International Embryo Transfer Society
Parent Committee on Companion Animals,
Non-Domestic & Endangered Species
(CANDES)
Report to the IETS Board of Governors
1 July 2007

Report from the Mid-Year Meetings and Activities

Joint Regulatory (Co-Chairs: Justine O'Brien & Linda Penfold) & Health & Safety (Co-Chairs: Bill Holt & Naida Loskutoff) Subcommittee Meetings

Two development workshops were held in 2007 to further progress in having a comparative disease risk analysis tool for live animal versus biomaterial international transport. The goal is to give regulatory officials a means of assessing the relative disease risks in importing live animals versus biomaterials (e.g., semen). The model being worked on currently is the potential risk of importing foot and mouth disease virus into the US from a Boran (*Bos indicus*) bull in Kenya versus importing frozen semen from the same bull. The workshops were sponsored jointly by the White Oak Conservation Center and the Henry Doorly Zoo and hosted by Dr. Linda Penfold.

Present at the first workshop were Dr. Bill White (Veterinarian, MS Epidemiology), USDA APHIS Plum Island representative in charge of import/export disease issues, and Mr. Thomas Manybe (MS Pathology), Kenya Wildlife Services. They were incredibly helpful in providing the details to complete the Precision Tree model for the live bull, including sensitivities and specificities on required and approved tests by the USDA for foot and mouth disease. However, it was realized that these same assays used for sera may not be effective for semen; therefore, one goal identified was to conduct a series of experiments using bull semen to validate the specific PCR and virus isolation tests. Current research is underway to validate the tests for semen and/or washed sperm so that the information can be used to complete the semen aspect of the comparative disease risk analysis. Detailed minutes from these meetings can be found in Appendix 1 of this report.

Joint Research (Co-Chairs: Rebecca Krisher & Monique Paris) & Technology (Co-Chairs: Damien Paris & Gabriela Mastromonaco) Subcommittee Activities

As mentioned in my last report, the Research and Technology Subcommittees have progressed on a special issue of Reproduction, Fertility and Development entitled: A Perspective on the Role of Emerging Technologies for the Propagation of Companion Animals, Non-Domestic and Endangered Species (Guest Editors: Rebecca L. Krisher and Monique J. Paris). We are very happy to report that it is completed, published and now available: http://www.publish.csiro.au/nid/44/issue/3368.htm. Please do take the time to see it when you have the time to do so.

Damien Paris of the Technology Subcommittee has been fine-tuning the workshop we would like to have considered as a pre-conference (Saturday) workshop at the 2009 IETS conference. Attached is a draft of the workshop program (Appendix 2). If approved by the

IETS Board of Governors, the CANDES Committee will continue to fine-tune this program, select speakers, and search for alternative sources of funding besides the registration fees and contribution from the Henry Doorly Zoo. After the final program is confirmed and approved by the Board (January 2008), the program will be sent to the list-serve for the CANDES Committee members as well as published in the Embryo Transfer Newsletter.

Respectfully Submitted,
Naida M. Loskutoff, Chairman of the IETS CANDES Parent Committee

APPENDIX 1

June 2007

IETS CANDES Biomaterial versus Live Animal Transport Disease Risk Analysis Development: Minutes from the Workshops held in 2007

2-3 March 2007 Workshop

Sponsored by White Oak Conservation Center and the Henry Doorly Zoo

Present:
Dr. Linda Penfold, White Oak Conservation Center, CANDES Regulatory Subcommittee Co-Chair
Dr. Bill White, USDA APHIS Plum Island representative in charge of import/export disease issues, Veterinarian, MS Epidemiology
Mr. Thomas Manybe, Kenya Wildlife Service. MS Pathology
Dr. Naida Loskutoff, Henry Doorly Zoo, CANDES Chairman and CANDES Health and Safety Subcommittee Co-Chair
Dr. Andy Teare, Jacksonville Zoo, Veterinarian, expertise in computer modeling
Lara Metry, research intern of Linda Penfold, MS Behavior/Reproduction
Dr. Laura Hungerford, Maryland School of Medicine, Veterinarian, MS Public Health, Phd Epidemiology, expertise in computer modeling, risk assessment
Mr. Brock Blevins, Henry Doorly Zoo, research on semen disinfection, interest in computer modeling
Dr. Phil Miller, Senior Program Officer CBSG, 12 years experience with tools for risk assessment , overall workshop facilitator

Regrets:
Dr. Justine O'Brien, U. Sydney and Sea World San Diego, CANDES Regulatory Subcommittee Co-Chair
Dr. Bill Holt, Zoological Society of London, CANDES Health and Safety Subcommittee Co-Chair

Workshop Program (Day 1):

Linda Penfold: welcome and introductions. Presentation given from the 2006 SSR satellite symposium on CANDES (see July 2006 symposium proceedings on the CANDES website) as well as her direct experiences following USDA regulations in importing gerenuk semen from free-ranging animals on a Kenyan wildlife reserve to the White Oak Conservation Centre.

Phil Miller: Introduction to CBSG workshop process.

Bill White: Update on literature demonstrating the potential of cross contamination of biomaterials stored in liquid nitrogen (e.g., Tedder et al., Lancet 346). He also described an

example of the dire consequence of first case of BSE in US – beef markets were shut down and the US lost billions revenue in just the next few weeks after the announcements.

Summary of conclusions from Day 1: acceptable risk decisions will be politically driven. In the absence of data for the disease risk model, scientists will act as *de facto* decision makers. Public perception of the trustworthiness of the scientist will be important to assure regulatory official trust

Import of semen and embryos logically should be less likely to introduce disease than the import of whole animals. Bill explained that one problem with Linda's past experience (gerenuk semen import from Kenya to the US) is that there was no constant supervision by USDA-approved officials. Phil commented that we need more justification and explanation of values inserted into model. This would make it more believable and reliable that may help acceptance from regulatory officials.

Phil described the CBSG role in "disease risk analysis". It has only been about 20 years that epidemiology/pathology has been presented as an important discipline of conservation biology (importance to conservation). In the early days: November, 1992, a preliminary conference was held focusing on the implications of infectious disease transmission in captive propagation and reintroduction programs of threatened species in California. Working groups addressed disease issues in captive wildlife, free ranging wildlife, reintroduction programs, risk assessment, population dynamics and diagnostic technology. The program addressed directly concept of zero risk tolerance, which was agreed to be unattainable. Ullie Seal and others decided at that time that tools need to be developed to facilitate disease risk analysis for animal transportation.

In the Journal of Zoo and Wildlife Medicine (Volume 24, September 1993) a major recommendation, stemming from a symposium on the topic was to develop a set of quantitative risk assessment tools. The first workshop to develop these tools was held at the Henry Doorly Zoo in 2000 to explore the nature of the problem and the current availability of tools to address the problem. The participants were divided into four working groups: disease information workbook, decision tolls, information modeling, "Vortex" modeling. Dr. Richard Jokob-Hoff (Regulatory Official from New Zealand), who already has worked out a disease risk analysis program for that country, demonstrated their tool in a plenary session. The second development workshop was held in New Orleans in 2001 and the working groups focused more directly on specific case studies. The third development workshop was held at the White Oak Conservation Center in 2002 where the participants continued with intensive testing and refinement of the analytic tools being development using single case study approaches, how to incorporate GIS information and tools, information from past CBSG Population and Viability Assessment workshops with the goal of developing a disease risk (epidemiology) module for the Vortex software. Richard was extremely helpful with his past experiences in designing the initial worksheets adopted by the participants.

Dr. Dominic Travis (Veterinarian and Epidemiologist from the Lincoln Park Zoo) and Laura described the disease risk analysis process by defining the big picture as far as problem policy and correctly identifying potential hazards. The CBSG "Outbreak" software was then introduced and described as an individually based simulation for wildlife diseases. It was

emphasized that several factors, such as specific epidemiology, outbreaks, prevalence, disease and demographic dynamics all need to be provided and evaluated if a link to the Vortex software can be made for a more sophisticated method to categorize the demographics of specific populations. An initial workbook was developed in 2002, then a disease risk analysis workshop for animal transport was for conducted in Mexico in 2002, then in Costa Rica in 2003 as training workshops that dealt with specific case studies, e.g., the Channel Island fox. The latest application to date was a workshop held in Japan in 2006 focusing on the Tsushima leopard cat and its interaction and hybridization with domestic and feral cats. A graphical model was developed by Dr. Alberto Paras (a Veterinarian from Mexico who has been a voluntary member of the CBSG team conducting the disease risk workshops). The major question was: what is probability of the wild leopard cats becoming infected with FIV from domestic cats. Their conclusions actually helped local wildlife officials develop measures to change management procedures in their wildlife reserves.

In 2003, the first development workshop was held (sponsored by the Henry Doorly Zoo on behalf of the IETS CANDES) to develop and apply tools to assess the comparative risk of transporting biomaterials versus live animals. A preliminary workbook was published and appears on the IETS CANDES web page. This was a function of both the CANDES Regulatory and Health and Safety Subcommittees. The first example that is now being focused on is the differences in the probabilities of importing the foot and mouth disease virus from semen versus a live gerenuk from Kenya to the US. Literature shows that the prevalence of foot and mouth disease in Kenya is anywhere from 0-50% in domestic bulls, in the case of this model decided to start with worst case scenario. Although rinderpest is worse for animals, foot and mouth disease is more politically and economically devastating for animal or semen transport, especially for the US. It is the most contagious disease and affects most species and, therefore, can be an enormous problem if introduced (10 times worse than UK). Nevertheless, for a complete disease risk analysis, all endemic diseases not occurring in the country of importation must also have a separate disease risk analyses performed.

A second development workshop was held at White Oak Conservation Centre in January 2005, hosted by Linda Penfold and sponsored in part by the Henry Doorly Zoo. At that time the model was changed from a gerenuk to a domestic (*Bos indicus*) bull breed (Boran) from Kenya owing to the fact that there is ample data available for cattle but scarce information available for wildlife on foot and mouth disease that is crucial for conducting the comparative disease risk analysis.

Laura then reviewed for the participants modeling and risk analysis, and the OIE emerging framework emphasizing scientifically based risk assessment, management and communication. Modeling allows you to get through this information quantitatively or qualitatively. Hazard characterization, release assessment, exposure quantification and risk characterization were defined and discussed. She explained why using models are important to the process to organize and store knowledge about disease risk. Laura emphasized the importance of explaining all assumptions incorporated into models satisfactorily because transparency is critically important to result in a model that appears valid. She mentioned that historically, this has been a problem with regulatory acceptance of a model. Models can predict the effectiveness of interventions – being proactive rather than reactive. They can predict consequences of an action. They should include logical or unexpected sequelae of our

assumptions. Modeling exercises can show what doesn't look accurate or may show something inserted into the model that may not be correct. Models can identify gaps and important factors or factors that really do not matter. This can hopefully lead to policy changes based on acceptable, transparent information. For this problem we are facing (sperm versus live animal transport from Kenya to the US and the probability of introducing foot and mouth disease virus, the Precision Tree, At Risk with Excel computer programs are the most appropriate.

Example: Decision of chance of event as the problem? Is the biomaterial contaminated in Africa? (no); prevalent in source herd? (no); good quarantine practice? (yes); etc. These are the steps in designing tree for live animals as well as biomaterials. It is important to identify and list all possible alternatives (initially using a flow chart). Information that is available is the collected for the Precision Tree. This includes types of analysis for the disease and types of treatment. Aspects must be decided as to what are the most important to avoid too many branches on the Precision Tree that are irrelevant or already assumed. It is also important to consider non-target effects (often outside the initial bounds of the problem), e.g., the billions of dollars lost to BSE in the US in the example Bill gave earlier – we must consider the outcomes of outside effects. Transform openly what should be done into a series of closed, more completely defined questions (need experts) considering a specific decision problem. Need to be sure to capture in the model critical points (areas where the biomaterial or live animal can potentially be infected during the entire transport process). Need to identify uncertainties and how to deal with them, also potential variability (e.g., time of shedding – we know it is there but it can change for different diseases). There is also the need to separate the types of variables from uncertainties. At the outcome, what is the key question to manage risk? Factor in what regulatory viewpoints exist that are bound to consider certain things and allow them to include those. Revise tree to allow for all these. Most decision makers (regulatory officials) are more risk averse so it it crucial to be comprehensive and transparent.

Central question for analysis at the March 2007 development workshop:

Initial model: What are the comparative risks of introducing foot and mouth disease (FMD) into a naïve Boran bull captive population in the US via importation of either the whole animal versus semen From Kenya? Once a disease risk analysis can be completed, then all other diseases of concern for this species (cattle breed) need to be completed following this model.

Following first meeting, Linda and Laura started with four bulls, and went through all the questions and inserted probabilities, e.g., prevalence. The range would be the same for semen and live animals. FMD carriers (28 days after infection) with potentially 7 serotypes and probably 60 sub-serotypes once infected, will only shed in semen when there are clinical signs. The virus is typically found in epithelial cells reportedly coming from the sheath and not from the seminal glands or testes. Bill presented an important reference from (Alexandersen et al., The pathogenisis and diagnosis of foot and mouth disease, J. Comp. Path. 129:1-36, 2003) which said that if the animal demonstrates clinical signs, the semen will be contaminated with the virus.

The first Precision Tree model that was worked on and completed was for the transport of a live Boran bull from Kenya to the US and the probability of introducing foot and mouth disease. One difference made from the previous model is that a pre-quarantine screening is to be performed on the farm for animals showing no clinical signs and blood work and Probang (esophageal scraping) are negative. They are then sent to a USDA-approved quarantine facility. At entry, they must not show any clinical signs or titers from blood and Probang testing. They are held for 10 days to see if any became infected (or carriers showing no clinical signs) within four days during after pre-screening.

Therefore, bulls are selected from the prescreening (pre-quarantine) process, separated for 10 days from the rest of the herd and tested (USDA APHIS on Plum Island and Kenya Wildife Service: both blood and Probang). Clinically negative bulls are then transported to the quarantine facility by truck (1% may be false negatives) for 60 days (a USDA official must be present for the entire quarantine period).

Bill was extremely helpful and a vital participant in our March 2007 development workshop in providing the list of tests that are required and approved by USDA APHIS for detecting foot and mouth disease using various blood ELISAs (3ABC); Probang; vn, va for antibodies (a 4 fold infection means active infection) PCR for virus in blood, and semen. A positive on antibodies indicating infection/exposure on any animal would mean those bulls would be excluded.

Bill was also extremely helpful in providing us with the actual specificities and sensitivities of several tests conducted by USDA APHIS on Plum Island for the detection of foot and mouth virus: virus neutralization (VN) 95% (OIE prescribed); virus infection associated antigen (VIAA) with a specificity of 95% and sensitivity of 85% (used by APHIS for 30-40 years, but will be replaced by 3ABC ELISA (the VIIA not as effective in vaccinated animals). VIIA looks for antibody to the 3D protein; 3ABC protein by ELISA can differentiate infection from vaccination. A third test virus neutralization test with a sensitivity of 95%, specificity of 100% (these measure antibodies of any type (vaccine or field infection). Finally, virus isolation – on Probang, blood and semen: sensitivity of 95%, specificity of 20% (but suspected to be higher for semen). If we are looking for antibodies, we would not expect animals positive on VN to be positive on the other tests. PCR would be more likely to find virus. Combine PCR and VN would be the most sensitive protocol as we would be testing for antibodies and antigen. PCR should be performed on Probang (esophageal scraping), semen and blood.

PCR on Probang was reduced from 96% to 30% for the live animal transport Precision Tree due to the fact that animals only shed intermittently. One would expect perhaps 50% of carriers would shed virus at time of testing. One reason semen may be better is because testing semen directly for virus isolation (depending on whether or not there are inhibitory factors in semen that affects PCR results, which is a possiblity).

One important conclusion reached at this meeting is that experiments are needed to validate the serum PCR assays for semen determine if semen contains inhibitory substances that will affect PCR results (a fact known with other viruses known to be shed in semen). Currently, Linda is working with Bill by taking fresh domestic bull semen at Plum Island, spiking it with live foot and mouth virus and conducting the assays to be sure there are not inhibitory factors

that can interfere with results. It is also known that seminal plasma contains factors that destroy tissue culture cells, which make virus isolation tests not possible. Future experiments will include first washing/treating semen to remove seminal plasma before subjecting the sperm to these assays. Another possibility is that there are methods available in the literature (especially for HIV patients) for removing inhibitory factors from semen (using column chromatography) before subjecting the samples to PCR analysis.

Conclusions

Thanks especially to Bill White and Thomas Manybe for their invaluable contributions for providing the accurate values and estimates to put into the model, and Laura Hungerford for her expertise in designing the Precision Tree model, we have now completed the Precision Tree for the live animal transport, all according to current USDA regulatory practices. We now need to determine the most appropriate method to either insert the semen branch into this tree, or create another separate Precision Tree for semen to be able to do a comparative analysis of the risks associated with introducing foot and mouth virus. Once this is completed, Linda Penfold will publish the information in a reputable journal that will have qualified epidemiologists and disease risk modelers as reviewers. Once accepted, we plan to publish the paper not only in the journal but also request posting it on the OIE newsletter.

25 June Workshop

Sponsored by White Oak Conservation Center and the Henry Doorly Zoo

Present:
Dr. Linda Penfold, White Oak Conservation Center, CANDES Regulatory Subcommittee Co-Chair
Dr. Andy Teare, Jacksonville Zoo, Veterinarian, expertise in computer modeling
Mr. Brock Blevins, Henry Doorly Zoo, research on semen disinfection, interest in computer modeling
Dr. Phil Miller, Senior Program Officer CBSG, 12 years experience with tools for risk assessment , overall workshop facilitator

Regrets:
Dr. Naida Loskutoff, Henry Doorly Zoo, CANDES Chairman and CANDES Health and Safety Subcommittee Co-Chair
Dr. Laura Hungerford, Maryland School of Medicine, Veterinarian, MS Public Health, Phd Epidemiology, expertise in computer modeling, risk assessments.
Dr. Justine O'Brien, U. Sydney and Sea World San Diego, CANDES Regulatory Subcommittee Co-Chair
Dr. Bill Holt, Zoological Society of London, CANDES Health and Safety Subcommittee Co-Chair

Over the course of this one day organizational meeting, participants focused on detailing the specificity and sensitivity of a variety of proposed FMD tests (3AB ELISA, 3ABC ELISA, RT-

PCR, VI, and VIAA (virus infection associated antigen) to be incorporated into the cattle Disease Risk Analysis Model. Validation for FMD tests on raw semen at USDA facilities as well as the effect of semen disinfection protocols on the removal of the virus were discussed yet remains in the early stages. At present, the most appropriate method to incorporate semen into the cattle importation disease risk model has not been determined. Further workshops are planned to finalize this model for publication.

Respectfully Submitted by:
Naida M. Loskutoff, Ph.D.
Linda Penfold, Ph.D.

APPENDIX 2

International Embryo Transfer Society Parent Committee on Companion Animals, Non-Domestic & Endangered Species (CANDES)

Workshop Series: Implementation of established assisted reproductive technology in CANDES

Purpose:
In addition to the publication of a *Reproduction Fertility & Development* special issue on emerging technology (August 2007), we propose to hold workshops to get member input on the role of established reproductive technologies for the propagation of CANDES species. Experts across the taxa will be asked to prepare extended abstracts, deliver presentations and lead discussions. The CANDES Research & Technology Sub-committee Co-chairs will facilitate the development of a consensus position statement highlighting the value of the respective technologies, their limitations, appropriate target species, and priority areas/taxa in which further research is required.

Topic:
For an initial workshop we propose to focus on one of the more fundamental technologies that has increasing success in CANDES species: Artificial Insemination (and endocrinology and spermatology specifically related to achieving successful AI) (see proposed scientific program and detailed content below).

Format:
The workshop shall attempt to cover the above topic for a broad range of CANDES species including: birds, reptiles, amphibians and mammals (specifically carnivores, ungulates, non-human primates, marine mammals and marsupials). Within each session the presentation/discussion will focus on:

(i) Current status of knowledge (development of the technique – early disappointments, successes, accidents and pitfalls; success in domestic and CANDES species).
(ii) Advantages (applications/benefits for CANDES).
(iii) Complications (problems with application of technique in any species, but particularly foreseeable issues with CANDES species).
(iv) Future Research Priorities (basic reproductive knowledge; technological developments; appropriate/inappropriate target species).

The morning will consist of traditional presentations focusing on ancillary approaches (Session 1: Monitoring & Manipulation of Female Reproduction; Session 2: Collection & Preparation of Spermatozoa) required to successfully implement artificial insemination in CANDES. The afternoon will consist of more applied talks/demonstrations/discussions split into two sessions (Session 3: Artificial Insemination Taxon Workshops; Session 4: Consensus Discussion on Research Priorities) to demonstrate current approaches, and identify limitations and priority areas for further research at the taxa level. Posters will be on display throughout the day but presenters will deliver a 3 min rapid poster communication in Session 3 (see proposed scientific program and detailed content below).

Location:
If approved by the Board of Governors, we propose to hold this first workshop as a pre-conference satellite symposium associated with the International Embryo Transfer Society (IETS) Annual Conference in Denver, Colorado, USA in January 2008. It is important that input is obtained from as many stakeholders as possible in this field to ensure output from the workshops is representative and credible. We will endeavor to ensure research presented is internationally representative and the range of species are well represented, while remaining economically and logistically prudent.

Outcomes:
The intended outcome of this workshop is to develop a consensus position statement highlighting the value of the respective technologies, their limitations, appropriate target species, and priority areas/taxa in which further research is required. It is hoped that the proceedings – papers, discussion points and summaries will be published and it is possible that a multi-author review paper or a journal special issue would be a natural result of this workshop. We are still considering the following:

(i) The format of the workshop output (proceedings, white paper/position statement, multi-author review paper, working minutes, meeting summary or techniques manual)

(ii) Means of disseminating the output (scientific journal, SSPs, TAGs, CANDES website, IETS newsletter, funding agencies)

35th Annual Conference of the International Embryo Transfer Society

Post-conference Satellite Symposium:
"Implementation of Artificial Insemination in CANDES"
January 2009

Scientific Program:

8:45am Opening remarks and welcome
(CANDES Research & Technology Sub-committee Co-chairs:
Damien Paris & Monique Paris, University of Utrecht, Netherlands; Gabriela Mastromonaco, University of Guelph, Canada; Rebecca Krisher, Purdue University, USA)

9:00am Session 1: Monitoring & Manipulating Female Reproduction
(i) non-invasive hormone monitoring
Franz Schwarzenberger or Janine Brown
(ii) ovulation induction by exogenous hormones
Morney de la Reye or Naida Loskutoff or JoGayle Howard
(iii) alternative monitoring & ovulation induction strategies (ultrasonography, behavior, male pairing – pheromones, removal of pouch young – lactational arrest, etc.)
Cheryl Asa or Thomas Hildebrandt

10:30am Coffee Break & Poster Viewing

11:00am Session 2: Collection & Preparation of Spermatozoa
(i) collection strategies (source & method)
Bill Swanson or Budha Pukazhenthi
(ii) determinants of sperm quality (morphology, motility, number, membrane integrity, competitive fertilization ability, high quality sub-populations)
Montserrat Gomendio or Bill Holt
(iii) sperm preservation strategies (freezing, prolonged survival in ambient temperatures)
Stanley Leibo or Budha Pukazhenthi or Linda Penfold

12:30pm Lunch

1:30pm Session 3a: Artificial Insemination Taxon Workshops
(Concurrent sessions including poster rapid communications)

(a) Birds/Reptiles/Amphibians	(b) Ungulates	(c) Marine Mammals/Marsupials
Juan Blanco	Thomas Hildebrandt	Justine O'Brien
J. K. Mattson	Dennis Schmitt	Damien Paris
Andy Kouba	Julian Skidmore	Frank Molinia
	Steve Monfort	

3:00pm Coffee Break & Poster Viewing

3:30pm Session 3b: Artificial Insemination Taxon Workshops
(Concurrent sessions including poster rapid communications)

(a) Carnivores	(b) Primates
Jo Gayle Howard	Naida Loskutoff
Wenche Farstad	Gabriel Sanchez-Partida
Cheryl Asa	

5:00pm Session 4: Consensus Discussion on Research Priorities (Expert Panel – Chaired by CANDES Research & Technology Sub-committee Co-chairs)

5:30pm Conclude

Content:

Session 1. Monitoring & Manipulating Female Reproduction: This session will be delivered in a traditional lecture style format of 30 min duration each (incl. 5 min discussion). It will focus on the importance of determining the time of ovulation in females for successful outcomes in artificial insemination in CANDES. It will concentrate on traditional hormone monitoring and manipulation of female reproduction by exogenous hormone regimes, but also explore alternative monitoring & manipulation strategies that may be appropriate for a number of species. Specific areas may include: sample collection (feces, urine, saliva, milk); non-invasive endocrine monitoring techniques/assays (RIA, EIA); hormone profiles; other monitoring techniques (ultrasonography, behavior, etc.); ovulation induction by hormone treatment (regimes, species specificity, induction, synchronization/down regulation, and superovulation); other ovulation induction techniques (pheromonal induction by pairing with male; synchronization by removing lactational stimulus, etc.).

Potential experts:

- *Janine Brown, National Zoo, USA (hormone monitoring – elephants & cats)*
- *Steve Monfort, National Zoo, USA (hormone monitoring & induction – deer & antelope)*
- *Todd Robeck, Sea World, USA (hormone monitoring & induction – marine mammals)(unwilling to participate)*
- *Katey Pelican/Rose Bauer, National Zoo, USA (hormone induction – cats)*
- *Helen Bateman, Cincinnati Zoo, USA (otters)*
- *Norman Rawlings, University of Saskatoon, Canada (hormone monitoring – bison, etc).*
- *Terri Roth, Cincinnati Zoo, USA (hormone monitoring – amphibians, reptiles)*
- *Franz Schwarzenberger/Erich Möstl/Rupert Palmer, University of Veterinary Medicine, Austria (hormone monitoring – CANDES)*
- *Frank Molinia, Landcare Research, New Zealand (hormone induction – marsupials)*
- *Lyn Hinds, CSIRO, Australia (hormone monitoring & induction – marsupials)*
- *Monique Paris (hormone induction – marsupials)*
- *Thomas Hildebrandt, IZW, Germany (ultrasound monitoring – CANDES)*
- *Other: Duane Kraemer (Texas A&M University); Cheryl Niemuller (Toronto Zoo, Canada); Nancy Czekala (San Diego Zoo, USA); SK Wasser/M Dehnhard (IZW, Germany); Nadja Wielebnowski (Brookfield Zoo, USA).*

Session 2: Collection & Preparation of Spermatozoa: This session will be delivered in a traditional lecture style format of 30 min duration each (incl. 5 min discussion). It will focus

on the collection, evaluation and preparation of spermatozoa required for artificial insemination in CANDES. Specific areas may include: source (ejaculated, epididymal) and method of collection (AV, EEJ, manual donation, biopsy); traditional and emerging determinants of sperm quality (morphology, motility, number, membrane integrity, competitive fertilization ability, high quality sub-populations); preservation strategies (cryopreservation, membrane integrity & cryodamage, prolonged survival in ambient temperatures in the presence of oviductal proteins).

Potential Experts:

- *David Taggart, University of Adelaide (collection & cryopreservation – marsupials)*
- *Bill Holt, IoZ, UK (sperm quality & ambient/cryopreservation – CANDES)*
- *Juan Blanco, CERI, Spain (collection & cryopreservation – raptors)*
- *Boris Dzyuba, IPCC, Ukraine (collection & cryopreservation – fish)*
- *Linda Penfold, White Oak, USA (collection – birds, antelope)*
- *Budha Pukazhenthi, National Zoo, USA (collection & cryopreservation – carnivores)*
- *Justine O'Brien, University of Sydney, Australia (sex-sorting & cryopreservation – birds, marine mammals)*
- *Bart Gadella, University of Utrecht, The Netherlands (sperm membranes, capacitation & flow cytometry - domestics)*
- *Monica Stoops, Cincinnati Zoo, USA (rhinos)*
- *Andy Kouba, Memphis Zoo, USA (amphibians)*
- *Rebecca Spindler, Taronga Zoo, Australia (pandas, cats)*
- *M. Tourmente, Argentina (snakes)*
- *Others: Genevieve Magarey (Cincinnati Zoo); Julio de la Fuente (Spain); Bill Swanson (Cincinnati Zoo); Theresa Abaigar (Spain).*

Session 3: Artificial Insemination Taxon Workshops (Concurrent sessions): These will be delivered as two concurrent sessions grouped by taxon and will consist of more applied talks/discussions led by 2 or 3 experts within each taxon. The format may include explanations, demonstrations or videos of techniques and a consensus discussion to identify priority areas/species for further research. We plan to have posters on display throughout the day (grouped by taxon) however, during the concurrent sessions poster presenters will be given 3 min to summarize their poster to each taxon group. Specific areas in artificial insemination may include: method (surgical, non-surgical); anatomical considerations & insemination site (vaginal, uterine); timing (pre or post ovulation). Specific areas for the consensus discussion may include: current limitations, priority research areas (basic reproductive biology/physiology, technology development, taxa/species).

Potential Experts:

- *Thomas Hildebrandt/Robert Hermes/Frank Goeritz, IZW, Germany (elephants, rhino)*
- *Dennis Schmitt, Southwest Missouri State University, USA (elephants)*
- *Monica Stoops, Cincinnati Zoo, USA (rhino)*
- *Jo-Gayle Howard, National Zoo, USA (cats, ferrets, pandas)*
- *Wenche Farstad/Ragnar Thomassen, Norwegian School of Veterinary Science, Norway (canids)*

- *Naida Loskutoff, Omaha Zoo, USA (gorillas)*
- *Damien Paris, University of Utrecht, The Netherlands (marsupials)*
- *Frank Molinia, Landcare Research, New Zealand (marsupials)*
- *Steve Johnston, University of Queensland, Australia (marsupials)*
- *Todd Robeck, Sea World, USA (marine mammals) (unwilling to participate)*
- *Justine O'Brien, University of Sydney, Australia (marine mammals?)*
- *Jonathan Daly, Melbourne Aquarium, Australia (sharks)*
- *Andrea Pilastro, University of Padova, Italy (guppies)*
- *Juan Blanco, CERI, Spain (raptors)*
- *Graham Wishart, Dundee Abertay University, UK (Houbara bustards)*
- *Others: D. Zambelli (Italy), Nei Moreira (Brazil), Henry Jabbour (Edinburgh, UK), Barb Wolfe (USA), Debbie Berg (New Zealand – ungulates/deer), Cheryl Asa (USA - canids/felids).*

Session 4: Consensus Discussion on Research Priorities: This session will consist of an open discussion in the presence of an expert panel (consisting of invited speakers) and mediated by the CANDES co-chairs. It will focus on discussing and compiling opinions on research priorities for the different taxa developed during session 3. The intended outcome of this workshop is to develop a consensus position statement highlighting the value of the respective technologies, their limitations, appropriate target