The Main IETS Program 2003 (New Zealand on January 12-14)

The main theme of the sessions for the main program 2003 is

"The contribution of animal biotechnology to medical and life sciences."

Sunday Program (January 12)

7:30-10:00 Poster Set-Up

7:30-8:30 Past President's Breakfast

8:00-10:00 Registration

10:00-10:30 Welcome and Introduction

Session I: Fundamental Aspects
(Chairs Keith Campbell and Poul Hyttel)

10:30-11:00 Gene expression and chromatin structure in preimplantation embryo. Jiri Kanka (Institute of Animal Physiology and Genetics: Academy of Science of the Czech Republic)
The preimplantation period of mammalian development includes the formation of the zygote, the activation of the embryonic genome and the beginning of cellular differentiation. During this period protamines are replaced by histones, the methylated haploid parental genomes undergoes demethylation after formation of the diploid zygote, and maternal control of development is replaced by zygotic control. Superimposed on this activation of the embryonic genome is the formation of a chromatin-mediated transcriptionally repressive state requiring enhancers for efficient gene expression. The development of transcriptionally repressive state is likely at the level chromatin structure since inducing histone hyperacetylation relieves the requirements for enhancers. Characterization of zygotic mRNA expression patterns during preimplantation period, and their relation to successful development in vitro and in vivo, will be essential for defining optimized culture conditions and nuclear transfer protocols. The focus of this review is to summarize recent advances in this field and to discuss their implications for developmental biology.

11:00-11:30 Epigenetic reprogramming and imprinting in mammalian development. Wolf Reik, co-authors: Wendy Dean, Fatima Santos. (Laboratory of Developmental Genetics and Imprinting, Developmental Genetics Programme, The Babraham Institute, Cambridge CB2 4AT)
Epigenetic modifications such as DNA methylation and chromatin modifications are crucial for mammalian development. One such epigenetic system is genomic imprinting, by which some genes in the mammalian genome are marked to be active or inactive in somatic tissues of the offspring. Epigenetic modifications are generally relatively stable in somatic tissues, but there are two phases in mammalian development in which there is genome wide reprogramming of DNA methylation. This occurs by active demethylation (replication independent), passive demethylation (replication dependent), and de novo methylation some time later. One reprogramming cycle occurs in primordial germ cells and its consequence is the erasure and later reestablishment of sex specific parental imprints. The other cycle takes place in preimplantation embryos, but importantly does not affect imprinted methylation patterns. Reprogramming in the early embryo may be important for removing gametic epigenetic information in order to allow embryonic gene expression and restore totipotency. In this respect, we have found that most cloned mammalian preimplantation embryos show a failure to reprogramme properly and completely the methylation patterns that are characteristic of somatic nuclei used as donors for cloning. This suggests that failure to reprogramme limits severely the potential of cloned embryos for normal development. We are now investigating possible interactions between the DNA methylation system and other epigenetic marking systems such as histone modifications, both in normal development and in cloned embryos.

11:30-12:00 Nuclear Reprogramming of Cloned Embryos Produced In Vitro. Young-Mahn Han (Korean Research Institute of Bioscience and Biotechnology, Korea)
Despite animal clones derived from somatic cells have been successfully produced in several mammalian species, developmental potential of the reconstructed embryos after nuclear transfer is still low. To develop the cloned embryos to normal animals, differentiated somatic nuclei should be reprogrammed during early embryogenesis. We analyzed methylation patterns in cloned bovine embryos to monitor the epigenetic reprogramming process of donor genomic DNA. It has been known that epigenetic modification such as DNA methylation relates to nuclear reprogramming. In mammals, DNA methylation is an essential process in the regulation of transcription during embryonic development and associated with gene silencing. A genome-wide demethylation may be a prerequisite for the formation of pluripotent stem cells that are important for the later development. In this study, we have hypothesized that the anomalies in cloned

animals may be due to incomplete epigenetic reprogramming of donor genomic DNA. Our findings demonstrated that aberrant methylation patterns were observed in various genomic regions of cloned bovine embryos except single-copy gene sequences. The overall genomic methylation status of cloned embryos was quite different from that of normal embryos produced in vitro or in vivo, but it closely resembled that of donor cells. There were also significant variations in the degree of methylation among individual cloned embryos. These results suggest that the developmental failures of cloned embryos may be due to incomplete epigenetic reprogramming of donor genomic DNA. To figure out the developmental defects of cloned embryos, reprogramming of donor genome should be looked at more extensively.

12:00-13:00 Lunch

Session II: Assisted Reproductive Technologies I
(Chairs Chang Kyu Lee and Xianghong (Jerry) Yang)

13:00-13:30 Cloning Advances. D.N. Wells (Reproductive Technologies Group, AgResearch, Ruakura Research Centre, PB 3123, Hamilton, New Zealand)

Cloning mammals by somatic cell nuclear transfer is a technically demanding multi-step process with potential perturbations in development occurring at any or all steps. In current practice, less than 6% of reconstructed embryos typically result in viable offspring. More troubling are the high rates of attrition throughout pregnancy, parturition and the post-natal period with cloned animals that contribute to the present low efficiencies. It is also important to evaluate the long term health and productivity of cloned livestock and their subsequent progeny. However, the fact that nuclear transfer is successful in producing some physiologically normal animals is in itself remarkable and provides encouragement for eventually elucidating the molecular mechanisms for effecting complete epigenetic reprogramming. A more fundamental scientific approach aimed towards understanding and gaining control over the reprogramming of donor nuclei will be necessary for developing a robust and safe procedure. This in turn will improve the utility and acceptability of cloning technology.

Because of the present inefficiencies, any commercial opportunities in cloning livestock are limited to high value breeding animals. Of interest in this niche area is the generation of cloned sires from elite males to more widely disseminate their top genetics following either increased semen production for artificial insemination or natural mating. Validation of this cloning application comes from recent reports that even where aberrant phenotypes were observed in clones these were not transmitted to their offspring following sexual reproduction; even from matings between two cloned parents. Cloning is being combined with genetic modification of donor cells to produce transgenic livestock and data will be presented on the production of transgenic heifers producing milk with enhanced protein composition. Cloning livestock on a larger scale will only become feasible and tolerated with substantially improved efficiencies that are ideally comparable to those achieved following artificial insemination.

13:30-14:00 Bringing up small oocytes to eggs in pigs and cows. Takashi Miyano (Faculty of Agriculture, Kobe University, Japan)

While a large number of small oocytes are present in the mammalian ovary only a small number of oocytes in the primordial follicles grow to their final size, mature, and are ovulated. In vitro growth of small oocytes would provide a large population of mature eggs for livestock production. It could also open a new window on assisted reproduction in humans and in endangered species. Before that can be accomplished key questions remain to be answered. These include: 1) How do oocytes initiate their growth? . 2) Is the initiation of growth, factor dependent?. 3) Why do mammals restrict the number of oocytes that are ovulated?. 4) What is the unique role of the antrum in mammalian folliculogenesis?. 5) How do oocytes acquire the meiotic competence during their final growth phase? These key questions will be addressed, and recent results of oocyte-culture experiments in mice and domestic species using oocyte-culture, and oocyte xenotransplantation into immunodeficient SCID mice, will be discussed.

14:00-14:30 Short Communications (chosen from Abstracts)

14:30-15:00 BREAK

Session III: Assisted Reproductive Technologies II
(Chairs TeruhikoWakayama and Ian Wilmut)

15:00-15:30 Prospects for Recapitulating Spermatogenesis In Vitro. John E. Parks, co-authors Dong Ryul Lee, and Michael T. Kaproth (Gamete Physiology and Cryobiology, Dept. of Animal Science, Cornell University, USA)

In recent years, extraordinary progress has been made in a broad range of reproductive technologies including spermatogonial transplantation in the male. However, effective procedures for the complete

recapitulation of spermatogenesis in vitro, including meiosis, remain elusive. Such procedures have the potential to facilitate (1) mechanistic studies of spermatogenesis, (2) directed genetic modification of the male germ line, and (3) treatment of male factor infertility. Early studies demonstrated the importance of germ cell-Sertoli association for germ cell survival in vitro but evidence for male germ cell survival and progression through meiosis has been reported only in a few species. We demonstrated the expression of spermatid-specific genes (protamine and transition protein 1) by alginate-encapsulate neonatal calf testis cells after 7-10 weeks in culture, suggesting that meiosis occurred. While identifiable germ cells in these cultures were very sparse, some indication of acrosome development was observed in some cultured cells. Following ROSI with presumptive spermatids produced in vitro, 50% of blastocysts produced were diploid and 37% were Y-chromosome positive. Improved culture conditions for germ cell survival, proliferation, and differentiation are essential for in vitro spermatogenesis to be useful on a routine basis. Other approaches to male germ cell manipulation and spermatid production will be discussed.

15:30-16:00 New microinsemination techniques in laboratory animals. Atsuo Ogura (The Institute of Physical and Chemical Research (RIKEN), Bioresource Center, Japan)
In several mammalian species, not only mature spermatozoa, but also spermatogenic cells at certain stages in the testis, can be used to construct diploid zygotes, some of which subsequently develop into normal offspring. These mature and immature male germ cells can be cryopreserved in simple cryoprotectant solutions for later use. The recent technical advances in microinseminaation, especially those for laboratory species, provide us with valuable information on the mechanisms of fertilization (e.g., sperm-inducing oocyte activation) and reproductive genetics (e.g., genomic imprinting). Furthermore, they increase the chance of rescue of infertile animals, conservation of invaluable genetic resources, and transgenesis. In the mouse, normal pups have been obtained using round spermatids retrieved from males aged from 17 days to 33 months, or using primary spermatocytes before meiosis I. In my lecture I will review the field and also describe our recent work on applications of microinsemination techniques to genetics of laboratory species.

16:00-16:30 Short Communications (chosen from abstracts)

16:30-17:00 BREAK

17:00-18:30 Student Competition

19:00 Social Event

Monday Program (January 13)

Session IV: Transgenic Technology
(Chairs Karen Moore and Mark Nottle)

9:00-9:30 Development of efficient strategies for the production of genetically modified pigs. Hiroshi Nagashima (Meiji University, Faculty of Agriculture, Japan)
Demands on efficient reproductive technology have been increasing rapidly with the shift of transgenic pig production from basic research to practical application. Pronuclear DNA microinjection has been the most reliable method for producing transgenic pigs for long years, though it’s efficiency needs to be improved. Novel methods such as sperm mediated gene transfer and nuclear transfer are expected to enable more efficient and economical production of genetically modified pigs. These sophisticated technologies can maximize their potentiality when the fundamental technologies including super ovulation, in vitro oocyte maturation, embryo culture, cryopreservation and embryo transfer are further developed. This presentation will review current status and future prospect of the reproductive technologies which are essential for efficient production of genetically modified pigs.

9:30-10:00 Artificial Chromosome Vectors And Expression Of Complex Proteins In Transgenic Animals. (Jim Robl, Hematech LLC, USA)
Pronuclear microinjection and, more recently, fibroblast cell transfection followed by nuclear transplantation have been used to produce transgenic livestock. Both of these methods have significant limitations. The maximum size of DNA that can be inserted limits the transfer of large, megabase (Mb)-sized transgenes and the transgene DNA must integrate into a host chromosome to be retained through development. The requirement for integration is associated with a risk of inducing adverse mutations and unpredictable transgene expression. Mammalian artificial chromosome (MAC) vectors may be a better choice for generation of transgenic livestock for some applications because they replicate as independent chromosomes without integrating into a host chromosome. Owing to their larger insert capacity, MAC vectors could carry genomic transgenes, rather than cDNA transgenes, including their intrinsic regulatory elements, such as promoter and enhancer sequences. One example of where MAC vectors would be

particularly useful is in the transfer of the Mb-sized human immunoglobulin (Ig) loci into livestock for production of large quantities of human polyclonal antibodies

10:00-10:30 Transgenic swine for biomedicine and agriculture. (Randall Prather, University of Missouri-Columbia, Animal Research Center, USA)
Technologies for germline transmission of transgenes in swine first included only pronuclear injection, but later evolved to include sperm-mediated transfection, oocyte transduction (also referred to as transgametic gene transfer), and nuclear transfer (NT)-mediated transgenic pig production. More recently, a specific genetic modification was made to the alpha (1,3)-galactosyltransferase gene prior to creation of the pigs by NT. Demonstration of the technology to make specific genetic modifications in pig opens a new door in the potential to use genetic modification for biomedical and agricultural purposes. Biomedical applications include xenotransplantation, understanding basic biology, and the creation of models of human disease. Agricultural applications include creating a more healthy product, creating that product more efficiently in an environmentally friendly fashion, and animals that are resistant to disease. Applications are limited by one's imagination. The techniques to produce these animals are still inefficient and some offspring are not phenotypically normal. Further research will not only improve the efficiencies, but also provide a better understanding of the biology of the system.

10:30-11:00 Short Communications (chosen from abstracts)

11:00-11:30 BREAK

11:30-12:00 Presentation of IETS Pioneer Award

12:00-13:00 Lunch

13:00-13:15 A report from IETS Data retrieval Committee

Session V: Commercialization
(Chairs Tanja Dominko and Steve Stice)

13:15-13:45 Commercialization of Animal Biotechnology. David Faber DVM (Trans Ova Genetics, Sioux Center, IA)
Commercialization of animal biotechnology is a wide ranging topic for discussion. In this paper, I will attempt to review embryo and related technology as they relate to food producing mammals. A brief review of the history of advances in biotechnology will provide a glimpse to present and future applications.
Commercialization of animal biotechnology is presently taking two pathways. The first application involves the use of animals for biomedical purposes. Very few companies have developed all of the core competencies and intellectual properties to complete the bridge form lab bench to product.
The second pathway of application is for the production of animals used for food. Artificial insemination, embryo transfer, cloning, transgencs, and genomics all are components of the tool box for present and future applications. Individually these are powerful tools capable of providing significant improvements in productivity. Combinations of these technologies coupled with information systems and data analysis will provide even more significant change in the next decade.
Any strategies for the commercial application of animal biotechnology must include a careful review of regulatory and social concerns. Careful review of industry infrastructure is also important. Our colleagues in plant biotechnology have helped highlight some of these pitfalls and provide us with a retrospective review.
In summary, today, we have core competencies which provide a wealth of opportunities for the members of this society, commercial companies, farmers, and the population. Successful commercialization will benefit all of the above stakeholders.

13:45-14:15 New commercial opportunities for the embryo transfer industry in small animals. (Charles R Long, Genetics Savings and Clone, USA)
As advanced reproductive technologies become repeatable and more efficient in livestock species, new opportunities develop to apply these techniques and expand the market for these services to companion animals. The companion animal market represents a unique business model that requires addressing issues of animal welfare and market acceptance on a different level than the livestock sector. Application of advanced reproductive technologies will be applied to more than just the pet owning population and should be considered a useful tool for conservation of genetic material from endangered or unique animals as well as production of biomedical models of human disease.

14:15-14:45 Short Communications (chosen from abstracts)

14:45-16:15 Poster Session #1

16:15-17:00 IETS Annual Business Meeting

17:00-18:30 Practitioner's Forum of the 2003 International Embryo Transfer Society
Session Chair: Charles R. Looney, OvaGenix, Bryan, Texas, USA

Synopsis of Continuous Plagues of Bovine Embryo Transfer
Embryo Transfer Practitioners will enjoy a fundamental discussion of current, prevailing solutions in two major problem areas: 1) Fertilization failure in super-ovulated donors, and 2) Inconsistent pregnancy rates with Direct Transfer freezing technology.

Discussion of Semen Quality and Its Affect on Fertilization Rate
The theory that all frozen semen is of good quality has consistently overwhelmed flushing results. The age of the bull has also been overlooked at the initial onset of the embryo transfer program – more specifically the use of unproven, young bulls; infertile bulls; and older, inefficient bulls. Data from several labs will be used to compare oocyte fertilizable quality using IVF technology and Superovulation using the same bull. Data comparisons of Superovulation drugs with and with out progesterone priming and follicle wave synchronization will also be given.

Inconsistencies Observed in Direct Transfer Pregnancy Rates
Many practitioners have recently shown concern with inferior pregnancy results than initially projected using Direct Transfer (Ethylene Glycol cryoprotecant) for embryo freezing/thawing. Discussion will be shown from several geographical areas using bovine genetics of many diverse breeds. Practitioners in warmer climates appear to have more difficulty with Direct Transfer technology than those in cooler climates. Results will be presented indicating that some of the variability can be attributed to temperature increases at the time of equilibration, the time of freezing, the time of thawing, and the time of transfer. Comparisons from within one herd using the conventional (Glycerol) vs. Direct Transfer (Ethylene Glycol) methods will be presented along with data from commercial media with and without sucrose.

19:00- HASAC and CANDES Committee Open Meetings

Tuesday Program (January 14)

8:30-10:00 Poster Session #2

10:00-10:30 Student Awards

10:30-11:00 BREAK

11:00-11:40 Trade and Societal Constraints in national and international embryo movements:
the purpose of HASAC, a report of IETS HASAC. M. Thibier and D. S. Stringfellow

11:40-12:10 CANDES Committee Update. N. Loskutoff

12:10-13:10 Lunch

Session VI: Current Status of Embryo Technologies in horses, sheep and deer
(Session Chairs, Jeremy Thompson and Robin Tervit)

13:10-13:40 Current Status of Embry Technologies in the Horse. E.L. Squires (Animal Reproduction and Biotechnology Laboratory, Colorado State University, Fort Collins, CO 80523 USA)
Embryo transfer is becoming more commonplace in the horse industry. However, there are still both manmade and biological limitations to some of the embryo technologies. Equine embryos are collected and transferred non-surgically generally from single ovulating mares. The development of techniques for cooling and storage of equine embryos has prompted the development of several large embryo recipient stations where embryos are shipping for unavailability of equine FSH and the poor response in mares to both porcine and ovine FSH.
In vitro production of embryos in horses has been quite difficult. Two major barriers have been oocyte maturation and sperm capacitation. Recently, progress has been made in both of these areas, which will result in in vitro production of equine embryos on a more consistent basis.
Oocyte transfer and gamete intrafallopian tube transfer have both been used to produce pregnancies from subfertile mares. Factors affecting the success of these techniques will be presented.

Success of freezing equine embryos is dependent upon the size of the embryo and the developmental stage. Embryos <300μ that are at the morula or blastocyst stage freeze as well as bovine embryos. Techniques have also been developed for preservation of equine oocytes.

13:40-14:10 Current status of Embryo Technologies in sheep and goat. Y. Cognie et al. (INRA, Physiologie de la Reproduction, France)
This paper reviews the technical bases of in vivo and in vitro embryo production in sheep and goat. In both species, the new strategies to overcome the current limitations of in vivo embryo production: the variability of response to the hormonal treatment, the fertilization failure in females showing a high ovulatory response and the importance of premature regressed corpus lutea are described. The new prospects offered by ovum pick-up and in vitro oocyte maturation in defined medium are presented with their influence on developmental competence up to the blastocyst. The recent improvements of embryo transfer and embryo technologies will allow in the efficient propagation of valuables genes in small ruminants populations.

14:10-14:40 New Developments in Reproductive Technologies in Deer. Debra K. Berg (Reproductive Technologies Group, AgResearch, Ruakura, Hamilton, New Zealand)
Red deer have been farmed in New Zealand since 1971. They are an agriculturally important species with 2 million deer currently farmed for venison and velvet production. This industry has provided valuable resources to elucidate basic reproductive physiological parameters that have enabled us to develop, adapt and trial in vitro technologies that may be used to propagate endangered deer species. Farmed red deer habituate well to research conditions and elite groups of animals are available to trial the developed reproductive technologies.
Several studies in various deer species have trialled standard ruminant in vitro production systems. It is difficult to compare these results because of differences in species, sources of ova, sperm, and culture conditions. However, they share a common feature, the majority of oocytes fail to develop to the blastocyst stage. We have developed a semi-defined fertilisation and culture media based upon the analysis of cannulated red deer oviduct fluid collected from several animals, Deer Synthetic Oviduct Fuid (DSOF). In vitro matured oocytes fertilised and cultured in DSOF consistently produce 25 to 30% blastocysts from abattoir oocytes while 10 to 15% of the trans-vaginal ovum pickup oocytes develop to the blastocyst stage. Blastocyst production appears to be seasonally dependant with no development in the latter portion of the breeding season. These reproductive tools will allow further investigation into follicular wave patterns, changes in oocyte competence and early embryonic development. 14:40-15:10 Short Communications

15:10-15:40 Closing

Social Event