspring could be derived from many different cell types (Wilmut et al. 2002). However, several limitations soon became apparent. Some species have proved refractory to cloning and it has been difficult to increase the proportion of embryos that produce viable and healthy offspring. To date, no cloned non-human primate offspring have been produced from adult cells, despite a considerable effort by experienced teams. The derivation of an embryo stem cell line following transfer of nuclei to 300 oocytes provides the strongest indication of technical success for that group of animals. There are other cases, such as the rat, in which a small number of offspring have been obtained (Zhou et al. 2003), but a repeatable procedure has not yet been established.

An increase in the proportion of cloned embryos that become live offspring has been achieved by attention to detail, the optimisation of procedures for each species and an introduction of steps to assist at the time of birth (Wells et al. 2004). However, it remains true to say, as we did in 2002, that NT is a repeatable, but fundamentally inefficient, procedure (Wilmut et al. 2002). It seems likely that this reflects the inability of the oocyte cytoplasm to reprogramme gene expression appropriately to support normal development to term, as suggested by studies of gene expression (Yang et al. 2007b) and of chromatin organisation in early cloned embryos (Beaujean et al. 2004b; Kishigami et al. 2006). Recent results (Kishigami et al. 2006) lend encouragement to the thought that increased efficiency will be possible by intervention to assist in the process of reprogramming gene expression, perhaps by facilitating the activity of oocyte factors. It is disappointing that even now little is known of the active proteins. These were all identified by searches in amphibian oocytes (Kikyo et al. 2000; Gonda and Kikyo 2006; Tamada et al. 2006; Koziol et al. 2007).

Many aspects of somatic nuclear remodelling observed in nuclear cloning are recapitulated in somatic nuclei incubated in amphibian eggs or egg extract and an in vitro nuclear remodelling assay has been used for the purification of two nuclear remodelling activities: imitation switch (ISWI) and Xenopus Y-box protein (FRGY2) and FRGY2b, two proteins capable of nucleolar disassembly. The chromatin-remodelling nucleosomal ATPase ISWI was shown to have a critical role in the exchange of proteins between the transferred nuclei and the cytoplasm. (Kikyo et al. 2000). In addition, the egg protein nucleoplasmin (Npm) caused decondensation of chromatin in undifferentiated mouse cells (Tamada et al. 2006). This change was associated with modifications to histones, including phosphorylation of histone H3, acetylation of Lysine 14 in histone H3, and with loss of heterochromatin protein (HP)1 and transcriptional intermediary factor (TIF)1β from the nuclei. Confirmation of the role of Npm was provided by the demonstration that pretreatment of mouse nuclei enhanced activation of four oocyte-specific genes in the nuclei injected into Xenopus oocytes (Tamada et al. 2006).

A search for proteins in Xenopus oocytes that activate Oct4 expression in mammalian nuclei has identified two proteins: (1) the retinoic-acid-receptor γ, which is a known repressor of Oct4 transcription; and (2) ‘tumour translationally controlled protein’ (Koziol et al. 2007). Tumour protein translationally-controlled 1(Tpt1) is widely expressed and conserved and is associated with reversion of tumours. It is strongly expressed in tumour cells, but is downregulated upon tumour reversal. Reduction of Tpt1 transcripts in oocytes reduced Oct4 transcription in HeLa nuclei, whereas increases in Tpt1 level accelerated the onset of Oct4 (Koziol et al. 2007).

In our recent research, we have used two approaches to try to identify the factors that reprogramme gene expression in early development. In the first we have analysed the ability of oocyte and embryonic stem (ES) cell cytoplasm to reprogramme gene expression in somatic cells. In the second, an RNAi approach has been used to examine the role of a key gene in early sheep embryos. In other research, we have started to use the iPS cell methodologies in order to study inherited human diseases.

Factors in oocyte cytoplasm that reprogram gene expression

A considerable technical challenge facing those who wish to identify active factors in mammalian oocytes is the very small quantity of material that is available. A successful approach in identification of the key factors has been to examine the ability of extracts of Xenopus oocytes to modify gene expression of mammalian nuclei. Mammalian nuclei can be reprogrammed by amphibian oocytes (Gurdon 1976) and, because these are much larger and more readily available in large numbers, they offer an attractive alternative. The effects of extracts can be measured by transiently permeabilising the plasma membrane of cells with streptolysin-O (SLO) before immersing them in extracts of Xenopus oocytes, in the approach pioneered by Collas et al. (Hakelien and Collas 2002; Collas 2003). To our surprise, we were unable to detect any repeatable changes in gene expression of 293T cells that were permeabilised and then immersed in Xenopus extract (Bru et al. 2008). The only effect that was
observed was the removal of lamin A/C from the nuclear membranes, which has been observed independently by another group (Alberio et al. 2005). We then decided to examine the effects of extracts from ES cells. Cell fusion experiments have demonstrated that ES cells are able to reprogramme gene expression of somatic cells (Tada et al. 1997). Because they are available in large numbers, cytoplasm required for these studies is readily available.

Collas et al. demonstrated that stably reprogrammed colonies, expressing a range of pluripotency genes, could be established if differentiated SLO-permeabilised somatic cells were incubated in ES cell extracts and then returned to cell culture for several weeks (Taranger et al. 2005). This approach demonstrates the dramatic ability to reprogramme gene expression, but has limitations as the basis of an assay for critical factors. In particular, long incubations in culture make it time-consuming to identify factors involved in the reprogramming of gene expression. In our search for a suitable assay, we have examined changes that occur directly after exposure of SLO-permeabilised somatic cells to ES cell extract. Full details of the procedures have been described elsewhere (Bru et al. 2008).

Permeabilised 293T cells, which are human in origin, were incubated in extracts of mouse ES cells and gene expression monitored using species-specific primers. Transcripts of Oct4, Klf4, Nanog and c-Myc mRNA were detected following 8 h incubation in extract, but were not apparent in untreated cells. Modest upregulation of Sox2 was also detected. All polymerase chain reaction (PCR) products were verified by restriction analysis to confirm that they were derived from a human transcript. Incubation of permeabilised 293T cells in somatic 3T3 cell extract caused no significant change in the expression of the pluripotency genes. In contrast with other reports, there was no significant change in gene expression if the somatic cells had not been permeabilised before incubation in ES cell extract (Hansis et al. 2004; Bru et al. 2008).

Because binding of RNA polymerase II to a promoter is an essential step in the commitment to initiate transcription, chromatin immunoprecipitation (ChIP) was used to analyse the loading of RNA polymerase II onto promoters of pluripotency genes. This revealed a modest increase in the binding of RNA polymerase II at the Nanog and Oct4 promoters. Increased loading of RNA polymerase was also detected on the promoter of the Cripto gene, which is specifically expressed in undifferentiated cells. Induction of pluripotency genes was blocked by the addition of an inhibitor of RNA polymerase II to the ES cell extract, namely α-amanitin. Furthermore, addition of cycloheximide, an inhibitor of protein synthesis, to the ES cell extracts reduced the efficiency by which gene expression was reprogrammed. A possible explanation for this observation is that translation of key transcriptional regulators is necessary for efficient activation of pluripotency genes (Bru et al. 2008).

Post-translational modification of histone H3 was monitored with ChIP using antibodies against acetylated and methylated lysines to perform ChIP in 293T cells before and after exposure to ES extracts. In order to provide control information, pluripotent (NCCIT) and differentiated (293T) cells were examined. Treatment of 293T cells with 46C mouse ES cell extract reduced the methylation of H3K9 and H3K27 on the Oct4 promoter and reduced methylation of H3K9 on the Nanog promoter, which is consistent with them becoming de-repressed. A slight reduction in the methylation of H3K9 and H3K27 was observed on the Cripto promoter. However, there was little evidence of the activating chromatin modifications, trimethylated H3K4 and acetylated H3K9, on these promoters; although some transient changes were detected, they were not maintained (Bru et al. 2008). These results suggest that treatment of somatic nuclei with ES cell extract lowers the level of certain repressive chromatin modifications, but does not strongly induce activating modifications. This is consistent with a partial chromatin remodelling that may be sufficient to allow the regulatory regions of some pluripotency genes to recruit RNA polymerase II.

To determine whether exposure to ES cell extract elicits a long-term upregulation of pluripotency genes, 293T cells were incubated in 46C ES cell extract for 6 h, resealed and returned to cell culture for 48 h. There was a continued upregulation of Nanog, Oct4, Sox2, c-Myc and Klf4 mRNA over the entire 48 h in culture. The continued and increasing expression of pluripotency genes suggests that exposure of 293T nuclei to ES cell cytoplasm can induce a long-term induction of gene expression towards a more pluripotent state (Bru et al. 2008).

This study provides the first insight into the earliest molecular changes that take place during reprogramming of gene expression in mammalian somatic cells exposed to ES cell extracts (Bru et al. 2008). This may open up a range of biochemical approaches to investigate mechanisms involved in this process; for example, fractionating ES cell extracts to identify the factors involved. There is an informative contrast in the speed of change in two different systems in which reprogramming occurs. Reprogramming brought about by the introduction of specific transcription factors took approximately 10 days for activation of the endogenous pluripotency genes (Stadtfeld et al. 2008). In contrast, during cell fusion (Han et al. 2008) and in the present studies (Bru et al. 2008), changes in expression of these genes were detected in a few hours. In the latter circumstances, the nucleus is being exposed to many more factors and they are able to promote more rapid change. It may be possible to enhance the speed and efficiency of direct reprogramming of cells by identification and the use of more factors than are used at present.

Developmental-specific isoform of sheep Dmnt1

In an investigation of molecular mechanisms that regulate early sheep embryo development and may have an important effect during NT, a novel ovine Dmnt1 exon, theoretically encoding 13 amino acids, was found to be expressed in sheep oocytes, preimplantation embryos and early fetal lineages, but not in the adult tissue (Taylor et al. 2008). Knockdown of this isoform in oocytes and early embryos by an RNA interference (RNAi)-mediated approach resulted in embryonic developmental arrest at the late morula stage, suggesting an essential role for this isoform in sheep early development.

The cytosine-specific DNA methyltransferase (EC 2.1.1.37; Dmnt1) is an essential participant of an epigenetic silencing pathway that maintains the transcriptional profile of different cell types and is essential for normal development in the mouse (Li et al. 1992). However, species differences in the changes
in DNA methylation in early embryos have become apparent in recent years. By the eight-cell stage, mouse embryos are severely hypomethylated. This reflects passive loss of methylation from the oocyte-derived chromosomes, but a rapid, active demethylation of the hypermethylated spermatozoon-derived pronucleus. In contrast, sheep embryonic nuclei lose less than 50% of their methylation before the eight-cell stage and maintain a comparatively high level of methylation at least until the expanded blastocyst stage (Beaujean et al. 2004a). A pattern that is similar to that in sheep has been described in other species, including pig, human, rabbit and rhesus monkey (Fulka et al. 2004, 2006; Shi et al. 2004; Yang et al. 2007a). These observations question the validity of the hypothesis that genome-wide demethylation is necessary to reprogramme mammalian pronuclei (Santos et al. 2002).

In an investigation of these mechanisms, a sheep Dnmt1 clone was identified from cDNA of sheep lung tissue (Taylor et al. 2008). Sequencing of the sheep PCR products from oocyte tissue revealed a 330-bp fragment that corresponded to the sheep Dnmt1 sequence obtained from adult lung. In addition, a 369-bp product contained a 39-bp insertion between nucleotides 709 and 1036 (i.e. exons 12 and 13, respectively), which remained in frame and encoded 13 amino acids. Oocytes and cleavage stage embryos were found to express high levels of the transcript of this novel isoform, designated Dnmt1(12b), whereas morulae and blastocysts did so to a lesser extent. It also revealed the presence of both Dnmt1 transcripts in Day 14 blastocyst, Day 21 fetuses and placenta. In contrast, Day 60 and Day 125 fetal heart and kidney predominantly expressed the somatic Dnmt1 transcript, whereas adult tissues expressed only the somatic form.

In order to investigate the requirement for the novel isoform in early development, oocytes were injected with short interference RNA (siRNA) to Dnmt1(12b). A reduction in Dnmt1(12b) mRNA levels was observed compared with oocytes injected with buffer alone or non-injected oocytes, whereas expression of somatic Dnmt1 remained undisturbed. Treated oocytes were then fertilised in vitro and cultured to the blastocyst stage. Control oocytes were injected with buffer only. There were no significant differences between any of the treatment groups with respect to cleavage or development to early morula stage. Approximately 50% of injected oocytes cleaved in both siRNA- and buffer-injected treatments compared with non-injected control oocytes, which showed a cleavage rate of 50–60%. However, none of the oocytes injected with siRNA progressed beyond the precompaction morula stage to form blastocysts, whereas those from the buffer-injected and non-injected groups developed to fully expanded and hatching blastocysts (Taylor et al. 2008).

That study provided important evidence for diverse species differences in early epigenetic reprogramming events. In addition, it revealed an unexpectedly early requirement for the gene. Further analyses are required to fully understand the role(s) of the sheep developmental isoform of Dnmt1 and to extend the observations to other species, in particular other ungulates.

**New opportunities to study inherited human diseases**

In our haste to use cells for therapy we often overlook the enormous potential benefit to be gained by their use in studies of inherited diseases and in toxicology. In particular, the ability to obtain pluripotent cells from specific patients is providing important new opportunities to study inherited diseases when the causative mutation is not known. The initial objective of this research is not cell therapy, but to use cells with the characteristics of those in a patient who has inherited the disease to establish a high-throughput screen to identify drugs that are able to prevent the development of symptoms of the disease.

In principle, derivation of ES cells from cloned human embryos would provide this opportunity, but this has not yet been achieved. There are many reports of the derivation of ES cells from cloned mouse embryos. It is striking that the proportion of embryos from which stem cell lines can be obtained is much higher than the proportion that would have become offspring had they been transferred to recipients (Wakayama et al. 2005) and that gene expression of these cell lines is very similar to that in cell lines derived from embryos produced by fertilisation in vivo (Wakayama et al. 2006). Until it is possible to mature human oocytes from the primordial follicles that are present in very large numbers or even from ES cells, there will always be the practical difficulty of obtaining a sufficient number of oocytes to obtain ES cell lines by NT.

Many laboratories are now using direct reprogramming through the use of viral vectors as an alternative means of producing disease-specific cell lines, as was first demonstrated by Yamanaka and colleagues (Takahashi and Yamanaka 2006). Research is in progress with cells from amyotrophic lateral sclerosis (ALS) patients, but many other inherited diseases may be studied in this way, including cardiomyopathy and schizophrenia (Park et al. 2008a). In many cases, the molecular basis of the disease is not yet known, although new understanding is being provided by detailed genetic analysis and access to the human genome sequence.

Many factors will determine the effectiveness of this approach. The greatest potential benefit will arise if the mutation is not known, because if it is known disease models can be created by modification of the candidate gene in experimental animals or in cells derived from an existing ES cells line. However, if the mutation is not known it will not be possible to be absolutely certain that the disease has been inherited rather than being the result of environmental effects. Use of cells as disease models also depends upon being able to derive cells equivalent to those that are affected by the condition and maintain them in culture. In some cases, aspects of the disease depend upon interactions between two different types of cell and it would be necessary to culture these together in order to mimic those circumstances. Perhaps the most important factor will be the time in culture that is required to reveal an aberrant phenotype. In many cases of ALS, which is actually a family of diseases (Vivekananda et al. 2008), the first clinical symptoms are not seen until the person is more than 50 years old. It remains to be seen whether differences that are associated with the disease can be detected after culture for a few days or weeks.

**Conclusions**

As is often the case, the benefits arising from new discoveries take a long time to become apparent and may not be


HASAC Report

A note from the HASAC Regulatory Subcommittee of IETS

Dr L Delver, Chairman of the HASAC Regulatory Subcommittee

This year was an exception as there was no meeting of the Regulatory subcommittee in 2009 as such due to the proximity of the meeting to the holiday season. Normally the Regulatory subcommittee meets the day following the one day meeting of the Research subcommittee because so much of the deliberations depend upon information provided by the research group but this year, they were unable to meet as a committee prior to the conference sessions.

However, a few issues of some urgency were discussed at the open meeting of the Health and Safety Advisory Committee and the decisions taken are noted below.

Several relevant Chapters of the Terrestrial Animal Health Code of the World Animal Health Organization (OIE) have been rewritten by staff at OIE so those were reviewed and several changes proposed. Those were discussed by an ad hoc group and recommendations were made which were endorsed by the Board of Governors and submitted to the OIE for consideration.

1. In vivo collected embryos (reference to the OIE Code: chapter 4.7)

The titles of the relevant chapters of the OIE Code were not thought quite appropriate because of the increasing commercialization of ET in species other than the bovine. Yet, all mention ‘bovine’ in chapter headings, so it was proposed to have them replaced by ‘production livestock and horses’.

The concern was raised that in some chapters wording stating freedom from specific diseases such as foot and mouth disease and rinderpest was not appropriate. It was hence recommended that in sentences referring to diseases not included in Category 1 the phrase ‘and which are of concern to the importing country, is added.

The cleanliness of all equipments having contact with embryos has always been a matter of concern and particularly those that may be hard to wash thoroughly. It was then recommended that used equipment such as laparoscopes which may not be effectively disinfected, not be transferred between countries for re-use by embryo collection teams.

Because storage of flushing and washing samples which are rarely requested to be tested may be felt as useless by many practitioners, it was recommended that they be collected and stored only if specific tests are requested by the importing country.

In the current wording of the relevant article of the OIE Code, it is supposed that collection and processing facilities are at the same location. Yet, this is frequently not the situation when collections are done on farm and processing is done in the fixed laboratory. Therefore it was recommended that it should be stated that they need not be co-located.

Regarding the micromanipulated embryos, it was proposed the following: “Micromanipulation should only be done after completion of disinfection by washing of the embryo/oocytes”.

2. In vitro produced embryos (reference to the OIE Code: chapter 4.8)

A recommendation was made that only the collection of ovaries/oocytes should not be done in a zone in which animals infected with a disease of concern but processing is done in a biosecure laboratory and this may be safely accomplished in an infected zone.

1Categorization of diseases and pathogenic agents referring to in vivo collected embryos.
Minor changes of the current wording of the OIE Code were proposed in order to accommodate abattoir collection of oocytes/ovaries as differentiated from OPU and a risk management series was inserted. The issue regarding abattoir derived tissue that was raised two years ago following European Union challenged interpretation was finally resolved by the efforts of the Research and Regulatory subcommittees this year with the insertion of the sentence ‘Ovaries which are collected at an abattoir should be collected, transported and processed according to the recommendations laid down in Chapter 4.8., article 4. This seems to arise any time oocytes are collected at abattoirs to provide tissues for cloning or for oocyte maturation and in-vitro fertilization to produce embryos for export.

The efficacy of washing oocytes clean of cumulus cells (CFO’s) was discussed. The committee called attention to Abstract #149 by I. Plejaeva et al of Viagen at the conference and previous work reported by Hematech for cattle. The group strongly encouraged researchers to generate more data on the risk of transferring virus through the oocytes. For example, there is a need for more research with transferring oocytes from persistently infected animals.

The utilization of non veterinarians in embryo production was discussed and the current position of the IETS/HASAC is that the team veterinarian is responsible for supervision and training of all personnel involved in the processing of embryos that will be presented for international shipment. The team veterinarian must be approved by the veterinary authority where the laboratory is located.

A recommendation was made that disposal or disinfection of equipment must be done between each batch of oocytes/embryos rather than between each embryo/oocyte.

The word quarantine should be deleted when referring to stored embryos. They are stored under official supervision which is the equivalent of quarantine and does not require a formal release from quarantine to move them.

3. The chapters of Specific diseases

It was reported that the new literature review on EIA (Equine Infectious Anemia) is complete with some information from the broader lentivirus group and suggestions will be provided at the 2010 meeting on wording respecting this disease.

Articles on lumpy skin disease will be reviewed and presented for discussion at the 2010 IETS meeting because the information was not available for this meeting.

Finally and more broadly, sexed semen was discussed with respect to the possibility of pathogen transmission and it was suggested that sexing equipment (ie. Cell sorters) should be cleaned and disinfected between batches from different donors and that this must be part of the laboratory routine.

In conclusion, quite a bit of work has eventually been done at this last IETS conference in San Diego from HASAC regarding the regulatory aspects of moving embryos between farms, regions or countries. Those recommendations here summarized have been well accepted by the Terrestrial Animal Health Code Commission of the World Animal Health Organization (OIE) and is currently in the process of being finally adopted by the net OIE General Assembly.

Respectfully submitted,

J. Larry Delver, chairman
36th Annual Conference of the IETS/
23rd Annual Meeting SBTE
Sheraton Córdoba Hotel
January 9-13, 2010, Córdoba, Argentina
Preparation of Abstracts for Poster Presentations

2010 Annual Conference of the International Embryo Transfer Society (IETS) and the Sociedad Brasileira De Tecnologia De Embrioes (SBTE)
January 10-12, 2010
Cordoba, Argentina

General Information: Free communication will be presented as posters, abstracts of which will be published in the January 2010 issue of Reproduction, Fertility and Development. The first author or a representative is required to present an expanded report of the data in poster format at the Annual Conference. Students may enter their abstract in a competition sponsored by the IETS Foundation and also are eligible to compete for travel awards. Details on eligibility, judging procedures, and prizes are available on the IETS website.

Abstract Requirements: Abstracts must be in English and prepared strictly according to the instructions for submission. The language should be concise and avoid jargon. Abbreviations must be defined and should be used sparingly. Abstracts should consist of unpublished, original data that contains objectives, experimental methods (including statistical methods), results, and conclusions. The Program Chairman and two other referees will subject each abstract to rigorous scientific review. Rejection by the Program Chairman is final. If withdrawal of an abstract becomes necessary, notify the Program Chairman by September 15, 2009.

Submission Restrictions: Due to space restrictions, submissions are limited. An individual may not be first author of more than one abstract. Space limitations allow a maximum of 3250 keystrokes (including characters, spaces, and punctuation). Begin count at the title and end count with the last word. Abstracts that are too long will be rejected automatically by the system. Coding for special characters is not included in the total keystroke count. If you must use boldface, italic, or sub-/superscript type anywhere in the abstract, consult the Text Attributes Coding information below. This special coding is not counted in the 3250 allowed keystrokes. Special characters such as Greek letters and math symbols are available on the electronic submission form itself.

If your abstract includes a table, the keystroke limit will be 2750 for the abstract and 500 for the table. The electronic submission form requires using the Table Wizard for preparation of tables. The printed abstract width allows for 70 keystrokes per line, including spaces; keep this in mind when formatting the table. Tables that are too wide are not printable. You must also include coding for text attributes in the table. This includes the italicization, superscripting, and subscripting of numbers and letters in the table and the footnotes.

Submission Deadlines: Abstracts must be submitted via the web site only. No fax or email copies will be accepted. The firm deadline for receipt of the abstract via the web site is July 30, 2009 by 11:59 PM Central Daylight Saving Time. Abstracts received after this date will be rejected.

Author Agreements: By submitting an abstract, the author verifies (1) that the information in the submitted manuscript has never been published and is the work of the named authors, who all agree to be listed as co-authors, and (2) that the first author or a representative will present an expanded report of the data in poster form at the annual conference. Failure to present a poster after your abstract has been published in the conference proceedings can result in rejection of abstracts submitted by your laboratory in future years. Authors need to be aware of patent considerations and copyright considerations before submitting an abstract for publication.

All questions and correspondence should be directed to:

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June 2009
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36th Annual Conference

IETS ANNUAL CONFERENCE 2010
January 9-12, 2010
Sheraton Hotel, Cordoba, Argentina

CO-PROGRAM CHAIRS: Matthew B. Wheeler and Gabriel Bo
THEME: Back to the Basics, from the bench to the field

Thursday, January 7, 2010
9:00 - 18:00  IETS Board of Governors Meeting

Friday, January 8, 2010
9:00 - 18:00  IETS Board of Governors Meeting
9:00 - 18:00  Health and Safety Advisory Committee (HASAC) - Research Subcommittee
16:00 - 19:00  Registration (pick up of pre-registrations only)

Saturday, January 9, 2010
8:00 - 18:00  Registration
8:00 - 18:00  Health and Safety Advisory Committee (HASAC) - Food Safety Subcommittee
8:00 - 18:00  Pre-Conference Symposium: New Developments in the Practice of Embryo Transfer.
13:00 - 18:00  Poster Setup
13:00 - 17:00  IETS Foundation Board of Trustees Meeting
13:00 - 18:00  Commercial Exhibit Setup
14:00 - 17:00  Health and Safety Advisory Committee (HASAC) - Regulatory Subcommittee
14:30-18:00  DABE Pre-Conference Workshop - “Current progress in domestic animals embryonic stem cell research”
17:00 - 19:00  Health and Safety Advisory Committee (HASAC) - Forms & Certificates Subcommittee

Sunday, January 10, 2010
7:30 - 8:30  Past President’s Breakfast
7:30 - 8:30  Student Competition Breakfast with Foundation Education Committee
7:00 - 18:00  Registration
8:00 - 17:00  Commercial Exhibition
8:00 - 9:30  IETS Foundation Education Committee
8:00 - 17:00  A/V Library/Speaker Preparation

Main Program

8:45 - 9:00   Opening and Welcome (M. Wheeler & G. Bo)

9:00 - 10:30  Session I – The Donor Animal

9:00 – 9:45    Recent Progress in Folliculogenesis
B.D. Murphy, University of Montreal

9:45 - 10.30   Oocyte Competence
Trudy Fair, University of Dublin

10:30 – 11:00 Refreshment Break/Exhibition

11:00 – 12:00 IETS Foundation Student Competition Presentations

12:00 – 13:30 Lunch Break

12:00 - 13:30 IETS Board Luncheon with Affiliate Society Representatives

13:30 - 15:00 Session II – Control of Environmental Conditions in the Lab and Field

13:30 – 14:15 Is the zona pellucida an efficient barrier for viral infection?
Ann van Soom, Belgium

14:15 – 15:00 Embryo/oocyte culture in microenvironments
Rebecca Krisher, University of Illinois

15:00 – 15:30 Short presentations from submitted abstracts

15:30 - 16:00 Refreshment Break/Exhibition

16:00 – 17:30 Session III – Embryo Manipulation

16:00 -16:45  Embryo Manipulation to Enhance Fertility
Henrik Callesen, Denmark

16:45 – 17:30 Applications of RNA interference based gene silencing in animal agriculture
Charles Long, Texas A& M

17:30 – 18:00 Short presentations from submitted abstracts

18:00 – 19:00 Health and Safety Advisory Committee (HASAC) Open Meeting

20:00 Reception

June 2009  
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Monday, January 11, 2010

7:00-8:00 Exhibitors Breakfast with Board of Governors
7:30 - 16:00 Registration
8:00 - 18:00 Commercial Exhibits
8:00 - 17:00 A/V Library/Speaker Preparation
9:00 - 10:30 Poster Session I/Refreshment Break/Exhibition

10:30 – 12:00 *Session IV – Production of pregnancies*

10:30 – 11:15 Enhancing Pregnancy Rates by embryo selection and manipulation
Peter Hansen, University of Florida

11:15 – 12:00 Recipient Synchronization and Management
Pietro Baruselli, Univ. Sao Paulo

12:00 -12:30 Short presentations from submitted abstracts
12:30 - 14:00 IETS Data Retrieval Committee Meeting
12:30 - 14:00 Lunch Break

14:00 - 15:30 *Session V – Production of live offspring*

14:00 – 14:45 Pregnancy Maintenance and Large Offspring Syndrome
Charlotte Farin, North Carolina State University

14:45 – 15:00 Delivery of offspring
Flavio Meirelles USP-Pirasununga

15:30 -16:00 Short presentations from submitted abstracts

16:00 - 16:30 Refreshment Break/Exhibition
16:30 - 17:00 IETS Pioneer Award Presentation
17:00 - 18:00 IETS Annual Business Meeting
18:00 - 19:00 Companion Animal, Non-Domestic & Endangered Species (Candes) Open Meeting
18:00 - 19:00 Domestic Animal Biomedical Embryology Committee (DABE) Open Meeting

19:00 - 21:00 Wine and Cheese Reception – Cash bar with Exhibitors
Tuesday, January 12, 2010

7:00 - 8:30   Organizational Meeting of the IETS Board of Governors.
8:00 - 15:00  Registration
8:00 - 13:30  Commercial Exhibits
8:00 - 17:00  A/V Library/ Speaker Preparation
9:00 – 10:30  Poster Session II/Refreshment Break/Exhibition

10:30 -12:30 Practitioners’ Forum – Commercial IVP – SBTE and Fernando Garcia

12:00 - 13:30 Lunch break
12:00 – 13:30 Organizational Lunch Meeting of the IETS Foundation
13:30 - 17:00 Commercial Exhibit & Poster Teardown
13:30 - 14:00 IETS Distinguished Service Award Presentation
14:00 - 14:45 IETS Foundation Student Competition Awards, CANDES & HASAC Updates

14:45 - 15:30 Session VI - Keynote Address

15:00 - 15:30 Early Embryo Development a Systems Biology Approach
              Nathan Price University of Illinois
15:45 – 16:00 Closing Ceremony
16:15 - 17:15 IETS Annual Running Competition
20:00 - ?   Closing Party

Wednesday, January 13, 2010

8:30 – 17:00 Workshop for Authors: Publishing Scientific Papers in English (day 1)

Thursday, January 14, 2010

8:30 – 17:00 Workshop for Authors: Publishing Scientific Papers in English (day 2)
Bioniche Animal Health is again generously sponsoring a symposium on new developments in the practice of embryo transfer for practitioners and clinical scientists, immediately before the Annual Meeting of the IETS in Cordoba, Argentina, January 9, 2010. Eight leading researchers and practitioners from Argentina, Brazil, Australia and the USA have been invited to present new and previously unpublished information. Topics will include new approaches to superovulation in the cattle and small ruminants, gonadotropins and receptor populations, embryo transfer in high-producing dairy cattle, fixed-time AI and embryo transfer, the application of research findings in embryo transfer practice, the development of new and improved media and embryo diagnostics utilizing DNA technology. The day will conclude with an open discussion in which everyone can participate. You won’t want to miss this one. Detailed information on the program will be published in the next newsletter.

Scientific Program

Gabriel Bo – New approaches to superovulation in the cow

Alejo Menchaca – New approaches to superovulation and embryo transfer in small ruminants

John Hasler – The development of synthetic media and vitrification media for bovine embryos

Mark Bryan – Experiences with fixed-time AI in seasonal breeding dairy cattle

Ciro Barros – The use of knowledge on changing receptor populations in the superstimulation of cattle

George Seidel - Embryo diagnostics: the use of DNA technology in genetic selection

Charles Looney - The application of new research information in the practice of bovine embryo transfer in the USA

Roberto Sartori– Embryo transfer in high-producing Holstein cattle in Brazil

Reuben Mapletoft – Moderator of general discussion at the end of the program
Workshop for Authors
Publishing Scientific Papers in English

January 13-14, 2010

John P. Kastelic, DVM, PhD, Co-Editor-in-Chief, Theriogenology

Many manuscripts are delayed or rejected due to poor experimental design, analysis and presentation of data, and writing. This workshop covers how to plan and conduct research, analyze and present data, write a paper, and interact with editors and reviewers. In addition to presentations of principles and common errors, there will be exercises and interactive discussions. Course notes and certificates of attendance will issued.

This workshop is primarily designed for those for whom English is a second language. Therefore, English syntax, grammar and punctuation will be reviewed. However, this workshop will also be valuable for those for whom English is their native language, especially students and young scientists.

The workshop will be held from 08:30 to 17:00 on Wednesday and Thursday, January 13 and 14, at the Universidad Católica de Córdoba, Obispo Trejo 323, Cordoba. This university is downtown and is walking distance (approximately 12 blocks) from the main conference hotel for the 2010 IETS meeting.

Class size is limited, so please register early to ensure your place. Registration fees are payable to IETS via the registration form (www.iets.org). The reduced early registration fee (US$150 for IETS members listed in current membership directory; US$200 for nonmembers) must be received before 1 December 2009. The On-Site registration fee is US$200 for IETS members and US$250 for non-members (if space is available). Student registration (undergraduate or graduate students and post-doctoral fellows) is US$125, pre-paid or US$175 at the door. The registration fee does not include meals.

This workshop will be conducted by Dr. Kastelic. He has more than 20 years of experience conducting research and publishing papers, and is author or co-author of >100 peer-reviewed articles and >200 abstracts, proceedings and other articles. In addition, he has considerable experience as a reviewer and editor, including serving as Co-Editor-in-Chief of Theriogenology since 2003. He has done presentations and workshops on science and science writing in nine countries on four continents. It has been his privilege to visit South America on several occasions, primarily to conduct collaborative research and to deliver lectures and workshops on science and science writing.

For information about the workshop, contact Dr. Kastelic: therio@shaw.ca
403-317-2236 (voice) 403-382-3156 (fax)

Registration and fees: www.iets.org iets@assochq.org or 217-398-4697
Classifieds

Canadian Embryo Transfer Association (CETA/ACTE) &
American Embryo Transfer Association (AETA)

2009 Joint Scientific Convention
September 17–19, 2009
Hilton Montréal Bonaventure
Montréal, Québec, Canada

For information, please visit: