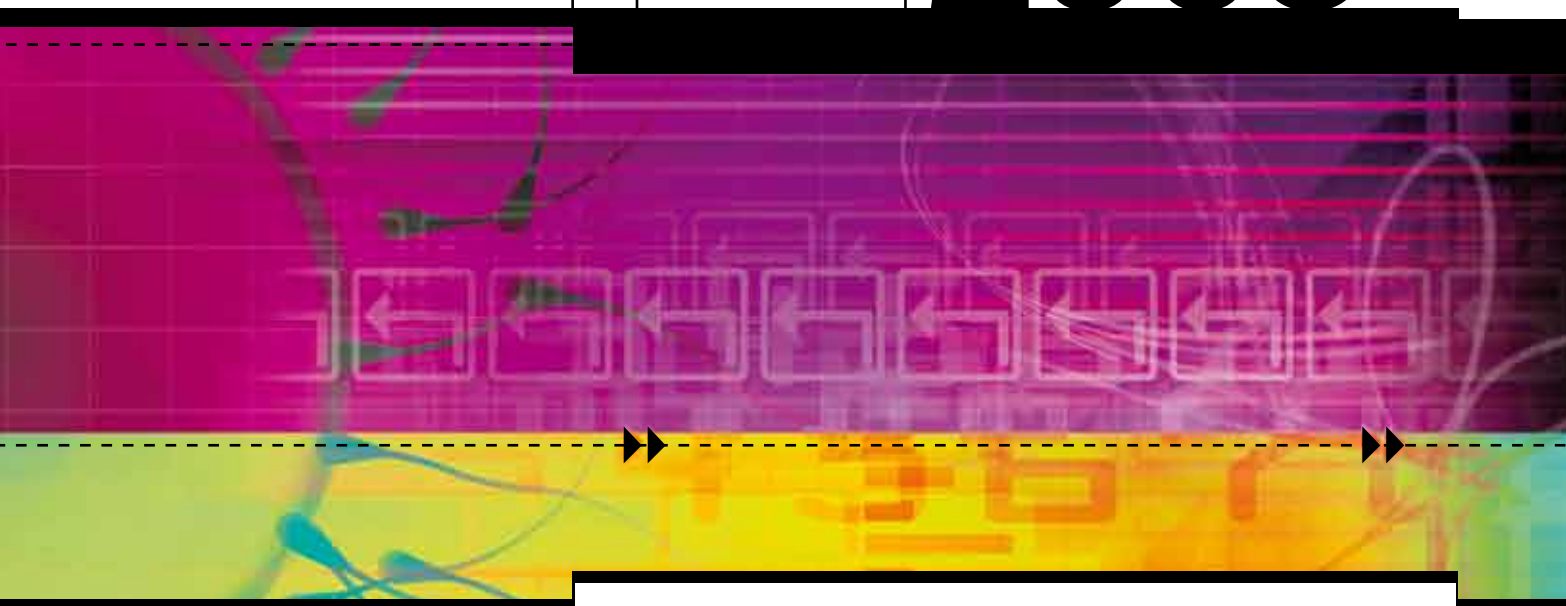
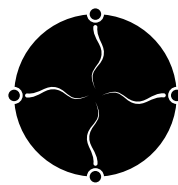


Annual Report

# 2008



The ARC Centre of Excellence in Biotechnology & Development



**CBD**

ARC Centre of Excellence in  
Biotechnology & Development

## Prospectus

Molecular mechanisms driving  
the specification and differentiation  
of male germ cells

An area of genome to phenome research

The Australian Research Council Centre of Excellence in Biotechnology and Development

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## Strategic Vision

The Centre for Biotechnology and Development brings together a unique group of outstanding chief investigators from four leading Australian Universities to address a central issue in developmental biology – the specification and differentiation of the male germ line.

Our mission is to dissect the complex developmental networks underlying male germ cell development and to use this information to address a number of key goals that are of immediate relevance to the development of the Australian Biotechnology Industry, protection of the Australian Environment and the health and well-being of the Australian people.

## Our Goals

Our overall goal is to generate information that will be valued by end-users in the biotechnology industry and medicine. The relationship between our research activities and our goals is set out below:

### GOAL 1:

#### ELUCIDATE THE CAUSES OF TESTICULAR CANCER

**Significance:**

This is one of the most common cancers in young Australian males. Its incidence is rising rapidly in every State in the Federation.

**Research Activities:**

Discovering the molecular mechanisms by which primordial germ cells become specified in the embryo.

Identifying the cues that govern how primordial germ cells proliferate, migrate and colonize the genital ridge.

Delineate the processes by which primordial germ cells form sperm precursors and then differentiate into spermatogonial stem cells.

Defining the control mechanisms that are central to the aetiology of testicular cancer.

**Collaborators:**

Professor Ewa Rajpert-de Meyts, Professor Niels Erik Skakkebaek, Rijshospitalet, University of Copenhagen

### GOAL 2:

#### NOVEL APPROACHES FOR THE CONTROL OF MALE FERTILITY IN MAN AND ANIMALS

**Significance:**

There is an urgent requirement and significant commercial market for human male fertility regulation. There is also a pressing need to develop non-surgical methods for controlling the fertility of domestic and pest animal species. No reversible, drug-based methods of male fertility regulation are currently available.

**Research activities:**

Use of advanced proteomics to discover possible contraceptive targets.

Analysis of the fundamental molecular mechanisms that regulate gonadal differentiation and the subsequent production of functional spermatozoa.

Development and analysis of novel mouse models of infertility.

Screening of infertile patients for mutations in candidate infertility genes.

**Commercial collaborators:**

CONRAD (Contraceptive Research and Development Branch of the US Agency for International Development), Pestat, Invasive Animals CRC, Starpharma

### GOAL 3:

#### RESOLVE CAUSES OF MALE INFERTILITY AND DNA DAMAGE IN HUMAN SPERMATOZOA

**Significance:**

Infertility affects 1 in 20 Australian males for reasons that remain largely unresolved but probably contain a large genetic component.

DNA damage is also commonly encountered in human spermatozoa.

DNA damage is associated with impaired embryonic development, miscarriage and genetically mediated disease in the offspring, including childhood cancer.

**Research Activities:**

Identify key genes involved in spermatogenesis, epididymal maturation and sperm function.

Establish the clinical relevance of genetic changes to reproductive failure through interrogation of the Monash DNA Repository.

Use advanced proteomics to examine the causes of defective sperm function.

Determine how oxidative stress and impaired chromatin remodelling contribute to defective sperm function and DNA damage.

**Commercial Collaborators:**

NuSep, IVF Australia, Westmead Fertility Centre, Monash IVF

### GOAL 4:

#### RE-PROGRAMMING GERM CELLS FOR APPLICATIONS IN BIOTECHNOLOGY

**Significance:**

Development of technologies for introducing commercially valuable transgenes into the male germ line of large domestic animals (gene pharming) would open up a biotechnology market worth millions of dollars a year.

**Research Activity:**

Analysis of the fundamental cell biology of spermatogonial stem cells and development of methodologies for their isolation, transfection and transplantation

**Collaborators:**

CSIRO

## Director's Report



The last year was another record year for the Centre. The Annual Scientific Meeting at Werribee was, by common consent, the most successful scientific meeting yet. This was, in no small part, due to the fact that on this occasion we gave the responsibility for organizing the scientific program to our postdoctoral fellows and Associate Investigators. The result was a vibrant, exciting cross section of our scientific research activities that was warmly appreciated by our Scientific Advisory Board. The Annual Scientific Meeting was also the venue for a Centre workshop on the preparation of competitive grants that was as entertaining as it was informative. Grateful thanks are due to Peter Koopman, Phil Robinson and Andrew Sinclair for orchestrating this event. Although the content was primarily aimed at the more junior members of our research community, I am certain that we all learnt something of value that evening. While we are on the theme of being informative and entertaining, these descriptions perfectly capture the lecture delivered by our 2008 guest speaker, Professor Rob McLachlan from Prince Henry's Research Institute. Rob is Director of Andrology Australia and a close colleague of many CIs. His lecture on 'Male Hormonal Contraception: A brief History, Physiology and Personal Perspective' was yet another highlight in an excellent meeting.

The Annual Scientific meeting was also the venue for the delivery of an important report by Biolink, a biotechnology commercialization company, on the commercial opportunities presented by the Centre's research. I am very grateful to Christian Toouli and his colleagues at Biolink for conducting this audit in such a highly proficient and professional manner. The outcome was a detailed record of our commercialization potential, highlighting not only the areas where we already have commercial linkages established but also indicating potential areas for future exploitation including proprietary mouse models, genes and targets, molecules, tools, methods and biological materials. In light of Biolink's report the Centre will establish a Commercialization management committee containing representatives from the four University nodes in order to allow a properly co-ordinated development of our commercial potential without impeding the momentum of our fundamental scientific research program.

The Centre contributed to a major andrology milestone last year with the completion of the 5th Edition of the WHO laboratory manual for the Examination of Human Semen and Semen-Cervical Mucus Interaction (Cambridge University Press). Despite its rather uninspiring title, this book is universally acknowledged as the reference text in diagnostic laboratory andrology. This revised version is the most complete account to date on how to create a conventional semen profile and includes invaluable reference limits for specific aspects of semen quality based on the analysis of over 1 900 recent fathers. This volume will therefore not only instruct laboratory scientists on the protocols that should be adopted for the analysis of human semen, but also enable clinicians to use these data to present patients with an estimate of the 'percentage probability of conception'. This is a major step forward for laboratory andrology and the Centre was centrally involved in the preparation of this text on behalf of the World Health Organization. It is anticipated that the manual will be published in the middle of 2009.

The Centre's CIs continue to win national and international accolades for their research performance. This year Peter Koopman was admitted to the Australian Academy of Science and also won the Lemberg Medal from the Australian Society of Biochemistry and Molecular Biology. Moira O'Bryan was also elected the Young-Andrologist-of-the-Year by the American Society of Andrology. This is the second time a Centre CI has won this prestigious award in the last 5 years. Given the weight of competition from all of the major laboratories in the United States and elsewhere, this is a truly remarkable achievement.

Of particular importance this year has been the achievements of our student population. We have recruited 10 new PhD students, 3 of whom have received Faculty Medals and one a VC's award for Outstanding Research Candidature as well as a University medal. Of our existing postgraduate population several have won important awards. For example Yun Hua Lee won the Merck-Serono ART Young Investigator award for 2008 at the Combined ASPIRE2008 conference in Singapore as well as the Third year PhD student Research Excellence Award at University of Newcastle Post Graduate Conference. Matt Dun won the 2008 Oozoa

Student Award at the annual meeting of the Society for Reproductive Biology. Adam Koppers was awarded the Second year PhD student Research Excellence Award at University of Newcastle Post Graduate Conference as well as a Faculty award for Outstanding Postgraduate Research Student Achievement. Duangporn Jamsai continues her distinguished career by winning a 2008 Outstanding Trainee Investigator Award from the American Society of Andrology as well as prestigious travel awards from both the International Society of Andrology and the American Society of Andrology. These and other awards to our student population demonstrate the attractiveness of the Centre as a rich, exciting training environment for postgraduate scientists. The fact that we are attracting such excellent students also bodes well not just for the future of our Centre but also for the future of reproductive/developmental biology in Australia. We live at a time when at least 1 in 20 of the male population is infertile and diseases of the male reproductive tract, particularly testicular cancer, are increasing at unprecedented rates. We also live at a time when 1 in every 35 babies born in Australia is the product of assisted reproductive technology and when significant paternal contributions to the risks associated with this form of treatment has been discovered. The lack of research into fertility regulation also means that the contraceptive needs of some 200 million couples go unmet every year and our environment is being overrun by pest animal species whose reproductive capacity is beyond our control. To address such issues we need to recruit and train a new generation of reproductive biologists who are passionate about unraveling the molecular mechanisms that regulate the differentiation and function of germ cells. In this context, our Centre of Excellence is playing an extremely important role.



**Professor R.J. Aitken**

PhD, ScD, FRSE

Director

## Governance and Structure

### Scientific Management Group

This Group is composed of the Centre Director, Chief Investigators and Associate Chief Investigators participating in the Centre.

#### University of Newcastle



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John Aitken graduated from the University of London in 1969 and subsequently undertook a PhD in reproductive biology at the University of Cambridge under the supervision of RV Short. Following post-doctoral positions at the Institute of Animal Genetics, University of Edinburgh and the University of Bordeaux, John accepted an invitation to join the World Health Organization in Geneva, where he acted as manager of two task forces dealing with embryo implantation and ovum transport respectively. In 1977, John Aitken took up a position with the MRC Reproductive Biology Unit, University of Edinburgh to establish a research group in gamete and developmental biology. In 1992 he was awarded an Honorary Professorship within the Faculty of Medicine of Edinburgh University, and in 1995 was elected a Fellow of the Royal Society of Edinburgh. In 1998 he received a ScD degree from the University of Cambridge and in the same year moved to the University of Newcastle, NSW, as chair of Biological Sciences and Foundation Director of the Centre for Life Sciences. He was subsequently appointed as Head of the School of Biological and Chemical Sciences before becoming Director of the ARC Centre of Excellence in Biotechnology and Development in 2003.

He has published more than 400 articles, which have received more than 9000 citations, given over 200 invited lectures and filed 8 patents. He has held industrial consultancies with a number of major pharmaceutical companies, including Organon, Schering and London International and is actively engaged in the establishment of commercial entities associated with the ARC Centre of Excellence. Examples of professional awards include the Walpole prize (Society for the Study of Fertility) in consecutive years (1986, 1987), the Puvan Memorial Lecture (Royal Malaysian College of Obstetrics and Gynecology) Bruce Stewart Memorial Lecture (American Society of Reproductive Medicine) the Amoroso lecture (Society for the Study of Fertility) the Jennifer Hallam Memorial Lecture (Family Planning Association of the United Kingdom) and the MJ Edwards lecture (Australian Birth Defects Society). In 2003 he gave the Lloyd Cox Memorial lecture to the University of Adelaide, and in 2004 delivered the annual Founders Lecture to the Society for Reproductive Biology in Sydney. In 2005 he received the ST Huang-Chan Memorial Medal from the University of Hong Kong, was appointed a Laureate Professor by the University of Newcastle and received the annual award for research excellence from the Hunter Medical Research Institute. In 2006, he delivered the Keynote Address to the American Society of Andrology in Chicago and was awarded the 2006 Award for Research Excellence by the Faculty of Science and IT, University of Newcastle. Last year he gave a Keynote address to the Frontiers in Bioscience Symposium convened to celebrate the 120th Anniversary of the Faculty of Medicine, University of Hong Kong and recently delivered the Inaugural Anne McLaren Lecture at the Fertility 2009 conference in Edinburgh.

## University of Newcastle



### **Eileen McLaughlin**

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Eileen McLaughlin is Deputy Head of the School of Environmental & Life Sciences at the University of Newcastle and a Deputy Director of the Priority Research Centre in Reproductive Science. She brings to the group extensive research experience in the cellular and molecular aspects of reproduction as well as a deep knowledge of assisted conception practices in both human and animal contexts. Eileen has published over 70 peer review papers and prepared a number of book chapters on aspects of male and female reproduction. Her research interests cover the cell biology of male germ cells and the molecular mechanisms regulating the development of primordial follicles within the ovary. Within the Centre she is part of the team of scientists driving our research program in spermatogonial stem cells. She also provides the Centre with valued technical expertise in confocal microscopy, second messenger imaging and phage display technology.

Prior to arriving in Newcastle, Eileen was a Lecturer in the Division of Obstetrics & Gynaecology at the University of Bristol, UK where she was a founder member of the Centre for Reproductive Medicine initiated by the late Professor Michael Hull. During her postdoctoral training, Eileen completed a Wellcome Trust Research Fellowship with Professor Len Hall in the Molecular Genetics Unit, University of Bristol, and a Research Fellowship at the Pest Animal Control CRC within the CSIRO Sustainable Ecosystems Biotechnology for Agriculture programme. In addition to undergraduate teaching at University of Bristol she has been a guest lecturer in Reproduction for the University of Warwick, UK and the University of Krems, Austria and lecturer and external examiner for University of Nottingham, UK.

Outside of the laboratory, her scientific and administrative skills have been recognised by election as Chairman of the British Andrology Society (1997-2000) and appointment as a Director of the Journal of Reproduction & Fertility (Ltd.). Additionally, she has served as member of the British Fertility Society Committee, Association of Clinical Embryologists Executive Committee and as Meetings Secretary of the Society for Low Temperature Biology. With a strong interest in Reproductive Medicine, she has edited multiple peer-reviewed clinical guidelines and was an advisor to the UK Government Department of Health and the Human Fertilisation and Embryology Authority. Currently Eileen serves on the Council of the Society for Reproductive Biology and is a member of the Program Organising Committee. Eileen has received research based awards from the British Fertility Society and the Society for Reproductive Biology from whom she received the 2007 Research Centre for Reproductive Health Award for Excellence in Reproductive Biology Research for outstanding contributions by a researcher to the discipline of reproductive biology.

## University of Newcastle



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Shaun Roman obtained his PhD in Medicine in 1996 from the UNSW for studies undertaken at the Garvan Institute of Medical Research and the Department of Medical Oncology, Westmead Hospital. His doctoral studies focused on the role of Vitamin A signalling in breast cancer. In subsequent postdoctoral research at Cornell University Medical College (NY, USA) Shaun investigated the role of Vitamin A metabolism in embryonic stem cell differentiation. Shaun has an ongoing interest in cellular differentiation and gene expression. He has published in a series of leading journals including PNAS, JBC, Dev Biol and Cancer Research.

Shaun joined the University of Newcastle as Associate lecturer in Biological Sciences in 1998 and became one of the founding members of the Reproductive Science Group. Shaun is currently Program Convenor for B. Biotechnology at the University of Newcastle and coordinates the placement of third year undergraduates into the Biotechnology industry.

In 2000, he was nominated for the ASRB award for young researcher of the year. Dr Roman brings to the Centre extensive knowledge and experience in molecular biology and participates in two CBD programs dealing with the isolation, characterisation and differentiation of spermatogonial stem cells and the causes and consequences of DNA damage in the male germ line.

## Monash University



### Michael Holland

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Michael Holland graduated (1974) with First Class Honours in molecular genetics from the University of Sydney. He undertook a PhD in reproductive physiology studying spermicidal and embryocidal of IUDs at the same Institution. This research saw the beginning of an interest in sperm molecular biology. Michael pursued this interest as a World Health Organisation Fellow and then with a NIH Fogarty International Fellowship at the University of Pennsylvania where he became an Assistant Professor before returning to a lectureship at the University of Adelaide. Here Michael's interest in sperm molecular biology led him to research the membrane changes sperm undergo as they acquire fertilising capacity in the epididymis.

Michael returned to Vanderbilt University on a Mellon Foundation Faculty Development Award where he joined the NIH Centre in Male Reproductive Biology but after 8 years returned to Australia to CSIRO. He became a Chief Investigator on one of the first CRCs (Vertebrate Biocontrol CRC) funded in 1992. Within CSIRO Michael was promoted to Principal, and then Senior Principal Research Scientist. At the same time he became an adjunct Professor at Utah State University where he worked and taught in the College of Natural Resources and the Biotechnology Centre. In July 2002 Michael joined Monash Institute of Reproduction & Development as a Professorial fellow where he became initially Director of the Centre for Early Human Development.

Michael's research at Monash has focused around his role as Leader of the Reproductive Technologies Program in the Dairy CRC. His contributions have been in the area of cloning technology with a focus on epigenetic factors affecting the development of cloned embryos. The ARC Centre of Excellence has provided him to work on germ cell transplantation and his long term interest in the molecular biology of sperm maturation in the epididymis.

Michael has always maintained a strong commitment to professional service through involvement in professional societies, membership of editorial boards etc. Currently he is President of the (Australian) Society of Reproductive Biology and serves as a member of the Executive of the American Society of Andrology.

Michael has edited or co-edited three books and has over 100 peer reviewed papers and in excess of 250 conference presentations. He has held grants in excess of \$70 million from the NIH, the NSF, ARC and many private Foundations and Government schemes, including 3 CRCs.

## Monash University



**David Jans**

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David Andrew Jans graduated from the University of Melbourne, Department of Microbiology, in 1980 and then joined the Department of Biochemistry at the John Curtin School of Medical Research (JCSMR) to carry out his PhD studies with Graeme Cox and Frank Gibson on bacterial ATPase. His first post-doctoral position was at the Friedrich Miescher Institut in Basel (Switzerland), followed by a visiting fellowship at the Max Planck Institut für Biophysik in Frankfurt am Main (Germany), working in the area of phosphorylation and signal transduction in mammalian cells. In 1990, he became a Senior Scientist at the Institut für Medizinische Physik und Biophysik, Westfälische Wilhelms-Universität in Münster (Germany), turning his focus to the use of confocal microscopic techniques to investigate transport processes. He returned to the JCSMR in 1993, initially as a Fellow, to establish the Nuclear Signalling Laboratory with a focus on protein transport into the nucleus. He rose to Senior Fellow in 1998, Full Professor in 2000, and since 1998 has also held a conjoint teaching appointment (Associate Professor/Professor) at the James Cook University of North Queensland (Townsville, Australia). In 2001, he moved to the Department of Biochemistry and Molecular Biology at Monash University, where he holds an NHMRC Principal Research Fellowship and a personal chair.

Of a total of 13 awards, his most recent include the IRPC (International Research Progress Council) Eminent Scientist of the Year Award for Molecular Biology in 2000, the GE Healthcare Bio-Sciences Award for Innovation in Research from the ASBMB (Australian Society for Biochemistry and Molecular Biology) in 2005, and Japan Society for the Promotion of Science senior fellowship (2008). He has been awarded 34 grants since 1999 (c. \$ 23.5 million AUS.) including awards from the Australian Research Council, UICC (International Union against Cancer), NHMRC (National Health and Medical Research Council), and Wellcome Foundation. In collaboration with Professor Sobolev and his group at Moscow State University (Russia) he is co-author of a patent which won a Medaille d'Or avec Mention at the 45th World Salon of Inventions – "Brussels – Eureka 96". He has been invited to speak at 46 national/international meetings since 1998, including 9 plenaries, and has a total of 234 full publications in refereed journals in the fields of bioenergetics, signal transduction, biochemical and genetic analysis of membrane receptors, phosphorylation, nuclear protein transport, photodynamic and gene therapy, and the role of nuclear transport in development. He has also published a monograph ("The mobile receptor hypothesis: the role of membrane receptor lateral movement in signal transduction"). In 2005, he was elected as a committee member for the International Photodynamics Association and in 2006 as a full member of the editorial board of Biochemical Journal (UK).

## Monash University



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Kate Loveland is a Senior Research Fellow of the NHMRC, working as a Senior Scientist and Laboratory Head at the Monash Institute of Medical Research at Monash University. Her PhD studies at Duke University (USA) identified proteins on mouse sperm involved in fertilisation and demonstrated how they changed during epididymal maturation. She investigated mechanisms of intracellular trafficking during a postdoctoral fellowship at the Howard Hughes Medical Institute in Dallas (USA) and moved to Monash University, Melbourne Australia in 1989. In work beginning in 1994, she has investigated the mechanisms that underpin spermatogenesis and testis development. Her current research objective is to understand the basis of normal and disrupted postnatal testis development, examining how the coordinated actions of hormones and growth factors mediate communication between germ cells and their supporting Sertoli cells. Her lab group has uncovered discrete patterns of expression and function for molecules that affect cell fate decisions in germ and somatic cell lineages, including TGF-beta superfamily ligands, Bcl-2 family proteins, and nuclear transport components. In addition to holding continuous grant support from the NHMRC, she has held funding from the Australian Research Council, Victorian Health Promotion Foundation, and the Wellcome Trust Foundation. Kate received the Young Andrologist Award from the American Society for Andrology in 2004 for her contribution to this field. In addition to active involvement in undergraduate and postgraduate teaching, she has served as Program Organising Chair (2001-2003) and Treasurer (2003-2006) for the Society for Reproductive Biology, and she is co-Chair of the 2010 Program Committee for the American Society for Andrology Annual Scientific Conference. Her work within the ARC Centre of Excellence in Biotechnology and Development addresses the roles of nuclear transport in germ line genesis and male fertility and on regulation of spermatogonial differentiation cues.

## Monash University



### Moira O'Bryan

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Associate Professor Moira O'Bryan is a NHMRC Senior Research Fellow whose research is focused on the identification of key regulatory processes in sperm development and function. Through the marrying of mouse models of male infertility and the analysis of infertile human samples, this research has direct relevance to human health. Moira received her PhD from The University of Melbourne, Department of Medicine (St. Vincent's Hospital) in 1994 in the area of complement regulation and its relevance to human male fertility. She then moved to The Population Council in New York as an Andrew Mellon Foundation Post-doctoral Fellow. It was at this time that she commenced work on the production and biological significance of nitric oxide within the testis and sperm; work which eventually saw her return to Australia, at Monash Institute of Reproduction and Development (Monash University) in 1996 as a NHMRC Peter Doherty Fellow. Since this time Moira has received several fellowships and has published extensively in the areas of the interaction between the immune system and male fertility, endocrine regulation, the identification and characterization of novel sperm tail proteins and the genetics and epigenetics of male infertility. In 2009 Moira took up the position of Deputy Head of Department at the Department of Anatomy and Developmental Biology at Monash University.

Moira has received research based awards from the Australian Academy of Science, the Fertility Society of Australia, the Endocrine Society of Australia, and the Society for Reproductive Biology from whom she received the inaugural 2006 Research Centre for Reproductive Health Award for Excellence in Reproductive Biology Research for outstanding contributions by a mid-career researcher to the discipline of reproductive biology. Moira was also named as the 2008 "Young Andrologist of the Year" by the American Society for Andrology. Moira and colleagues also received the 2006 Monash University Industry/Community Engagement via Research Contract Award for their productive and prolonged interaction with Bayer-Schering AG (Berlin) in the area of men's health. Moira is the convener of The Australian Phenomics Network and sits on 'The Male Infertility Advisory Board' of Andrology Australia.

Within the ARC Centre of Excellence in Biotechnology and Development, Moira's work is focused on the development and analysis of ENU induced mouse models of infertility and the mechanisms of sperm maturation. This work has relevance for the diagnosis and treatment of human male infertility and the development of urgently required sperm based contraceptives.

## University of Melbourne



### **Gary Hime**

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Gary Hime received his PhD in 1994 from the University of Adelaide where he studied, with Professor R. Saint, the developmental control of cytokinesis in the *Drosophila* embryo. He entered the field of reproductive biology as a C.J. Martin Fellow at Stanford University where he studied aspects of *Drosophila* spermatogenesis in the laboratory of Dr Margaret Fuller, Department of Developmental Biology. While at Stanford, Gary also continued his interest in cell division and characterised a gene required for meiotic cytokinesis. As part of this work he developed a novel assay for detection of actin filaments in the *Drosophila* testis and assisted the laboratory of Dr Chris Bazinet, St. John's University, New York to adapt this assay for the study of sperm individualisation.

Upon his return to Australia Gary set up a *Drosophila* facility with Professor David Bowtell at the Peter MacCallum Cancer Institute. In this position he was able to utilise the genetic tools available in *Drosophila* to dissect oncogene function. He is now a Senior Lecturer in the Department of Anatomy and Cell Biology, University of Melbourne, where he uses *Drosophila* as a genetic model for the study of stem cells and human disease genes and, in his role as a Centre CI, the regulation of spermatogenesis.

## University of Melbourne



### **Andrew Sinclair**

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Andrew Sinclair is an NHMRC Principal Research Fellow, Professor in the Department of Paediatrics, University of Melbourne and Director of Early Development and Disease at the Murdoch Children's Research Institute, Royal Children's Hospital. In this latter role he has strategic and financial management of eight research groups with a total of 140 researchers. His own research focuses on understanding the molecular genetics of sex determination. He isolated the human Y-linked testis-determining gene, SRY to great international acclaim and showed genetically that it was required for normal testis development, as mutations in this gene resulted in dysgenic testis and XY sex reversal. Since then he has continued to work on this gene and unravelling the molecular network regulating gonad development. His group has identified new genes in this regulatory pathway and has assessed their roles in vitro, positioning them in the sex determination network in vivo. The role of these genes in human disorders of sexual development is also being assessed. This fundamental work is complemented and enhanced by clinical collaborations at the Royal Children's Hospital. His work continues to provide new insights into the molecular genetic regulation of testis and ovary development. He has won numerous awards and published extensively, including six papers in Nature. One of these publications has become a citation classic with over 1,000 citations. Andrew was President of the Australian Society for Medical Research in 2004, and led national advocacy efforts seeking increased Federal Government funding of health and medical research. In 2005, the Federal Minister for Health appointed Andrew to the Australian Biotechnology Advisory Council. This Council provides high-level independent advice to the Ministers for Health, Education & Science, and Industry.

## University of Queensland



### **Peter Koopman**

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Peter Koopman is head of the Division of Genetics and Developmental Biology at the Institute for Molecular Bioscience. His research focuses on genes that regulate embryonic development, with special emphasis on the control of sexual dimorphism and gonadal development. He was part of the team that discovered the Y-chromosomal sex – determining gene Sry: his work showing that Sry can sex-reverse an XX transgenic mouse was hailed as one of the most important breakthroughs in genetics of the 20th century. This work has led to the identification of many other genes important for normal sexual development. Professor Koopman's laboratory remains one of the major world centres of research on Sox genes, a family of genes related to Sry and important for diverse aspects of embryonic development. His team discovered Sox9, a critical gene for gonadal and skeletal development, and Sox18, a major control gene for blood and lymphatic vessel development. More recently his group have discovered the molecular pathway by which germ cell fate is specified, a discovery that may be applicable to modulating human or animal fertility in vivo and production of functional gametes from germ line stem cells in vitro.

Professor Koopman is author of over 160 papers, including 5 in *Nature*, 7 in *Nature Genetics*, 2 in *Cell*, and 1 in *Science*, which together have been cited 7000 times in the literature. He is on the editorial board of six international journals including *Developmental Dynamics*, *Nature Reviews Genetics*, *Sexual Development*, *Mechanisms of Development*, *Gene Expression Patterns* and *Biology of Cell*. Professor Koopman received the AMP Queensland Biomedical Research Award in 1992, the Julian Wells Medal in 1998, the Amersham-Pharmacia Biotech Medal in 2003, the President's Medal of the Australia and New Zealand Society for Cell and Developmental Biology in 2005, the GSK Australia award for Research Excellence in 2007 and the Lemberg Medal in 2009. He is a Federation Fellow of the Australian Research Council and a Fellow of the Australian Academy of Science.

## Associate Members



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Dr. Baker graduated at Monash University, Melbourne Australia, majoring in both Biochemistry and Chemistry. Mark accepted a position in the Biochemistry and Molecular Biology department undertaking an honours project with Dr Alfons Lawen, studying the biochemical mechanisms responsible for the mediation of apoptosis. After receiving an H1 degree and publishing his results in the journal "Apoptosis", he was awarded an Australian Postgraduate Award to embark on a PhD. Although continuing his studies with Dr Lawen, Mark decided on a complete change of project, this time researching plasma membrane electron transport systems. During his PhD, he purified, identified and then cloned, for the first time ever, a trans-plasma membrane NADH-reductase, namely Porin 1. This finding has excited many researchers in the plasma membrane oxido-reductase field and has led to extensive collaborations and invitations, worldwide. Mark was then invited to work with Professor John Aitken at the University of Newcastle on the role of such systems in the generation reactive oxygen species by male germ cells. He managed to identify the enzymes responsible for the NADH and NADPH-dependent reduction of lucigenin, work that has helped clarify many uncertainties in this area. To further enhance his skills, Mark has pioneered new areas of research within the Aitken Lab, down the path of advanced proteomics. In particular, he has introduced Difference in 2D-gel Electrophoresis (DIGE) and label-free LC-MS/MS quantitation to determine proteins that become functionally modified in spermatozoa. This work has led to numerous publications and two international awards – a Postgraduate Trainee Merit Award and a Lalor International travel award, which were presented at the 2005 American Andrology Society meeting in Seattle. The proteomic platform that Mark has created at the University of Newcastle is recognized worldwide and resulted in many invitations to speak at scientific meetings overseas including the Lorne proteomics conference, the prestigious Gordon conference on Fertilization and Activation of Development, the AOHUPO conference in Cairns and recently, the 2009 HUPO mass spectrometry convention in Berlin.

**Josephine Bowles**

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Jo Bowles received a PhD from the University of Queensland in 1993. She then joined the group of Professor Peter Koopman to undertake postdoctoral studies at the Centre for Molecular and Cellular Biology (now Institute for Molecular Bioscience), University of Queensland, working in the fields of mouse sex determination, gonad development and SOX gene biology. Twenty primary research publications, including two in *Nature Genetics* have resulted from this work. Jo was awarded an NHMRC Australian Postdoctoral Fellowship (1994-1997), and has subsequently held two NHMRC project grants as CIA.

In recent years, Jo has focused on the germ cell component of the developing mouse gonads. Her findings, that germ cell meiosis is induced by retinoic acid, and that this is prevented in the male gonad by the actions of a P450 enzyme, CYP26B1, were published in *Science* in 2006. Current studies are centered on understanding the cellular mechanisms of meiotic induction and avoidance in fetal germ cells.



**Duangporn Jamsai**

PhD, NHMRC Peter Doherty Postdoctoral Fellow  
Senior Research Officer  
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Duangporn was awarded a highly competitive Royal Golden Jubilee PhD Scholarship from the Thailand Research Fund and a grant from the Thalassaemia Society of Victoria to undertake a PhD at Mahidol University (Thailand) and at the Murdoch Childrens Research Institute (Melbourne, Australia). Her PhD research in the field of beta-thalassaemia has generated nine publications (five as first author). The outstanding quality of her PhD research was recognised by a number of prestigious accolades including the 2004 Mahidol University Distinguished Thesis Award and the Thai Society for Biotechnology Thesis Award. These awards recognise the best PhD thesis in the field of Medical Science. Moreover, she was awarded the 2004 National Research Council of Thailand Thesis Award which recognises the best PhD thesis in the field of Medical Science by a Thai citizen worldwide.

Following the completion of her PhD in October 2003, she then joined the laboratory of Assoc. Prof. Moira O'Bryan at the Monash Institute of Reproduction and Development as a Postdoctoral Fellow. Her research focuses on understanding the causes of male infertility by analysing the underlying genetics and biochemistry which control sperm formation and function using a number of infertile mouse models which has resulted in three recently publications. Her outstanding achievement as an early career scientist was recognised by the awarding of a NHMRC Peter Doherty Postdoctoral Fellowship for funding 2006-2010.

In addition to the awards associated with her PhD, she was awarded a number of travel awards (i.e. from the Faculty of Medicine and Nursing Health Sciences (Monash University) in 2005 and 2009, The North American Testis Workshop in 2006, The Endocrine Society of Australia (ESA) in 2007, The Ian Potter Foundation in 2007, The Contributing to Australian Scholarship & Science (CASS) Foundation in 2008, The International Liaison Committee of the American Society of Andrology (ASA) in 2008, and The Lalor Foundation in 2009) to present her research at several international conferences. Most recently, she was awarded the Outstanding Trainee Investigator Award at the ASA Annual Meeting held in USA in April 2008. The award is given to the ASA trainee member with the best abstract and research presentation at the ASA Annual Meeting.

**Brett Nixon**

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Brett Nixon received his PhD in 1999 from the University of Newcastle working in the area of epididymal sperm maturation and the identification of target proteins for immunocontraceptive development. He then moved to the Department of Cell Biology, Emory University (GA, USA) as an NIH Post-doctoral fellow where he initiated his research on the molecular basis of sperm-egg interaction. Since his return to the Discipline of Biological Sciences at the University of Newcastle as a Lecturer in 2001, Brett has established an active research program with a focus on identifying and characterising the proteins responsible for mediating sperm-zona pellucida recognition in both the human and mouse models.

Brett's contribution to the field has been recognised by the award of the Student Travel Grant for his presentation at the 23rd Annual meeting of the American Society of Andrology, Long Beach, CA, USA (1998) and the fact that he was a finalist in the Junior Scientist Competition at the 30th Annual Conference of the Australian Society for Reproductive Biology (1998). More recently, Brett was awarded the prestigious Lalor Foundation Travel Award for his presentation at the 31st Annual meeting of the American Society of Andrology, Chicago, IL, USA (2006). He was also an invited discussant in the inaugural Novartis Foundation symposium on 'The Biology of Extracellular Molecular Chaperones' (London, June 2007).

## Senior Executive

The Senior Executive is comprised of one representative from each of the core participating institutions. The four members of the Executive are responsible for the management of funds within their respective organizations and preparing annual reports on the expenditure of those funds.

University of Newcastle	R. John Aitken
University of Queensland	Peter Koopman
Monash University	Kate Loveland
University of Melbourne	Andrew Sinclair

This year Kate Loveland was elected Deputy Director of the Centre.

## Scientific Advisory Board

The Scientific Advisory Board met in conjunction with our Scientific Management Group during the Annual Scientific meeting at Sofitel Werribee Park Mansion Hotel and Spa in Melbourne during the period 7-10 September 2008. The Scientific Advisory Board provided extremely valuable, positive, feedback on the international competitiveness of our fundamental scientific research program.





**Sue Forrest**

Sue Forrest is Director/CEO of the Australian Genome Research Facility Ltd. She has in total published over 100 papers in international journals, one review article on Friedreichs ataxia, one invited book chapter on atopic dermatitis, one invited book chapter on Next-Generation Genome Sequencing and edited a book on mutation detection.

She is a Member of the Biotechnology Strategic Development Plan Infrastructure Working Group (BSDP) for the State Government of Victoria, the Chair of the Scientific Advisory Board, ARC Centre of Excellence in Biotechnology and development and the Genomics Platform Convenor of BioPlatforms Australia.

She has over 20 years experience in genetics and molecular biology including research into both single gene defects and complex traits including neuromuscular and behavioral disorders.

During her D.Phil, she cloned the gene responsible for Duchenne muscular dystrophy and then headed the DNA diagnostic laboratory at MCRI for seven years.

Her role as CEO of AGRF has enabled the growth of large scale genomic science in Australian including the first large genome sequencing project, that of the Tammar Wallaby, funded by the State Government of Victoria and the National Institutes of Health (NIH).



**Professor David de Kretser**

Professor David de Kretser is Governor of Victoria, and former Director of the Monash Institute of Medical Research, Andrology Australia and Associate Dean Biotechnology Development. He was the Founding Director of the Monash Institute of Reproduction and Development. He received his MBBS (1962) from the University of Melbourne and his MD (1969) from Monash University for a thesis entitled "Studies on the structure and function of the Human Testis". He is a Fellow of the Royal Australasian College of Physicians, a Fellow of the Australian Academy of Science and a Fellow of the Australian Academy of Technological Sciences and Engineering. He was admitted as an Officer in the Order of Australia in 2000 and was appointed a Sir John Monash Distinguished Professor in 2003.

His research in the field of reproductive biology, infertility and endocrinology is internationally recognised. Over the past decade he has co-directed a programme of research into the isolation and biology of the inhibin related proteins and has led to numerous studies of the role of these proteins in reproductive biology and other systems. His current research interests also include investigations into genetic causes of male infertility.

As Associate Dean Biotechnology, he sat on the Boards of several companies involved in commercialising biomedical and biotechnology applications. These include Monash IVF, CopyRat and IngenKO, the latter representing "start-up" companies involved in rodent transgenic technologies.

In April 2006 David took up a 5 year appointment as Governor of Victoria.

**Patrick Tam**

Patrick Tam is the Deputy Director and Head of the Embryology Research Unit at the Children's Medical Research Institute of the University of Sydney. He is an NHMRC Senior Principal Research Fellow, a Professor and Senior Principal Research Fellow in the Faculty of Medicine, University of Sydney and an Honorary Professor of the Faculty of Medicine, the University of Hong Kong. His research currently focuses on the molecular and cellular mechanisms of embryonic patterning, and the genetic and signalling activity controlling head, orofacial and gut morphogenesis. Patrick also studies the developmental basis of malformations of the eyes and X-linked congenital neurological diseases using mutant mouse models generated by transgenesis and gene targeting. Patrick is an Editor of *Development*, an Associate Editor of the *International Journal of Developmental Biology*, a member of the editorial board of *Developmental Cell*, *Developmental Biology*, *Developmental Dynamics*, *Genesis*, *Differentiation and International Journal of Biological Sciences*, a contributor of the *Faculty of 1000*, and *Highlights Advisor of Nature Reviews Neuroscience*. He has co-edited, with Janet Rossant, a book entitled 'Mouse Development: Patterning, Morphogenesis and Organogenesis' published by Academic Press. He serves on the Scientific Advisory Board of the Max Planck Institute of Molecular Genetics in Berlin, the Genome Institute of Singapore, and the Institute of Molecular Biosciences of the University of Queensland. He is a member of the Scientific Council of the RIKIN Centre for Developmental Biology in Japan, and the Chair of the Scientific Advisory Board of the Australian Stem Cell Centre. Patrick was the 2007 recipient of the President's Medal of the Australian and New Zealand Society of Cell and Developmental Biology and is a Fellow of the Australian Academy of Science.



**Rob Gilchrist**

Dr Robert Gilchrist is an oocyte biologist whose research encompasses basic and applied aspects of ovarian folliculogenesis, oocyte maturation and preimplantation embryo development. In 1996 he completed his D.Sc. Agr. (Magna cum laude) on oocyte maturation in marmoset monkeys at the University of Göttingen in Germany, and then returned to Australia to take up a post-doctoral position at the University of Adelaide. In 2006 he was awarded a NHMRC RD Wright Fellowship and is currently a Senior Lecturer based at the Robinson Institute, University of Adelaide. As CIA he has held three NHMRC Project Grants and an ARC Linkage Grant and is currently a Co-Investigator on a NIH Grant and a NHMRC Program Grant. In 2008 he was Co-Coordinator of the Centre's Honours course.

Dr Gilchrist is established as a fully independent career scientist, heading his own research group of ~6 staff and graduate students. Over the past 11 years he has supervised 8 Honours students and 6 PhD/Masters students to completion, and he currently has 5 PhD students. He conducts basic discovery research on the dynamic interactions between the oocyte and the somatic cells of the ovary as a determinant of subsequent embryonic development, and he also manages an applied research program with the objectives of improving oocyte IVM technologies in animals and women. Dr Gilchrist has published 40 peer-reviewed research publications including 5 reviews and he holds a patent in an oocyte IVM technology. He has a collaborative research agreement with Cook Australia Pty. Ltd., one of the world's largest medical device manufacturers for clinical IVF products, on the development of novel products, including oocyte collection and maturation media, for the treatment of human infertility. Dr Gilchrist is also a member of the Scientific Advisory Board of an infertility clinic, Repromed Pty. Ltd.



**Phil Robinson**

Phil Robinson is Head of the Cell Signalling Unit at the Children's Medical Research Institute, Sydney, an NHMRC Senior Principal Research Fellow and Professor at the Universities of Sydney and Newcastle. His BSc(Hons) was at Sydney University and he completed a PhD in Medical Biochemistry with Peter Dunkley at Newcastle University on protein phosphorylation in synaptic transmission. After an NIH Fellowship and a postdoctoral position at the University of Cincinnati he returned to Australia as an Australian Postdoctoral Fellow and a QEII Fellow. He developed an independent research team in 1990 in Newcastle, and then established his Unit in 1996 at the CMRI in Sydney. He now runs a team of 25 students and postdocs.

Phil has made major research contributions to understanding some of the multiple roles of signal transduction in regulation of synaptic transmission. He has been defining the biochemistry of endocytosis. This is now resulting in an understanding of how the protein phosphorylation network controls the protein machinery underlying nerve communication. The mechanisms controlling endocytosis have been shown in his laboratory to converge on the protein dynamin. Work in his lab contributed to the initial discovery of dynamin, cloning of the three dynamin genes, and in determining its role as a phosphoprotein that is rapidly dephosphorylated on stimulation of nerve terminals leading to the initiation of endocytosis. His lab together with that of A/Professor Adam McCluskey at the University of Newcastle has been developing a pharmacological-based approach to endocytosis. Their team has developed the world's first dynamin and endocytosis inhibitors. These compounds may ultimately have important applications for epilepsy and cancer.

He has received a number of awards during his career including the AW Campbell award for Excellence in Neuroscience from the Australian Neuroscience Society and the Amersham Pharmacia Biotechnology Medal from the Australian Society for Biochemistry and Molecular Biology. Phil was Chair of the Fellowships Committee (RFC) of the NHMRC and a member of the Research Committee (RC) and the Training and Awards Committee (TAC) for 3 years. He is on the editorial board of a number of journals including the Journal of Biological Chemistry (JBC).

## Steering Committee

The Steering Committee has been created to exploit the elements within our overall research program that have commercial potential or are of practical value to our end users – i.e. translational research.

- **David Adamthwaite**  
Adams Pluck, Patent and Trade Mark Attorneys
- **Brent Jenkins PhD**  
CEO, Tunra
- **Peter Illingworth MD (Hons),  
FRCOG, FRANZCOG, CREI**  
Medical Director of IVF Australia
- **Anna Martorana PhD**  
Adams Pluck, Patent and Trade Mark Attorneys
- **Tony Peacock PhD**  
CEO of the Australasian Invasive Animal  
Co-operative Research Centre
- **Christian Touli PhD**  
Business Development Manager, BioLink
- **Tim Wawn MA**  
CEO of Applimex Systems, a Sydney based  
biotech company spun out of Macquarie University

In the past year we undertook a major review of our commercialization potential with the aid of an audit conducted by **Biolink**, a biotechnology commercialization company based in Sydney and Melbourne. The results of this audit were presented at the Annual Scientific Meeting in Werribee. The survey amounted to an exhaustive review of our existing IP portfolio and a detailed description of the areas where commercialization potential exists.

The Steering committee will be instrumental in helping the Centre to exploit the commercial leads identified by Biolink.



## Centre Scholar

The current Centre Scholar is Professor Susan Suarez, Professor of Biomedical Sciences from Cornell University New York, USA. Susan is an internationally acclaimed expert on the cell biology of mammalian spermatozoa. When sperm reach the oviduct, they are trapped and held in a reservoir and stored there until ovulation. She has shown that sperm are held in the oviductal reservoir by binding to glycoproteins expressed on the surface of the epithelium lining the oviduct. The complementary proteins on spermatozoa that cause them to bind to these oviductal glycoproteins have also been characterized by Susan's laboratory. When sperm are released from the oviductal reservoir, their flagellar beating pattern switches from symmetrical to asymmetrical. This switch, known as hyperactivation, aids the sperm in penetrating mucus in the oviduct and the zona pellucida of the oocyte. Using a high speed imaging system, Susan has shown that intracellular calcium is increased in hyperactivated sperm from an internal calcium store that she has identified as the redundant nuclear envelope. Furthermore, the calcium released from this store has been shown to interact with calmodulin to activate calmodulin kinase II to switch on hyperactivation. She is now seeking to uncover the remaining elements of the signal transduction pathway in sperm that cause hyperactivation. For this purpose, Susan has decided to spend her sabbatical in the ARC Centre of Excellence in Biotechnology and Development in order to take advantage of our world-class resources and expertise in phospho-proteomics and sperm cell biology. Using chemical reagents to induce specific patterns of hyperactivated movement Susan wishes to establish the relationship between the patterns of flagellar beating and phosphoprotein expression in the axoneme. These experiments should shed new light on the way in which the flagellar wave form is controlled. Such information is strategically important because spermatozoa that are perfect in every respect except they lack the ability to hyperactivate are infertile. Clearly resolving the underlying biochemistry of this process might provide opportunities to develop novel approaches to male contraception and to understand the etiology of spontaneous male infertility, much of which is currently unexplained.



## Patent Activity

The Centre's CIs have established a number of provisional patents during the lifespan of the Centre including:

- **Aitken, R.J.**, Blackmore, D., and McLaughlin, E.A. (2004) Patent No. 2004903226. The University of Newcastle Research Associates. Provisional specification for invention entitled Method for reducing the reproductive potential of a female animal. Refiled in 2008.
- **Aitken, R.J.**, and Ainsworth, C. (2004) International patent publication PCT/AU2004/001367. The University of Newcastle Research Associates. Provisional specification for invention entitled 'Gamete Separation'
- **Loveland, K.L.**, O'Hehir, R., Phillips, D.J., and **de Kretser, D.M.** (2004) Australian Provisional patent application No. 2004902056 "Therapeutic Method" (to be cognated with Australian provisional patent No. 2003905461).
- **Loveland, K.L.**, Meachem, S., (PHIMR) and Skinner, M. (2004) (Washington State University, USA) United States Provisional Patent Application entitled "A method of diagnosis".
- **O'Bryan, M.K.**, Hedger, M.P. et al. (2004) Patent No 2004903689. Provisional specification for Apolipoprotein N.
- **Aitken, R.J.** and Griffith R. (2007) The University of Newcastle Research Associates Ltd. Patent no: 2007902839. Contraceptive and Microbicidal compositions. Refiled in 2008
- **Aitken, R.J.** and De Iullis, G.N. (2008) Newcastle Innovation Limited. Patent no: 2008902473. Assay for the assessment of oxidative stress in gametes or embryos

As indicated earlier in this report in 2008 the Centre contracted Biolink, a life sciences commercialisation company based in Sydney and Melbourne, to conduct an IP audit of the Centre's research. This review identified a number commercialization opportunities for the Centre based on:

- Proprietary mouse models
- Proprietary genes and targets
- Proprietary molecules
- Proprietary tools
- Proprietary methods
- Proprietary samples

The IP that will probably generate a return to the Centre in the shortest term is an International patent application [# PCT/AU2004/001367] covering an electrophoretic method for the rapid isolation of highly purified sperm suspensions for assisted conception therapy. The device that forms the subject of this patent has just successfully completed Phase 1 clinical trials at Westmead Hospital which were published in 2008. A commercial entity 'SpermGen' has now been created by the parent company 'NuSep' in order to orchestrate the scaled-up production of devices in 2009 and the initiation of international clinical trials in North America and Europe.

The Centre is actively involved in a number of ventures with commercial companies that should generate further IP including a linkage with the Invasive Animal CRC and its associated commercial entity 'Pestat' on non-surgical sterilization technologies and CSIRO in the area of transgenic animal production.



## ▶▶ **Review of Research Activities**

The research program instigated by the Centre has been divided into 4 major these. These themes have been defined in order to facilitate description of the CBD's Scientific Program. They are not rigid subdivisions and carry no funding, personnel or institutional connotations.

## Sperm Cell Biology Program

### Sub-program I: DNA damage in the male germ line

Aitken, Jans, Koopman, Roman, O'Bryan

#### Aim

This program of research is designed to determine the causes, nature and consequences of DNA damage in the male germ line.

#### Significance

Human spermatozoa are associated with abnormally high rates of DNA damage. Such damage has been linked with paternal impacts on human health including childhood leukemia, male infertility and testicular cancer. DNA damage in the male germ line is also associated with the etiology of most, if not all, *de novo* dominant genetic mutations in our species giving rise to such conditions as Apert syndrome and achondroplasia.

At present we have no idea how such DNA damage is induced, the specific nature of the damage or the mechanisms by which such damage brings about mutational change. There is strong evidence to suggest that paternal age and a plethora of environmental factors are involved. Clearly resolving these issues is relevant to the development of strategies to diagnose, treat and ultimately prevent these conditions from occurring.

#### Progress

1. Having developed and validated an assay for oxidative base adduct formation in human spermatozoa we have collaborated with IVF Australia to determine how the presence of the 8OHdG (8 hydroxy, 2 deoxyadenosine) correlates with DNA damage measured by other tests of DNA integrity. The results of this study, which have just been submitted for publication, demonstrated extremely tight relationships between DNA damage, the degree of DNA protamination and the formation of 8OHdG in the sperm nucleus.

These results have led us to formulate a 2-step hypothesis for the origins of DNA damage in the male germ line. The first step involves a defect in sperm chromatin remodeling during spermiogenesis. As a result of this defect the spermatozoa are released from the germinal epithelium prematurely and exhibit a compromised phenotype associated with poor morphology, the retention of excess residual cytoplasm and poorly protaminated chromatin. The lack of protamines also means that the DNA in such cells is poorly stabilized by disulphide bonds. Such

lack of stability renders the DNA highly vulnerable to attack. The proposed second step in this process is then a free radical attack on the DNA inducing oxidative base damage and DNA fragmentation (see illustration).

2. This hypothesis raises major questions about the source of free radicals associated with the induction of DNA damage. In the past year we have published a significant paper indicating that most of the free radicals that are damaging to spermatozoa are generated by the sperm mitochondria as a result of electron leakage from the mitochondrial electron transport chain. We have also published cause and effect data demonstrating that when mitochondrial free radical formation is artificially stimulated, then the cells suffer from a state of oxidative stress that compromises their functionality via mechanisms that can be reversed by the concomitant presence of antioxidants.

These data have subsequently prompted research into the mechanisms by which electron transport in sperm mitochondria might be so impaired as to generate a state of oxidative stress. Studies inducted over the past 12 months have identified at least 2 conditions that are associated with the stimulation of mitochondrial ROS (reactive oxygen species) generation in human spermatozoa. The first is the presence of amphipathic molecules such as free polyunsaturated fatty acids or certain retinoids that intercalate into the inner mitochondrial membrane and disturb the ordered flow of electrons. The latter are particularly interesting because the most active of these compounds, 13-cis retinoic acid, is currently used in high doses by dermatologists to treat young men with skin complaints, particularly acne. The impact of this treatment on the quality of their spermatozoa has not previously been investigated. A second scenario we have found to be associated with enhanced mitochondrial ROS generation is exposure of human spermatozoa to radio-frequency electromagnetic radiation.

Over the past 3 years a number of epidemiological reports have appeared suggesting a relationship between mobile phone use and human semen quality. We have recently completed a careful analysis of the impact of radio-frequency electromagnetic radiation on human spermatozoa the results of which have recently been submitted for publication. In this

study, purified human spermatozoa were exposed to radio-frequency electromagnetic radiation (RF-EMR) tuned to 1.8 GHz over a range of specific absorption rates (SAR) from 0.4 W/kg to 27.5 W/kg that cover the exposure levels associated with mobile phones. In step with increasing SAR, motility and vitality were significantly reduced after RF-EMR exposure, while the mitochondrial generation of ROS and DNA fragmentation were significantly elevated. Furthermore, we also observed highly significant relationships between SAR, the oxidative DNA damage bio-marker, 8OHdG, and DNA fragmentation after RF-EMR exposure. We conclude that RF-EMR in both the power density and frequency range of mobile phones enhances mitochondrial ROS generation by human spermatozoa, decreasing the motility and vitality of these cells while stimulating DNA base adduct formation and, ultimately DNA fragmentation. These findings have clear implications for the safety of extensive mobile phone use by males of reproductive age, potentially affecting both their fertility and the health and wellbeing of their offspring.

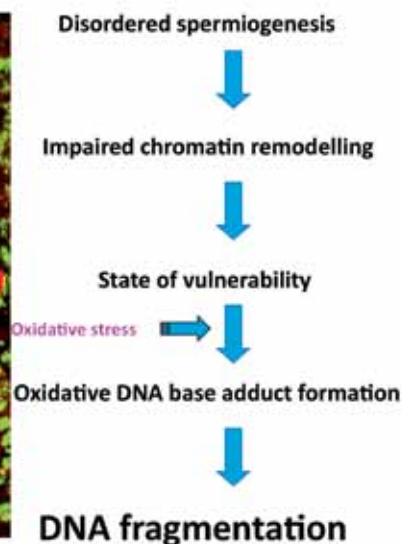
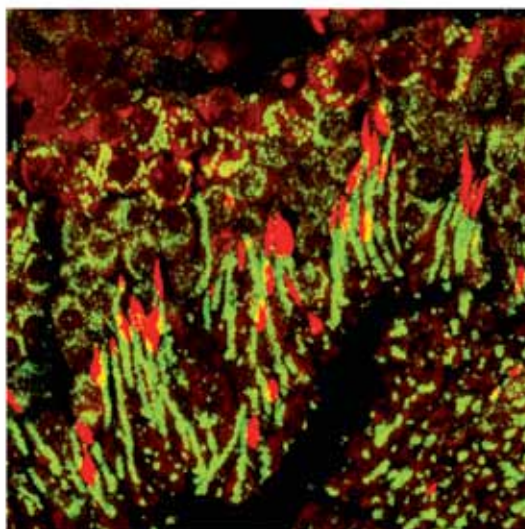
3. Sperm preparation device. If DNA damage in spermatozoa is so potentially harmful to embryonic development and the growth and well-being of the offspring, then it is essential that we develop methods for selecting spermatozoa free of such damage for assisted conception purposes. In this context we have developed and patented a device for the rapid isolation of high quality spermatozoa that successfully completed large scale clinical trials

at Westmead Hospital, Sydney, in the past year. A full commercial roll out of this device is scheduled in the coming year by our commercial partners, NuSep.

4. Many of the basic mechanisms underpinning the sperm development and DNA compaction remain unknown. Like many human conditions, it is predicted that some groups of the population will be more susceptible to DNA damage than others and that there will be a spectrum of susceptibility within the population as a whole. In an effort to improve the fields knowledge of the processes of sperm head formation and DNA compaction, we have screened our randomly mutated mouse libraries for lines displaying abnormal sperm nucleus morphology. To this end we have identified three lines, caused by recessive mutations. In the homozygous state, males are sterile. Over the current year research will focus upon identifying the causal genes and elucidating their role in DNA compaction and genome stability.

We are conducting a detailed analysis of the regions of the human genome that remain packaged by histones and thus susceptible to DNA damage. Using a modified version of the chromatin immunoprecipitation (ChIP) assay and genome tiling arrays we currently have data at a resolution better than one nucleosome across three chromosomes. We have identified numerous regions susceptible to DNA damage. We are currently modifying the ChIP assay even further to allow us to examine DNA packaging in a single ejaculate.

## 2-step Hypothesis



## Future studies

1. We have recently generated evidence for the induction of an apoptotic cascade in human spermatozoa that, while having none of the morphological features of apoptosis in somatic cells, does share some of the biochemical features of the intrinsic, mitochondrial, apoptotic pathway. Specifically, spermatozoa have a tendency to spontaneously default to this form of regulated cell death unless they are prevented from doing so by the activation of a pro-survival pathway regulated by PI3kinase and Akt-1. If either of these enzyme activities is inhibited, an apoptotic cascade is initiated that results in the generation of mitochondrial ROS, oxidative DNA damage, caspase release and DNA fragmentation. Although the endonucleases, CAD and endonuclease G are released and activated during this apoptotic cascade, these enzymes are physically prevented from translocating to the sperm nucleus because of the unique cellular architecture of spermatozoa. The sperm DNA damage characteristic of sub-fertile males must therefore originate from one of two sources; either the DNA fragmentation is entirely oxidative and completely driven by mitochondrial ROS emission or it is endonuclease-dependent and involves (potentially unique) sperm specific endonucleases that are already incorporated into the chromatin during spermatogenesis rather than having to translocate there as in somatic cells. In the next year we shall be addressing these fundamental issues.
2. If a default apoptosis pathway is responsible for loss of sperm cell viability and DNA damage then activators of PI3kinase /Akt-1 should prolong the survival of these cells. This line of investigation has led us to identify factors that dramatically extend the life span of human and murine spermatozoa *in vitro*. These findings have important implications for the storage of mammalian gametes and the establishment of an intellectual property position in this area. These pro-survival pathways will be the subject of intense research over the coming months.
3. We shall continue to examine the impact of environmental factors on DNA damage in the germ line, with emphasis on the mechanisms by which RF-EMR or molecules generated as a result of errors in endogenous metabolism, (excess polyunsaturated fatty acids) or as a consequence of chemical exposure (phthalate esters, heavy metals) stimulate ROS generation by mitochondria.
4. We have an ongoing collaboration with a variety of assisted conception clinics including Repromed, Monash IVF, Hunter IVF and IVF Australia to screen for various kinds of DNA damage in patients' spermatozoa. With these clinical partners we shall be examining the significance of oxidative stress in the etiology of male infertility, the sources of the free radicals that generate this stress and its impact on DNA integrity, fertilizing potential and pregnancy outcome.
5. Over the next 12 months we also intend to conduct a clinical trial focusing on the beneficial effects of antioxidant treatment for the treatment of male patients exhibiting high levels of DNA damage in their spermatozoa. We have developed and validated an assay for recruiting such patients to a trial, and monitoring their response to treatment, based on the formation of the oxidative base adduct, 8OHdG. The trial will be performed as a randomized cross over placebo controlled study in conjunction with several independent assisted conception clinics.
6. In 2009 we anticipate the full commercial roll out of the sperm separation device for the purification of mammalian spermatozoa free of DNA damage.
7. In the coming year we also intend validating an *in vivo* animal model for studying the effect of diabetes on DNA integrity in mammalian spermatozoa. Given the recently-discovered close association between diabetes and DNA damage in human spermatozoa.

## Sub-program 2: Molecular basis of sperm function

Aitken, O'Bryan, Baker, Nixon, McLaughlin

### Aim

To achieve an in depth understanding of the molecular mechanisms that control sperm function.

### Significance

Epididymal maturation and capacitation are of strategic importance in the design of post-testicular methods of male fertility regulation, the development of IVF systems for the preservation of endangered species and elucidation of the causes of male infertility.

### Progress

1. Proteomics platform. As a consequence of establishing the advanced proteomics facility at the University of Newcastle, the Centre has established the first published detailed proteomic profiles of mammalian spermatozoa, providing the scientific community with an invaluable resource of over 1000 proteins that are present in human, rat and mouse spermatozoa.
2. The Centre has also pioneered the use of DeCyder MS software to conduct comparative proteomic analyses and resolve the post-translational changes that take place in the male gamete during the processes of sperm capacitation and epididymal maturation. The first of these studies is now complete and has provided important new insights into the biochemical changes that take place in these cells as they complete their functional maturation. In addition, the Centre has developed the use of titanium dioxide columns to focus on the phosphoproteome since this particular form of post translational modification appears to be critically important for the functional differentiation of mammalian spermatozoa, and is potentially targetable for the purposes of contraception.
3. The proteomics platform is also being used to profile the oocyte and define those cell surface proteins that might be involved in the mediation of sperm-oocyte fusion. Similarly, proteomics is being used to map changes in the male germ line during the specification of primordial germ cells (in a collaboration between the Universities of Queensland and Newcastle) and to search

for biomarkers of reproductive tract cancers, particularly testicular cancer (in collaborations with the University of Copenhagen and Prince Henry's Research Institute, Melbourne).

4. Studies on the epididymal maturation of mammalian spermatozoa have recently been completed by a PhD student, Yun Lee. In these studies Yun demonstrated an important change in sperm mitochondrial function as these cells transit the epididymis. Immature cells in the caput and corpus epididymis have mitochondria that are silenced in that they have no capacity for developing a membrane potential. However as these cells complete their maturation in the cauda epididymis, their mitochondria develop both a membrane potential and a capacity to generate ROS. Such regulation is achieved by means of a soluble inhibitor of mitochondrial function that is present in the fluids of the caput epididymis but is lost/degraded as epididymal maturation proceeds. The effects of this factor are reversible since the addition of caput epididymal fluid to caudal epididymal spermatozoa turns off their mitochondria while washing caput spermatozoa free of epididymal plasma results in mitochondrial activation. The inhibitory factor can also cross species barriers because rat caput epididymal fluid will turn off mitochondrial membrane potential in both mouse and human spermatozoa. Identification of this factor is a major thrust of our current research on epididymal physiology.
5. Research on the molecular mechanisms for sperm-zona interaction has been conducted as a collaboration between Monash University and the University of Newcastle. This research is founded on a novel hypothesis that the ability of spermatozoa to recognize the surface of the zona pellucida during the early stages of fertilization involves the capacitation-dependent expression of a multimeric zona-receptor complex on the sperm surface through the mediation of molecular chaperones. During the past year we have published papers demonstrating that the zona receptor complex is assembled in lipid rafts and that in the mouse, one of the chaperones involved is assembling this complex in chaperonin 10.

6. Signal transduction. Our proteomics platform has allowed us to develop a deeper understanding of the signal transduction pathways that regulate mammalian sperm function. The Centre made a significant contribution to this field by demonstrating the key roles played by the tyrosine kinases, Src and Abl, in mediating the cAMP-induced tyrosine phosphorylation cascade that controls capacitation in the mouse. We now have papers in press that report on the downstream identity of phosphorylated proteins in this signal transduction cascade. In a similar vein we have mapped the totality of post translational changes that occur in spermatozoa as they transit the epididymis and initiated studies on that subset of proteins where the changes are due to alterations in their phosphorylation status.
  7. The proteomics platform has also been extremely valuable in comparing the proteomic profiles of spermatozoa from fertile individuals and patients with defined functional lesions in their spermatozoa, and specifically a failure of sperm-zona recognition. Such a comparison has revealed a number of highly significant changes, one of the most important of which is in the status of a chaperone (HSP2A), which has previously been implicated in the process of sperm-zona interaction.
  8. In the past year we have patented a novel approach to spermicide/microbicide development and submitted a paper for publication describing these results. The compounds we have identified immobilize millions of spermatozoa in a matter of seconds without inducing cell death. Moreover these molecules are also active against reproductive tract pathogens such as Chlamydia. We have found that these compounds alkylate key proteins in the sperm tail, specifically AKAP 3 and AKAP4 and prevent all PKA dependent signaling in these cells. Further specifying the mechanism-of-action of these spermstatic compounds is a major component of our future program.
- to undertake 'a systems biology' approach to understanding the cell biology of male germ cells through the careful integration of metabolomic and proteomic data. This research tool is already being used to extend our knowledge of sperm cell biology but ultimately this approach will be of relevance to many aspects of the Centre's research program.
2. Use our proteomics platform to characterize the targets of Src/Abl mediated tyrosine phosphorylation during capacitation and further resolve the molecular mechanisms responsible for driving the functional maturation of these cells.
  3. Complete our studies on the molecular basis of defective zona binding in male patients with emphasis on the involvement of HSP2A.
  4. Further develop our work on the mechanism-of-action of spermstatic compounds in order to understand the chemistry of the sperm immobilization process. Such information will not only help us to refine the chemical structure of the compounds we subsequently take forward for clinical assessment but will also enhance our IP position, since we shall not only be able to protect the compounds we have identified in this research program but also secure protection for their mechanisms-of-action.
  5. Use the technical resources and expertise located in Newcastle and Monash to resolve the assembly and composition of zona-receptor complexes on the surface of both human and murine spermatozoa, and to elucidate proteomic changes during epididymal maturation and capacitation coincident with the competence to engage the process of sperm-egg recognition.
  6. Characterize the epididymal factor responsible for controlling the development of mitochondrial membrane potential.
  7. For three ENU-induced mouse models of infertility we shall continue to map the causal mutations and the genes they are contained within. In parallel, we shall undertake detailed phenotypic analyses of each of the mouse lines to precisely define where in the spermatogenic process problems first arise. It is planned that mouse lines will be allocated to PhD students with supervisors in different nodes. For example, the 9CAT53 line contains a mutation which results in both male and female infertility. It will form the basis of a collaboration between the Monash University and Newcastle University nodes.

### Future Studies

In the coming year we shall:

1. Upgrade our advanced mass spectrometry facility to include two new systems that will not only improve our proteomics facility but also permit the Centre to provide a technology platform in the area of metabolomics. Through the enablement of this facility we shall advance the ability of the Centre

## Spermatogenesis Program

Our objective is to identify fundamental mechanisms and genes that control spermatogenesis and sperm function. We aim to define new targets and tools for fertility regulation, to facilitate infertility diagnosis and treatment and to create new contraceptive development opportunities. This work will provide new knowledge of general mechanisms that underpin normal and aberrant (e.g. oncogenic) development in many biological contexts as we develop new strategies for regulating the expression of genes critical for progressive cellular differentiation.

### Sub-program 1: ENU mouse mutagenesis program

O'Bryan, Aitken

The goals of the spermatogenesis program are addressed in a series of closely linked subprograms:

**Phenotype to genotype.** Through the test-breeding of libraries of mice with randomly induced mutations, we have identified 12 lines with male and/or female infertility. Of these, 9 lines are actively being characterized. They present with phenotypes ranging from a testis containing a Sertoli cell-only epithelium, to those with arrest at the spermatocyte (meiosis) or spermatid (haploid) stages of development, to those with apparently normal spermatogenesis but failures of sperm function. Interestingly, lines with male infertility present approximately four times more often than those with female infertility, perhaps reflecting the relative fragility of fertility in the male. Of these, the majority are overtly of testicular origin. During 2008 we identified the causal mutations in two lines: Mot1

and Joey. Both mutations were in genes previously unlinked to male fertility, and each results from a single amino acid substitution in what we have now shown are critical domains for protein function. For both lines, the identification of the causal mutation followed by an assessment of normal gene expression has led us to the identification of additional pathology in somatic tissues. These findings reinforce the perception that male fertility is often a sensitive barometer of general health. For both mouse lines, a phenotypically equivalent group of patients has been identified and will be screened for polymorphisms in the orthologous genes.

In addition to the two mouse lines described above, several other causal mutations have been mapped to small intervals. During the next year we shall focus on the identification of the causal genes and in-depth phenotypic analyses.

### Sub-program 2: Epigenetics and male infertility

O'Bryan, Aitken

It is becoming increasingly clear that male fertility is exquisitely sensitive to changes in epigenetic programming of the genome. While the mechanisms underlying spermatogenic abnormality and trans-generational effects are being elucidated in the mouse, it is anticipated that similar affects will occur in humans and will be influenced by both the genetic composition of the parents, and the manner in which their gametes are handled, for example, during in vitro fertilization procedures. During 2008 we have continued our analysis of the role of epigenetics in male fertility, with

a particular focus on meiotic and haploid germ cell development. Using a mouse line, we have defined the role of a key component of the DNA methylation pathway in the meiotic segregation of the X and Y chromosomes. We have shown that a perturbation in this pathway results in a significant increase in XY-bearing sperm. The equivalent pathway in the human may lead to Klinefelter's syndrome. In addition, we have used microarrays to illustrate that different splice variants of the same gene are involved in the epigenetic silencing and activation of many haploid genes.

### Sub-program 3: The affect of gene dosage on male fertility and testicular cancer

Sinclair, O'Bryan

Variations in the structure of the human genome take many forms ranging from microscopically visible aneuploidies to single nucleotide changes. It has recently become apparent that submicroscopic changes in DNA segmentation that range in size from kilobases to megabases are common and cover more than 12% of the genome. Such changes are commonly referred to as copy number variations (CNV), and result in structural changes to the genome, leading to phenotypic variance and, increasingly, disease. With the exception of several studies on gene copy number variations on the Y chromosome, the incidence and consequences of similar arrangements on the X-chromosome or autosomes for male fertility have not been studied. Using the Affymetrix Human GeneChip Arrays and custom written software, gDNA from men with highly refined sub-types of infertility are being screened for CNVs and compared to profiles from 'healthy' men.

Our first study on men with meiosis arrest, as defined following testis biopsy, has resulted in the identification of two putative meiosis CNV regions on chromosomes 11 and 3. The frequency of such CNVs in the fertile versus infertile Australian population is being assessed using multiplex ligation-dependent probe amplification (MLPA). Funding for this research on meiosis, along with that on an ENU-induced mouse model of meiotic arrest was successfully leveraged through the awarding of a NHMRC project grant to start in 2008.

Future studies will focus on the analysis of other discretely defined sub-groups of infertile male and the potential for synergy between clinical samples and our unique ENU-induced mouse models of male infertility.

### Sub-program 4: Developmental switches in Spermatogenesis

Loveland, Jans

#### 4a. Regulated transport protein synthesis linked to developmental switches.

This project examines the hypothesis that regulated nucleocytoplasmic trafficking governs differentiation, with spermatogenesis serving as an excellent developmental model due to the simultaneous presence of cells at multiple stages of maturation. We have previously defined the expression profiles of key transport proteins (importins) at distinct stages of spermatogenesis, demonstrating that several importins are themselves regulated through spermatogenesis in terms of their subcellular localization. Funding for the investigation of non-transport roles of importin proteins, including those in spermatogenesis, has been obtained separately through an ARC Discovery Project and Research Fellowship which supports Dr. Yoichi Miyamoto's work in this area.

We have employed yeast-two-hybrid screening to identify many cargo proteins in developing germ cells that selectively bind to IMP family members. Notably, key importin  $\alpha 2$ -recognised cargoes in the adult testis, such as lamin B1, Cdyl, and coilin exhibit differential subnuclear targeting when transported by either importin  $\alpha 2$  or importin  $\alpha 6$ . One additional importin  $\alpha 2$ -recognised cargo identified from screening a fetal mouse testis library is paraspeckle protein-1 (PSPC1), and it is mislocalized when importin  $\alpha 2$  function is reduced. The nuclear localisation of these cargoes in specific testicular cells seems to be important for spermatogenic progression and current work is examining this in greater detail. For example, nuclear access of Cdyl has been demonstrated to be critical for its function in histone H4 acetylation, and this is a step required for histone-to-protamine exchange during spermiogenesis.

Selective over – or under – expression of key transport proteins can drive and determine the phenotype of ES cells differentiated in culture (see Spermatogonia Program), and we have adopted this approach in our studies of mouse ES cells, based on our objective of driving these cells into the germline. We are measuring the impact of hormones and growth factors on the synthesis of individual nuclear transport proteins and their encoding mRNAs, and we have identified importin proteins that are sensitive to these experimental manipulations.

We have initiated the generation of several transgenic mouse models in which importin protein synthesis or function is altered. In collaboration with the Centre's Queensland node, we are generating mouse strains that each have a gene incorporated which will disrupt the normal transport function of importin  $\alpha 2$  and  $\alpha 4$  proteins in the mouse testis. The protein products of the incorporated transgenes will be under the control of the protamine promoter, which means they will only be activated in the late male germ cells (round and elongating spermatids). We predict that the expression of these dominant-negative importin  $\alpha 2$  and  $\alpha 4$  proteins will cause key importin  $\alpha 2$  and  $\alpha 4$ -recognised cargoes (identified in the initial yeast-two-hybrid screen and other pull-down/immunoprecipitation screens described below) to be sequestered in the cytoplasm and thereby inhibit their nuclear function and progression of spermatogenesis. We have 14 candidate founder mice and are currently examining further litters to obtain at least three lines of homozygous mice for each transgene. Preliminary data indicate that the transgene is expressed in elongating spermatids, as expected, and initial fertility trials suggest some of these lines may exhibit subfertility or be infertile. Biochemical and morphological analyses are underway to verify and understand these observations.

In a parallel paradigm, we have begun to test the potential importance of regulated nuclear transport via non-classical mechanisms in germ cell differentiation in vivo. Generation of a transgenic mouse that mis-expresses a key repressor of Hedgehog transcription factor nuclear transport signaling has been initiated, and candidate founders are under analysis.

#### **4b. Specific transport cargoes are proteins critical for spermatogenesis.**

We will determine the in vivo roles of proteins identified through our Y2H and other screens which undergo regulated movement into the nucleus at precise stages of spermatocyte and spermatid maturation (e.g. Cdy1, coilin and MAP2). Constructs encoding MAP2 isoforms which differ in their nuclear localization potential have been used for transgenic mouse generation at the Centre's Queensland node, and one founder line is under analysis. We also have established a collaboration with Greg Matera's laboratory (Cleveland, USA) whereby we will have access to coilin knockout mice to define its spermatogenic phenotype.

To explore our hypothesis further, we have begun to define the spermatogenesis "importome". In these experiments, recombinant proteins fused to Glutathione S-transferase (GST) are being used in pull-down assays with testicular lysates to define, for the first time, importin  $\alpha 2$ -,  $\alpha 4$ -, and  $\alpha 6$  – interacting proteins in adult rat testes. In addition, highly purified germ cells (>90% pure meiotic spermatocytes and haploid round spermatids) have been used in immunoprecipitation (IP) assays using anti – importin antibodies specific for three importins, 2 (RCH1),  $\alpha 3$  (QIP2) and  $\alpha 4$  (QIP1). Protein bands specifically precipitated by either a GST – importin protein or an anti-importin antibody have been identified using mass spectrometry by Dr. Mark Baker (Newcastle University). Using this approach in 2008, we have identified 30 candidate molecules in the GST pull down assay and 32 molecules in the IP assay as importin binding partners; some of these were isolated from a specific band associating with a single importin, while others were found to associate with two or more importin proteins. We are focused on 5 of these candidate importin-interacting proteins, including Structural Maintenance of Chromosomes 6 (SMC6), which is known to function in DNA repair, cloning all 5 genes from adult mouse testis into mammalian expression vectors. These are now being used to generate tagged fusion proteins to study the subcellular localization and binding interaction with each importin for each candidate. Confirmation of their functional importance and stage-specificity will be achieved using in vitro experiments in addition to our emerging set of in vivo models, thereby leading to our goal to identify key cargoes with stage-specific functions.

## Spermatogonia/Stem Cell Program

### Program Objectives and Strategies:

This program aims to address questions of spermatogonial stem cell (SSC) biology and technology in order to provide the scientific community with an understanding of the molecular regulation of SSCs and local development of stem cell technologies. The multi-faceted approach is designed to overlap with the other programs and provide a variety of systems to analyse stem cell biology that are also utilised in other collaborative efforts with the Centre. The prime goals of the spermatogonial program are divided into four subprograms.

### Sub-program 1: SSCs and the origins of testicular cancer

Hime, McLaughlin, Roman, Loveland, Koopman

Testicular germ cell tumours (TGCTs) are the most common malignancies to affect males between the ages of 15 and 44. During the last 50 years epidemiological studies in many developed countries including Australia have shown significant trends of increased rates of testicular cancer, particularly among adolescents and young adults. The origins of testicular cancer remain obscure but it is thought that they are derived from primordial germ cells which fail to properly differentiate into spermatogonia. Around the time of birth primordial germ cells migrate towards the periphery of the seminiferous tubule and transform into SSCs/spermatogonia. During this period the putative germ line stem cells "home" to the specific microenvironment on the basement membrane known as the SSC "niche". Establishment of the stem cell population from this group of primordial germ cells is not well characterised and is the source of much controversy, largely due to the inability to distinguish between the self renewing stem cells and their differentiated daughter cells. The lack of a set of appropriate marker genes has severely hampered the identification of the transition of primordial germ cells to SSCs and the subsequent production of differentiating spermatogonia. This transition process is crucial to the genesis of testicular cancer and hence our limited understanding has prevented development of new therapies for this devastating disease.

We have utilized biochemical and immunological technologies to identify new cell surface markers of spermatogonial cells. Because of the correlation between aberrant KIT expression and testicular cancer, these factors are also being examined in an *in vitro* human germ cell cancer (seminoma) cell line to determine their influence in a clinical model. We have also made use of cross-platform technologies within the Centre to conduct evolutionary comparisons of genes found to be expressed in spermatogonia of different species. This has allowed us to define a subset of genes that are expressed in spermatogonial stem cells and thereby provided unique methods of differentiating stem cells from their progeny. These stem cell markers will not only provide a valuable resource for stem cell isolation for a variety of biotechnological applications but analysis of human testicular tumour biopsies has indicated that these new stem cell markers may be up-regulated during tumourigenesis. Not only have we defined new markers of testicular tumour cells but they may also provide inputs for therapeutic intervention to target biochemical pathways required for testicular tumour growth.

## Sub-program 2: Dissection of SSC biology *in vivo*

Hime, McLaughlin, Roman, Loveland

We are utilising the power of a combination of *in vivo* models to provide a genetic and cell biological understanding of SSC maintenance and differentiation. Genetic studies in *Drosophila* identified the RNA-binding proteins Musashi (Msi) and Held Out Wings (How) families of translational regulators as controllers of stem cell differentiation. Loss of Msi disrupts the balance between stem cell renewal and differentiation, resulting in the premature differentiation of SSCs. Our recent data suggest that multiple members of the Musashi gene family act to regulate key cell signaling pathways during spermatogonial development. We have developed genetically-modified mouse strains that confirm the role of Musashi proteins in vertebrate stem cell biology.

We have also conducted a unique genetic screen in *Drosophila* to isolate novel genes that cause germ cell tumours when mutated. We have so far isolated 12 new mutants that cause development of testicular tumours and are in the process of mapping and cloning the associated genes. These genes will provide new regulators of germ cell proliferation that are potential targets of chemotherapeutics.

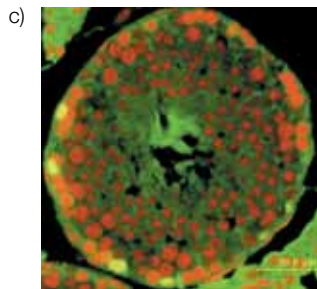
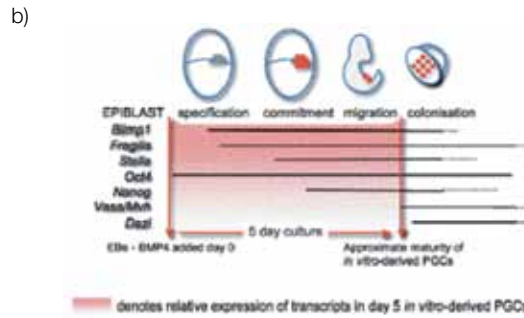
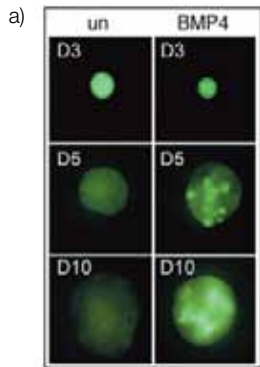
## Sub-program 3: Manipulation of growth and gene expression in SSCs

McLaughlin, Roman, Loveland, Hime

The objective of this project area is to enhance our understanding of germ cell differentiation in such a way that will enable us to systematically drive male germ cell differentiation. We have set up *in vitro* culture systems through which we can manipulate germ cell proliferation and differentiation, and develop a reliable method for germ cell transfection. Preliminary attempts to transfect isolated bovine germ cells with green fluorescent protein are underway. These transfected cells will be transplanted into the irradiated testes of bull calves to follow transplantation success and to ultimately produce green calves.

Working in the mouse system, we have defined a window of germline specification which holds promise as a means to reproducibly generate cells exhibiting a germline transcriptional profile. Cells are obtained in sufficient numbers for our co-culture and grafting

procedures. This approach provides us with a new platform for the study of spermatogenesis itself, and it should offer new strategies for generating genetically modified SSCs for production of transgenic animals. The technology developed for embryonic stem cell germline specification is currently being tested with induced pluripotent stem (iPS) cell lines. Its application to the study of ES cells from mice with reproductive phenotypes will enable investigation of these phenotypes at the earliest stages of germline development. As part of our effort to identify molecules that drive spermatogenesis, we are working with members of the Spermatogenesis Program to evaluate how changes in a cell's ability to import regulatory proteins into its nucleus may be crucial to controlling the earliest stages of germline development utilizing the new culture systems.



- a) BMP4 treatment induces a population of putative primordial germ cells (green) within differentiating embryonic stem cell aggregates.
- b) These cultures enable primordial germ cell differentiation up to the pre-colonisation stage.
- c) SSCs stained for a marker of pluripotency (green)

## Sub-program 4: Transplantation of cultured / manipulated SSCs into adult gonads

Holland, McLaughlin, Roman, Loveland

This subprogram not only aims to establish a technological base for efficient production of transgenic domestic animals but also explore areas of germ cell biology that could have profound consequences for design of future strategies in conservation biology. We have established the capacity to grow mixtures of bovine somatic and germ cells with mouse somatic and germ cells, both in vitro and in vivo. Bovine germ cells isolated using Staput technology and co-cultured with murine Sertoli cells can be maintained for at least 25 days in culture. Our set of cell and species-specific antibody markers enabled us to track the growth of each of these kinds of cells, and we are now trying to optimize the conditions required to support bovine spermatogenesis with mouse somatic cells for longer times. Intriguingly, the bovine Sertoli cell is quite amenable to transplantation and indeed may be required to support bovine germ cell maturation into sperm. Understanding the dialogue between somatic and germ cells is crucial for reaching the objectives of this program. For example, the process by which

sperm “mature” into fully functional cells is believed to occur as a consequence of interactions between secreted components from the male tract and the sperm. Particularly important are the species-specific proteins secreted into epididymal fluid. Since these proteins are species-specific it would be expected that the epididymis of a species could only mature sperm of its own species. We are testing this paradigm by seeding the testis of a host species (rat) with spermatogonia from a donor species (mouse) after endogenous spermatogenesis has been ablated by chemical agent. Preliminary experiments show that up to 50% of subsequent spermatogenesis in testis cross sections can be derived from the donor species (mouse) species. We then recover mouse sperm from the rat cauda epididymis and evaluate for motility and fertility, which are acquired during epididymal passage. Motile, fertile sperm would mean that the rat epididymis has somehow successfully matured mouse sperm. This would open new vistas in infertility treatment and conservation biology.

## Foetal Germ Cell Program

The major aim of this program is to discover and characterise genes and proteins important for determining the quality, quantity and behaviour of germ cells in the male fetus. We are interested in factors affecting the allocation of the germ cell lineage, the proliferation, migration and survival of primordial germ cells, the transition of primordial germ cells to spermatogonial stem cells, and the signalling between somatic cells and germ cells.

### Sub-program 1: Analysis of gene and protein expression in fetal mouse genital ridges

Koopman, Sinclair, Aitken

#### Gene expression screens

We have completed a number of RNA-based gene expression screens and have moved to a point where several genes or groups of genes have evolved into major research efforts in their own right (see sub-programs 2, 4 and 5 below).

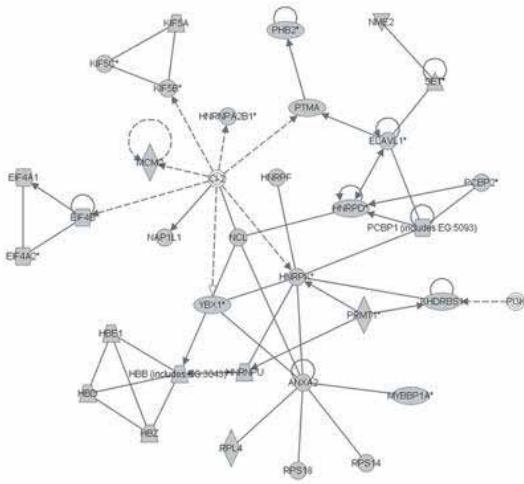
#### Proteomic screen

To complement this microarray analysis and advance our understanding of gonad and PGC development beyond the transcriptome level, we performed a proteomic screen of embryonic gonads. Briefly, proteins were extracted from male and female genital ridges before and during the period in which the soma induces male PGCs to commit to a spermatogenic fate. This commitment is signified by a possible inhibition of meiosis and a consequent arrest of the mitotic cell cycle in male PGCs. The timing of protein collection also corresponded with the initial stages of gonadal sex determination.

Two sets of data were generated: (1) a total proteomic screen of the combined male and female embryonic gonads and (2) a differential protein expression analysis of the embryonic testis during early gonad/PGC sex determination. To perform these studies we utilized 2D liquid chromatography (Ettan MDLC) coupled

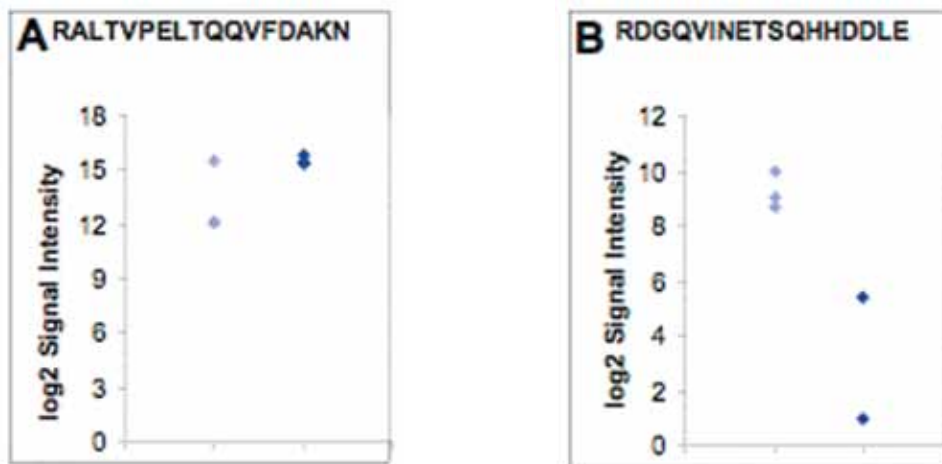
with tandem mass spectrometry (LTQ MS/MS), taking advantage of CBD platform technologies available in the Aitken laboratory. The differential expression screen results were analyzed with DeCyder MS software also available to the CBD in the Aitken laboratory.

The embryonic gonad total proteome survey identified over 1000 redundant proteins, many of which have known roles in gonad and germ cell development and function, such as steroidogenic enzymes, molecular chaperones and transcription factors [Ewen et al., manuscript submitted]. The screen highlighted the prevalence of networks of proteins involved in RNA post-transcriptional modification and trafficking, protein folding and synthesis, and post-translational modification processes (Fig. 1). Many proteins were also identified which have not previously been associated with the gonads. The role of these proteins in gonad/PGC sex determination and differentiation warrant further investigation. Moreover, a number of proteins were detected that map to regions orthologous to human loci associated with disorders of sexual development (DSDs). The corresponding genes represent candidates that potentially regulate gonadal development and sex determination.



**Figure 1:** Biological network constructed from gene products identified in the embryonic gonad proteome. Of the 35 proteins in this network, 33 were detected in the screen (in grey). Network functions include RNA post-transcriptional modification, gene expression and RNA trafficking.

Differential expression analysis identified a number of proteins being either up – (Fig. 2A) or down-regulated (Fig. 2B) in the testes over the time period being assessed (11.5 to 12.0 dpc). Expression profiling via Western blot analysis of a selection of these proteins was used to validate the proteomic screen results. These candidate proteins may provide new insights into the cellular mechanisms controlling PGC differentiation, germ-soma interactions and gonad development.



**Figure 2:** Differential expression of two peptides in the testes between developmental ages 11.5 (light blue) and 12.0 dpc (dark blue). A, the RALTVPELTQQVFDANK peptide (from tubulin b5) exhibited a significant increase in expression with time (+ 4.82 fold,  $p < 0.05$ ). B, the RDGQVINETSQHDDLE peptide (from vimentin) exhibited a significant decrease in expression with time (- 65.8 fold,  $p < 0.05$ ).

## Sub-program 2: Regulation of germ cell proliferation and testicular cancer

Sinclair, Koopman

### Cell cycle and pluripotency in the fetal germ line

There is a close biological relationship between germ cells and pluripotent cell types including embryonic stem cells, embryonic germ cells and embryonal carcinoma, the stem cells of testicular cancer. A small group of key developmental genes, which control differentiation potential, are restricted to pluripotent cells and the developing germ line. These genes are also typically reactivated in germ cell derived embryonal carcinoma and it has been hypothesized that these testis cancer cells are similar to germ cells present in the developing embryo. Despite the importance of understanding the developing male germ line, very little of this process has been elucidated. For example, it has been established that soon after specification of the male germ lineage, the germ cells enter mitotic arrest, but the timing and molecular control of this process remain almost completely unknown.

We have developed a powerful fluorescence activated cell sorting (FACS) protocol to examine the progression of developing germ cells through mitotic arrest. These technologies enabled us to identify several key cell cycle regulators that are strongly regulated during the initial stages of germ cell cycle arrest. In particular, we observed strong up-regulation of the INK4 (p<sup>15INK4b</sup> and p16<sup>INK4a</sup>) and CIP/KIP (p27<sup>kip1</sup>) inhibitors occurs during the initial stages of germ cell cycle arrest. The function of these proteins is to prevent the inactivation (by inhibiting its phosphorylation) of the G1-S phase checkpoint regulator pRB. This allows pRB to actively prevent cell cycle progression. In arresting germ cells we have observed that coincident with the up regulation of the INK4 and CIP/KIP cell cycle inhibitors, pRB becomes transiently activated (dephosphorylated) and the germ cells enter G1 arrest (Western *et. al.* 2008, *Stem Cells*). This contrasts with cell cycle mechanisms operating in female germ cells, which arrest in the G2/M phase of the cell cycle and express several G2/M proteins but do not up-regulate p27<sup>kip1</sup> or p16<sup>INK4a</sup> and pRB remains in an inactive state (D. Miles, J. van den Bergen, A. Sinclair and P. Western; manuscript in preparation).

Finally, we have shown that pluripotency associated genes are down-regulated at the transcriptional (e.g. *Sox2*, *Nanog*, *Esg1*) and post transcriptional (e.g. *Oct4* and *Dppa2*) levels coincident with mitotic arrest of male germ cells and the up regulation of the molecular machinery that inhibits G1-S phase progression (P.

Western, J. van den Bergen, D. Miles and A. Sinclair; manuscript in preparation). We are now examining the possibility that post-transcriptional control of *Oct4* and *Dppa2* may be mediated by specific micro-RNAs, some of which have been implicated in the regulation of pluripotency and are over-expressed in germ cell derived cancers.

Examination of the methylation status of the promoters controlling expression of the pluripotent genes shows that suppression of these genes is unlikely to involve methylation of their promoter regions (P. Western, J. van den Bergen, D. Miles and A. Sinclair; manuscript in preparation). This is in contrast to the current understanding for transcriptional control of these genes in somatic cells. Future work will determine the status of chromatin contained within the promoters of the pluripotent genes with the aim of defining the mechanisms underlying control of this important group of transcription factors.

Based on our data defining the control of the cell cycle in developing male germ cells and the reactivation of pluripotent genes in germ cell cancers, we hypothesize; (1) that an anti-proliferative signal results in up-regulation of specific CIP/KIP and INK4 cell cycle inhibitors, (2) that as a result of the action of these inhibitors pRB becomes de-phosphorylated (activated) and causes arrest at the G1-S phase check point and (3) that down-regulation of pluripotent genes is required for normal male germ cell differentiation and effective exit from the cell cycle.

To further define the differentiation of male germ cells we have examined their differentiation using expression micro-array screening. Preliminary analysis of these data indicates that differentiation of the spermatogonial lineage is a complex process involving activation and silencing of many genes. This screen has identified several novel gene families that are regulated during male germ cell differentiation and mitotic arrest and significantly expands our understanding of the genes regulated during this process.

Our analysis of mitotic arrest combined with recent studies of the activity of the testis tumor gene, *Dnd1*, suggest that DND1 may regulate early male germ cell differentiation through its control of specific micro-RNA/target gene interactions. Targets of DND1/micro-RNA complexes may include p27<sup>kip1</sup>, a key cell cycle repressor that we have shown is activated at the outset

of germ cell mitotic arrest. Further work will determine whether this hypothesis is valid.

In addition, research in human and mouse models of testicular cancer will enhance our understanding of the molecular basis of testis cancer and the development of the spermatogonial lineage. To this end we have characterized the progression of male fetal germ cells through mitotic arrest in testis cancer susceptible 129T2svJ mice. Our data show that mitotic arrest occurs approximately 24 hours later in 129T2svJ mice than in testis cancer non-susceptible outbred CD1 mice. However the timing of mitotic arrest in 129T2svJ is similar to that exhibited by testis cancer non-susceptible inbred C57Bl6 mice. This indicates that although timing of mitotic arrest in the overall germ cell population varies between strains, this variation is not necessarily associated with susceptibility to testis cancer (P. Western, R. Ralli, D. Miles, J. van den Bergen and A. Sinclair unpublished data)

### Hbp1 – role in XY germ cell phenotypic sex determination?

HMG box containing protein 1 (HBP1) is a high mobility group domain transcription factor that regulates proliferation in differentiated tissues. *Hbp1* expression is male-specific in the germ cells of the fetal mouse testis, and is maintained beyond the onset of spermatogenesis after birth. We previously showed that the expression of *Hbp1* in XY germ cells correlates with the onset of mitotic arrest in these cells, leading us to hypothesise that it may regulate male germ cell behaviour in the fetus. We now find by section in situ hybridisation (SISH) that *Hbp1* expression is maintained in the newborn male PGCs until 8-10 days *post partum*, the approximate time when the male PGCs re-enter the mitotic cell cycle in preparation for meiosis. These results support the hypothesised role of *Hbp1* as a mitotic arrest factor.

The previously unreported existence of a second splice variant raises the possibility of multiple functions for HBP1. As such the expression profile of this second variant has been performed and investigation into where this factor is localized within germ cells is in progress. Nuclear localization studies, in association with Professor David Jans, have identified opposing sub-cellular localizations for the two variants. This finding suggests that the complete transcript that gains access to the nucleus can function as a transcription factor, whilst the second truncated variant that remains in the cytoplasm may have an alternate role. We are now investigating interacting factors for each variant in cell culture using the GC2 cell line in order to understand their individual actions.

We previously obtained an *Hbp1*-gene-trapped ES cell line from Bay Genomics, which has since been used to generate mutant mice harboring *Hbp1* ablation in our transgenic facility. These mice have currently been analyzed on a mixed genetic background and both heterozygous and homozygous mutants appear viable, although fertility assessment of the homozygous mice is ongoing. Throughout embryonic germ cell development we could not detect any significant changes to germ cell gene or protein expression, and cell cycle state and cell number also appear normal. Despite this, we have observed one case of teratocarcinoma within a chimeric founder at 6 months of age, and so we are currently aging mice homozygous for the *Hbp1*-genetrap mutation to determine if this is as a direct result of *Hbp1* ablation. We are also in the process of crossing this mutant line onto pure C57Bl/6 and 129x1/sv backgrounds in order to identify a phenotype that may not be obvious on the mixed background analyzed to date.

Investigation into reported interacting partners for HBP1 has led to the interesting finding that Retinoblastoma is expressed within the germ cells at the correct time to also suggest a role in germ cell mitotic arrest. This is plausible given the established role Rb plays in cell cycle control. We have investigated this possible function by analyzing the Rb knockout model during germ cell development. Consistent with our hypothesis, we have identified the *in vivo* role for RB in regulating XY germ cell entry into G1/G0 arrest as in the absence of *Rb*, we could detect proliferating germ cells at a timepoint when all wildtype germ cells had arrested (14.5 dpc). This phenotype is rescued by 16.5 dpc however, presumably by the action of Rb family members p130 and p107 which were also upregulated at this time. This finding has highlighted the involvement of a well known cell cycle regulator in the process of XY germ cell differentiation and emphasized the complex nature of germ cell biology that uses multiple levels of cell cycle control to ensure correct development.

In order to investigate HBP1 in a wider cell cycle sense, a cell cycle array has been used to analyze a purified population of germ cells. The results of this array have identified many interesting candidates that become up-regulated during G1/G0 arrest. Specifically, we identified a role for the retinoblastoma family, already under investigation in our laboratory, in addition to the cell cycle inhibitor p21. We also observed a robust mechanism for maintenance of germ line integrity and apoptosis that is active within both XY and XX germ cell populations. The comprehensive profile of germ cell arrest is a valuable tool for identifying those factors that HBP1 may interact with during XY germ cell G1/G0 arrest.

### The role of the Nodal/Cripto signalling pathway in male germ cell development

From Affymetrix screening outputs, we found that genes encoding members of the Nodal pathway, including *Nodal*, *Cripto* and *Lefty1/2*, are transiently expressed in XY germ cells during embryogenesis. This occurs even before XX germ cells begin to express *Stra8*, suggesting to us that their expression might be regulated by FGF9 (see Sub-program 5). Our findings from organ culture and germ cell assays indicate that this is true. Expression of these important developmental genes has not been noted before in germ cells, although these genes are thought to be associated with pluripotency in human ES cells. Since XY germ cells in *Fgf9* null mice die by about 13.5 dpc, we hypothesise that an active Nodal pathway is associated with survival of germ cells in the RA-free environment of the developing male gonad.

Recently we have demonstrated that the Nodal pathway does indeed appear to be active in XY but not XX germ cells since phosphorylated Smad2/3 proteins can be detected in germ cells of embryonic testis but not of embryonic ovary. Since Nodal is essential for various processes early in development, we are currently attempting to ablate *Nodal* in germ cells only. To this end, we are crossing *Nodal* conditional null mice with a TNAP-*cre* recombinase mouse. Although the TNAP-*cre* recombinase mouse has been used successfully in many studies, we are detecting ectopic *cre* recombinase expression in this line and embryos are dying before we are able to assess the germ cell phenotype associated with Nodal deletion. We aim, therefore, to collect gonadal tissues from embryos at 14.5 dpc and culture for several days before assessing the germ cell phenotype. We have devised a second strategy that may allow us to address the question of Nodal function in XY germ cells and we will carry this out in parallel with studies on TNAP-*cre*<sup>+</sup>; *Nodal*<sup>fl<sub>ox</sub>/fl<sub>ox</sub></sup> embryos. In the second approach, we aim to treat cultured *Noda*<sup>fl<sub>ox</sub>/fl<sub>ox</sub></sup> gonads with a cell-permeable *Cre* recombinase and examine the effect on XY germ cell survival and phenotype.

## Sub-program 3: Functional analysis of genes identified in molecular and in silico screens

Koopman, Loveland, Roman

The gene targeting and transgenesis technology platform has undertaken a number of new and important projects to generate mouse models for a number of projects in the ARC Centre for Biotechnology and Development.

### Cyp26B1 and male germ cell sex determination

Previously, we showed that retinoic acid (RA), produced by mesonephroi of both sexes, causes germ cells in the ovary to express *Stra8*, enter meiosis and initiate oogenesis. We showed also that entry into meiosis is avoided in the fetal testis by the action of the retinoid-degrading enzyme CYP26B1, ultimately leading to spermatogenesis. *Cyp26b1* is expressed by somatic cells of the male gonad. In testes of *Cyp26b1*-knockout mouse embryos, germ cells enter meiosis precociously, as if in a normal ovary.

We are trying to generate a line of mice that over-express *Cyp26b1* in somatic cells of the female gonad. We would predict that XX germ cells in a *Cyp26b1*-expressing ovary would be prevented from going into meiosis. Our efforts to use a *Sf1* promoter-*Cyp26b1* transgene have not been successful, possibly because the *Sf1* promoter is driving expression of the CYP26B1 enzyme in non-gonadal locations at earlier stages during development, leading to embryo lethality. We are currently designing and constructing *Cyp26b1* over-expression constructs that are driven by the promoters of *Irx3* and *Dax1*. Both of these genes are highly expressed in somatic cells of female developing gonads.

In order to study the dynamics of RA up-regulation of the critical meiosis gene, *Stra8*, we are presently making *Stra8* promoter-eGFP transgenics. This construct has been used previously by others and they showed specific expression in male germ cells as they enter meiosis during puberty. Although we have generated lines showing expression in the adult male, we have not been able to show specific expression in female germ cells as they enter meiosis during embryogenesis. This may indicate that the *Stra8* promoter is regulated in a different manner during embryonic life. If true, this would be surprising since it is postulated that entry into meiosis is regulated in both fetal ovary and adult testis by RA acting through *Stra8* expression.

### Hedgehog signaling and germ cell tumourigenesis

We are creating a transgenic mouse model (*Pdha2 promoter-eGFP-SuFu*) to investigate the potential of the product of the SuFu gene to block cytoplasmic-to-nuclear translocation of GLI1 protein in mouse spermatocytes and its impact on adult germ cell maturation. This transgenic mouse model will assist in the identification of potential therapeutic targets in germ cell tumours in which *Hedgehog* signaling is dysregulated. *Gli1* is a key mediator of the *Hedgehog* pathway of activation, and its overexpression in germ cells is implicated in the aetiology of a subset of testicular germ cell cancers. We have identified new downstream targets of *Hedgehog* signaling in the foetal and adult mouse testis and will examine whether these genes are affected in these cancers.

### The role of BMP4 in male fertility

We are generating a transgenic mouse model to investigate the effect of over-expression of BMP4 on terminal spermatogenesis and male fertility. Our previous work (Baleato *et al.*, 2005) demonstrated that BMP4 is expressed maximally in spermatogonia, and declines when the cells differentiate into spermatocytes. Our working hypothesis is that a reduction in BMP4 expression is necessary for differentiation to take place. To test this hypothesis we have engineered a modifying transgene, *Ldhc promoter-BMP4*, which should induce ectopic expression of BMP4 in spermatocytes, and lead to induction of apoptosis, and ultimately, azoospermia and infertility.

### Generation of a Fragilis-Cre transgenic deleter mouse strain

As part of the PhD studies of CBD student Cassy Spiller, and in collaboration with Professor Patrick Tam (CMRI, Sydney, and a member of the CBD Scientific Advisory Board) we are currently developing a Fragilis-Cre deleter transgenic mouse model. Professor Tam has shown that specific regions of the *Fragilis 1 (Ftm3)* promoter are active and specific in all germ cells throughout all stages of their development. We have 10 founders to date for the Fragilis-cre line, each of which are currently being crossed to the ROSA26-LacZ and ZAP reporter lines for extensive analysis of Cre-recombinase activity. Once confirmed, the Fragilis-Cre transgenic mice will be bred with mutant mice harbouring a targeted gene flanked with loxP recombination sites. The germ cell-specific expression of Cre-recombinase in the offspring should specifically delete the targeted allele in the germ line and will therefore be a powerful tool in genetic analysis of germ cells.

### Gene targeting and transgenesis technology platform

This technology platform continues to offer a world class standard of service to colleagues within the ARC Centre of Excellence in Biotechnology and Development. In the last 12 months, the gene targeting and transgenesis technology platform has generated a range of transgenic and targeted mutant mouse models. Among these are mutants that perturb gonad phenotype, which shed light on the molecular regulators of gonad sex determination (*Wt1-Sox3*; *Wt1-Sox10*; *Rspo1*; *Dax1-Dax1*); and mice that permit studies of the role of microtubule-associated protein-2 (MAP-2) isoforms in spermatogenesis and in meiosis (*Pdha2-MAP2cN-EGFP* and *Pdha2-MAP2cNdeltaT-EGFP*). In collaboration with Professor Patrick Tam, the technology platform is in the process of establishing a colony of *Fragilis-Cre* transgenic mice that may be bred with any floxed mutant, to generate offspring with a phenotype in the germ line.

## Sub-program 4: Regulation of germ cell entry into meiosis

Koopman, Sinclair

### Regulation of germ cell fate by retinoic acid

As indicated previously the precise regulation of retinoid levels during fetal gonad development provides the molecular control mechanism that specifies germ cell fate. This dramatic finding overturned the previously accepted dogma that entry into meiosis is the default pathway of germ cell sex determination (Bowles et al, 2006).

The major progress we have made recently relates to our discovery that FGF9, a signaling molecule and growth factor that is male-specifically expressed in developing gonads, also appears to have an effect on germ cell fate. STRA8 appears to be the key molecule induced by RA and is essential for entry into meiosis in both embryonic and adult systems. We now know that FGF9 also affects *Stra8* expression, down-regulating it in opposition to the effect of RA in up-regulating it. The concept of a secondary means of ensuring appropriate germ cell fate is attractive because, in general, networks stabilised by both positive and negative regulatory mechanisms tend to be very robust. We propose that, in the male gonad, both *Fgf9* and *Cyp26b1* are up-regulated once male somatic fate is decided by the expression of *Sry*. This results in high levels of FGF9 and low levels of RA in the male gonad environment, whilst low levels of FGF9 and high levels of RA are present in the female gonadal environment. Hence, RA and FGF9 act antagonistically to determine the level of expression of *Stra8*, and hence whether germ cells will enter meiosis at 12.0 to 13.5 dpc.

A recurring theme in developmental biology is proving to be the co-regulation of developmental processes by RA and FGF ligands, working in an antagonistic manner. In general, RA induces differentiation and FGF maintains pluripotency, and it appears that this is the case in our system also. We are now trying to understand how the two signaling molecules RA and FGF9 impinge on the germ cells in a mechanistic sense. We have found that RA and FGF9 both affect expression of *Cdx2* gene in germ cells, RA inducing it

and FGF9 repressing its expression. *Cdx2* is a critical regulator of cell fate in the early embryo because up-regulation of *Cdx2* is the key change observed during the first cell fate decision of the blastocyst: *Cdx2* marks cells of the trophoctoderm lineage. In that and other systems, *Cdx2* is expressed in a mutually exclusive manner to the pluripotency marker, *Oct4*. We now believe that *CDX2* is critically involved in the decision of germ cell sexual fate. In other systems, it has been shown that *CDX* proteins act as transducers of both FGF and RA signaling and the related *Cdx1* gene is a known direct transcriptional target of RA. Another gene with expression apparently regulated by both RA and FGF9 is *germ cell nuclear factor* (*Gcnf*), an orphan nuclear receptor required for expression of pluripotency genes and a known target and effector of RA signaling. *Gcnf* is known to bind the *Oct4* promoter and to be critical in repressing *Oct4* and restricting its expression to the germ cells following gastrulation. In general, *Gcnf* expression responds in a positive fashion to RA and in a negative fashion to FGF9, although it appears that this gene is more sensitive to levels of these factors than are either *Stra8* or *Cdx2*. The likely importance of *Cdx2* and *Gcnf* in the determination of germ cell fate is underscored by our observations that 1) both genes show sex-specific expression in germ cells even earlier than is observed for *Stra8* and 2) like *Stra8*, both genes demonstrate a dose-dependent sensitivity to levels of RA, with up-regulation in both heterozygous and homozygous *Cyp26b1*-null embryonic testes.

We have demonstrated that the application of exogenous FGF9 to XX gonadal cultures leads to a strong up-regulation of genes associated with male germ cell fate such as *Hbp1*, *Dnmt3L* and *Nanos2*. For at least one of these genes, *Dnmt3L*, it appears that this is a direct effect on germ cells. Since *Dnmt3L* encodes a de novo methylase, it is possible that FGF9 triggers the re-methylation of the genome in XY germ cells, thereby effectively closing the window of susceptibility to meiotic induction.

Using isolated germ cell cultures and whole gonad organ cultures, we have amassed quantitative gene expression data that suggests that RA and FGF9 act antagonistically with respect to expression of *Stra8*, *Cdx2*, *Gcnf*, pluripotency genes and male germ cell fate genes (see Figure). We now plan to demonstrate this more complete picture of germ cell sexual fate determination using mouse models. We have imported *Cyp26b1* KO mice (from H. Hamada, Japan). Previously, we carried out some analyses of these mice, but were unable to fully study their phenotype with respect to germ cell fate determination because available samples were very limited. We now plan to carry out a much more comprehensive study of this mouse model. In addition, we now have *Fgf9* KO mice (from B. Capel, USA). We plan to analyse thoroughly the germ cell fate phenotype in each of these KO lines as well as in the double knockout.

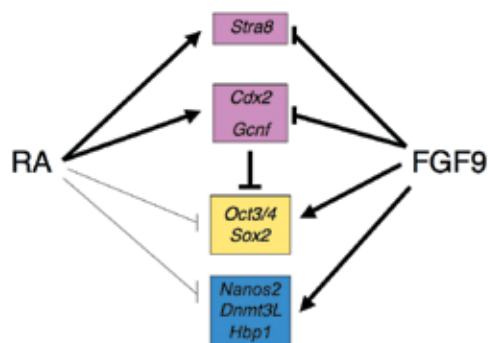


Figure. RA and FGF9 act antagonistically to regulate germ cell sexual fate. In a female gonad, RA levels are high and FGF9 levels are low. RA acts to up-regulate *Stra8*, *Cdx2* and *Gcnf* and appears to have a modest inhibitory effect on expression of pluripotency genes, *Oct4* and *Sox2*, and on male germ cell fate markers, *Nanos2*, *Dnmt3L* and *Hbp1*. CDX2 and GCNF may act to inhibit expression of the pluripotency marker *Oct4*, as they do in other systems. In a male gonad, in contrast, RA levels are low and FGF9 levels are high. FGF9 appears to inhibit *Stra8*, *Cdx2* and *Gcnf* expression, to enhance expression of *Oct4* and *Sox2* and to induce expression of *Nanos2*, *Dnmt3L* and *Hbp1*.

## MAP kinase signaling in the fetal testis

Our studies have highlighted the MAP kinases, and in particular the p38 MAPK pathway, as being of interest in male PGC development due to its potential involvement in inhibiting male PGC meiosis entry, a pivotal event in male PGC sexual differentiation. The p38 MAPK pathway is classically a stress response pathway known to regulate cell cycle progression in various cell types by directly phosphorylating downstream kinases and transcription factors or indirectly influencing gene regulation.

In support of this, expression analysis in developing gonads revealed that p38 MAPK is indeed present within the testis. Microarray data showed that p38 MAPK isoforms alpha and delta are present in the testis, and that the p38 delta isoform is sex-specifically up-regulated in the testis as early as 11.5 dpc. Quantitative real time PCR (qRT-PCR) analysis also confirmed the expression of p38 alpha and p38 delta in purified testicular PGCs. Immunofluorescence further revealed that p38 MAPK is activated specifically in male PGCs from 12.5 dpc, at which point the male PGCs do not enter meiosis like their female counterparts, instead they undergo mitotic arrest.

Expression studies were performed to determine which downstream targets of p38 MAPK are being directly activated or indirectly regulated in male PGCs. qRT-PCR showed a number of direct p38 MAPK targets to be more highly expressed in male versus female PGCs. The activation of these targets (including HBP1) needs to be confirmed with phospho-specific antibodies. Indirect targets of p38 MAPK were also found to be up-regulated in male PGCs from 13.5 dpc. These candidates should provide useful insight into the cellular mechanisms involved in male PGC meiosis inhibition and their overall development.

Cumulative data from functional studies of gonad explants using p38 MAPK inhibitors further indicate a role for this intracellular signalling pathway in inhibiting meiosis entry in male PGCs. Complementary *ex vivo* gain-of-function experiments using magnetofection to constitutively activate MKK6, the upstream kinase responsible for activating p38 MAPK, have been attempted. Further work is needed to overcome technical difficulties associated with this approach before conclusive data can be generated.

## Technology Platforms

One of the central functions of the Centre of Excellence is to add value to the network of scientists and Universities it represents through the effective integration of resources. In the present era very few individual research institutions can offer all of the advanced technologies needed to mount internationally competitive research programs in the biomedical sciences. The creation of a multi-institutional Centre of Excellence provides each University node with the opportunity to specialize in the provision of advanced technology platforms that benefit the entire network. Examples of this activity includes the siRNA technology being introduced at the University of Melbourne, the DIGE (Direct in Gel Electrophoresis) and Decyder MS systems for comparing complex proteomic profiles, operating at the University of Newcastle and the transgenic animal facility that the CBD is supporting at the University of Queensland. An indication of the array of invaluable resources and cutting-edge technologies that are available through the Centre of Excellence are given below:

### University of Newcastle

Advanced mass spectrometry facility featuring a 2D LC mass spectrometer and associated software for statistical comparison of complex proteomic profiles generated by either 2D-electrophoresis or 2D liquid chromatography. Recently updated by incorporation of ETD (Electron transfer dissociation) to facilitate peptide identification. In 2008 we have generated LIEF grant funding to the value of \$850,000 to further upgrade our mass spectrometry facility to improve our capabilities in proteomics and to develop metabolomics as a major new strand in our research armamentarium. This is a strategically important development for the Centre that will enable us to develop a 'systems biology' approach to resolving the molecular mechanisms that control the specification and differentiation of male germ cells. With this approach we shall integrate the proteomic and metabolomic profiles of germ cell at different stages of development in order to get a whole cell perspective on how the physiology of these cells is changing during development, rather than the reductive approach which focuses on the resolution of individual signal transduction pathways.

Advanced second messenger imaging facility enabling users to monitor 2 second messengers simultaneously. In 2008, we have generated a second LIEF grant funding to the value of \$650,000 to purchase a live cell imaging laser scanning confocal microscope. This will allow us to improve our capabilities in long term genetically modified germ cell culture and migration studies.

Electron microscopy. Ultrastructural immunolocalization. FPLC, HPLC.

FacsAria cell sorter and FacsCalibur flow cytometers

Also in 2008, we acquired funding from Cancer Institute NSW to support purchase of a real time, fast imaging system enabling acquisition of biologically relevant events within milliseconds (IVIS Kinetic) primarily for whole live animal imaging

### University of Melbourne

Drosophila genetics including phenotypic screens, generation of transgenic fruit flies. An ability to direct transgene expression to specific cell populations and to investigate genetic interactions of characterized and novel genes with genes of interest.

- Advanced bioinformatics.
- Biacore protein interaction analysis.
- siRNA technology.
- Gene delivery systems.

### Monash University

- Repository of DNA from men with a variety of infertility disorders.
- Quantitative microscopy.
- Confocal laser scanning microscopy including 2-photon excitation microscopy.
- Fluorescence polarization measurements to examine protein-protein interactions.
- Chemical labeling of proteins and oligonucleotides with fluorescent dyes.
- Expertise in phosphorylation studies eg. kinase assays, peptide/protein phosphorylation.

### University of Queensland

- Transgenic mouse facility including knockout production.
- Microarray analysis (Affymatrix system) assisted by bioinformatics capability within the ARC Centre in Bioinformatics at IMB.
- Robotic high-throughput in situ hybridisation and immunohistochemistry.



▶▶ **Key Result Areas and  
Performance Measures**

# 1. Research Findings

## 1.1. List of Publications

Articles Published or accepted for publication in 2008 = 64

Books = 2

Editorials: 2

Letters: 1

Book chapters = 4

Papers submitted to peer review journals = 55

Percentage with an IF of >5 = 28%

\* Indicates by invitation

### Books

1. **Aitken R.J.**, et al (2009) Editors. *World Health Organization Laboratory Manual for the Examination of Human Semen and Semen-cervical mucus interaction*. Cambridge University Press, Cambridge. (in press).

*This book is universally acknowledged as the reference text in laboratory seminology, now in its 5th edition. This revised version is the most complete account to date on the creation of a conventional semen profile and includes invaluable reference limits for specific aspects of semen quality based on the analysis of over 1 900 recent fathers.*

2. Habenicht U & **Aitken R.J** (2009) Editors. *Handbook of Experimental Pharmacology. Fertility Control – Today and in the Future*. Springer-Verlag (in press).

### Journal Articles and Book Chapters

3. **\*Aitken R.J.** (2008) **Invited Editorial** to accompany publication of the 5th edition of the WHO Laboratory manual. Whither must spermatozoa wander: the future of laboratory seminology. *Asian Journal of Andrology* (in press)
4. **Aitken, R.J.** (2008) **Invited Editorial**. Just how safe is assisted reproductive technology for treating male infertility? *Expert Reviews in Obstetrics and Gynaecology* **3**, 267-271. [No impact factor]
5. **\*Aitken, R.J. & Baker, M.A.** (2008) The role of proteomics in understanding sperm cell biology. Proceedings of the Florence-Utah International Symposium on Genetics of Male Infertility. *International Journal of Andrology* **31**, 295-302. [Impact factor = 2.7]
6. **Aitken, R.J., Baker, M.A., Doncel, G.F., Matzuk, M.M., Mauck, C.K. & Harper, M.J.K.** (2008) As the world grows: contraception in the 21st century. *Journal of Clinical Investigation* **118**, 1330-1343. [Impact factor = 16.4]
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8. **Aitken, R.J., Deluliis, G.N. & McLachlan, R.I.** (2008) Biological and clinical significance of DNA damage in the male germ line. *International Journal of Andrology* **32**, 46-56. [Impact factor = 2.7]
9. **\*Aitken, R.J.** Hughes, L.M., Griffith, R. & **Baker, M.A.** (2008) Bridging the gap between male and female fertility control. Contraception-on-demand. *Contraception* **78**, S28-S35. [Impact factor = 2.2]
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12. **Baker, M.A., Hetherington, L., Reeves, G.H. & Aitken, R.J.** (2008) The mouse sperm proteome characterised via IPG Strip prefractionation and LC-MS/MS Identification. *Proteomics* **8**, 1720-1730. [Impact factor = 5.8]
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42. **Mitchell, L.A., Nixon, B., Baker M.A. & Aitken, R.J.** (2008) Investigation of the role of SRC in capacitation associated tyrosine phosphorylation of human spermatozoa. *Molecular Human Reproduction* **14**, 235-243. [Impact factor = 3.2]
43. **\*Nixon, B. & Aitken, R.J.** (2008) Proteomics of human spermatozoa. In: *Immune infertility*. W. Krause, & R.K. Naz, Editors, Springer Verlag, Berlin. (in press)
44. **\*Nixon, B. & Aitken, R.J.** (2008) The significance of detergent resistant membranes in spermatozoa. *Journal of Reproductive Immunology* (in press). [Impact factor = 3.3]
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62. **Western, P.S., Miles, D., Van Den Bergen, J.A.,** Burton, M. & **Sinclair, A.H.** (2008) Dynamic regulation of mitotic arrest in fetal male germ cells. *Stem Cells* **26**, 339-347. [Impact factor 7.8]
63. **Zamudio, N.M.**, Chong, S. & **O'Bryan, M.K.** (2008) Epigenetic regulation and male fertility. *Reproduction* **136**, 131-146 [Impact factor = 3.0]
64. Zhang, Z., Hill, J., **Holland, M.K.**, Kurihara, Y. & **Loveland, K.L.** (2008) Bovine Sertoli cells colonize and form tubules in murine hosts following transplantation and grafting procedures. *Journal of Andrology* **29**, 418-430 (A figure from this paper was used as the cover photo for this volume). [Impact factor = 2.327]

### Publications by CIs supported by external funding

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66. Caly, L., **Jans, D.A.** & Piller, S.C. (2008) Cleavage at novel sites within virions and transfected cells of GFP-HIV-1 Vpr fusions: concerns for intracellular trafficking studies *Journal of Fluorescence*, in the press (#JOFL597R1)
67. Caly, L., Saksena, N.K., Piller, S.C. & **Jans, D.A.** (2008) Impaired nuclear import and viral incorporation of Vpr derived from a HIV long-term non-progressor *Retrovirology* **5**, 67 (<http://www.retrovirology.com/content/5/1/67>)
68. Daly, J., Galloway, D., Bravington, W., **Holland, M.K.** & Ingram, B. (2008) Cryopreservation of sperm from Murray cod, *Maccullochella peelii peellii*. *Aquaculture* **285** (2008) 117-122 [Impact factor = 1.735]
69. Dowling, D., Nasr-Esfahani, S., Tan, C.H., O'Brien, K., Howard, J.L., **Jans, D.A.**, Purcell, D.F.J., Stoltzfus, C.M. & Sonza, S. (2008) HIV-1 infection induces changes in expression of cellular splicing factors that regulate alternative viral splicing and virus production in macrophages. *Retrovirology* **5**, 18
70. Elliott, D.A., Kim, W.S., **Jans, D.A.** & Garner, B. (2008) Macrophage apolipoprotein-E knockdown modulates caspase-3 activation without altering sensitivity to apoptosis *Biochimica et Biophysica Acta* **1780**, 145-153
71. François, M., Caprini, A., Hosking, B., Orsenigo, F., Wilhelm, D., **Browne, C.**, Paavonen, K., Karnezis, T., Shayan, R., Downes, M., Davidson, T., Tutt, D., Cheah, K.S., Stacker, S.A., Muscat, G.E., Achen, M.G., Dejana, E. & **Koopman, P** (2008) Sox18 induces development of the lymphatic vasculature in mice. *Nature*. 456, 643-647.
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74. Kuusisto, H., Wagstaff, K.M, Alvisi, G. & **Jans, D.A.** (2008) The C-terminus of apoptin represents a unique tumor cell-enhanced nuclear targeting module. *International Journal of Cancer* **123**, 2965-2569.
75. Li, D-S., **Jans, D.A.**, Bardin, P.G., Meanger, J., Mills, J. & Ghildyal, R. (2008) Association of respiratory syncytial virus M protein with viral nucleocapsids is mediated by the M2-1 protein. *Journal of Virology* **82**, 8863-8870.
76. Maddika, S., Wiechec, E., Ande, S.R., Poon, I.K., Fischer, U., Wesselborg, S., **Jans, D.A.**, Schulze-Osthoff, K. & Los, M. (2008) Interaction with PI3 kinase contributes to the cytotoxic activity of Apoptin. *Oncogene* **27**, 3060-3065
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78. Ross DG, **Bowles J**, **Koopman P**, Lehnert S (2008) New insights into SRY regulation through identification of 5' conserved sequences. *BMC Molecular Biology* 9:85
79. Sinigalia, E., Alvisi, G., Mercorelli, B., Coen, D.M., Pari, G.S., **Jans, D.A.**, Ripalti, A., Palù, G. & Lorigian, A. (2008) Role of homodimerization of human cytomegalovirus DNA polymerase accessory protein UL44 in origin-dependent DNA replication in cells. *Journal of Virology* **82**, 12574-12579.
80. Wagstaff, K. M., Fan, J.Y., de Jesus, M.A., Tremethick, D.J. & **Jans, D.A.** (2008) Efficient gene delivery using reconstituted chromatin enhanced for nuclear targeting. *FASEB Journal* **22**, 2232-2242.
81. Yu, D.T., Ajami, K., Park, J., Gall, M., Soon, L.C., Evans, K.A., **McLaughlin, E.A.**, McCaughan, G.W. & Gorrell, M.D. (2009) The in vivo expression of Dipeptidyl Peptidase IV and related enzymes. *Journal of Histochemistry and Cytochemistry* (in press accepted 20th Jan 2009) [Impact factor = 2.6]

## 1.2 Number of patent applications

1. **Aitken, R.J.**, Blackmore, D. & **McLaughlin, E.A.** (2004) Patent No. 2004903226. The University of Newcastle Research Associates and Jurox Pty. Provisional specification for invention entitled 'Method for reducing the reproductive potential of a female animal'. **Refiled in 2008**
2. **Aitken, R.J.** & Griffith R. (2007) The University of Newcastle Research Associates Ltd. Patent no: 2007902839. Contraceptive and Microbicidal compositions. **Refiled in 2008**
3. **Aitken, R.J.** & De Iuliis, G.N. (2008) Newcastle Innovation Limited. Patent no: 2008902473. Assay for the assessment of oxidative stress in gametes or embryos.

## 1.3 Invitations to address and participate in international conferences

1. **Aitken, R.J. & De Iuliis, G.N.** (2008) *Biological origins of DNA damage in human spermatozoa*. Beyond the Light Microscope. Fertility Society of Australia, Sero International Symposia, Brisbane. Abstract L4.
2. **Aitken, R.J.** (2008) *Causes and origins of sperm DNA damage*. Symposium on Sperm DNA integrity. 5th European Congress of Andrology, Rome, Italy.
3. **Aitken, R.J.** (2008) *Dual purpose contraceptives*. Symposium of New concepts in Contraceptive Development. SRB/ESA Annual meeting, Melbourne. Proceedings of the Thirty-ninth Annual Conference of the Society for Reproductive Biology. Abstract: 054
4. **Aitken, R.J.** (2008) Session Chairman. 9th International Conference on Membrane Redox Systems. Wellington, New Zealand.
5. **Aitken, R.J.** (2008) Session Chairman. Building healthy oocytes and sperm. Australian Health & Medical Research Congress 2008, Brisbane Convention Centre.
6. **Aitken, R.J.** (2008) *The role of environmental factors in regulating reproduction in the human male*. Symposium on Environmental interference with gamete development – role in infertility? Australian Health & Medical Research Congress 2008, Brisbane Convention Centre. Abstract 075.
7. **Aitken, R.J.** (2009) **The Inaugural Anne McLaren Memorial Lecture**. *Advances in sperm cell biology*. International Congress 'Fertility 2009', Edinburgh International Conference Centre, UK. Conference Proceedings page 16.
8. **Aitken, R.J., Baker, M.A. & De Iuliis, G.N.** (2008) Plenary Lecture. *Sources of reactive oxygen species generation in mammalian spermatozoa*. 9th International Conference on Membrane Redox Systems. Wellington, New Zealand. Abstract page 21.
9. **Aitken, R.J., De Iuliis, G.N., Nixon, B. & Roman, S.D.** (2008) *Causes and clinical significance of DNA damage in the male germ line*. Symposium on DNA damage in sperm and effects on offspring. 2008 Society for the Study of Reproduction 41st Annual Meeting, Kailua-Kona, Hawaii. Abstract 440.
10. **Daggag, H., Svingen, T., Western, P.S., van den Bergen, J.A., McClive, P.J., Harley, V.R., Koopman, P.A. & Sinclair, A.H.** (2008). *The RhoX homeobox gene family shows sexually dimorphic and dynamic expression during mouse embryonic gonad development*. XX International Congress of Genetics, Berlin, Germany (poster presentation)
11. Fraser, B., Sobinoff, A., **Hime, G.R., Siddall, N., Roman, S.D., McLaughlin, E.A.** The role of the RNA-binding protein, *Musashi-1*, in murine spermatogonial stem cell maintenance. 41st Annual Meeting of the Society-for-the-Study-of-Reproduction, Kona, Hawaii USA Biology of Reproduction Meeting Abstract: 739
12. **Hime, G.R.** (2008) *Genetic analysis of a stem cell niche*. Germ cell-soma interactions in gonadal development and germ cell tumours, Baeza, Spain.
13. **Hime, G.R.** (2008) *Signalling in the Drosophila testis*. Australian Health and Medical Research Congress, Brisbane, QLD.
14. **Hime, G.R.** (2008) Invited speaker: *Repressor proteins prevent stem cell differentiation*. Hunter Cell Biology Meeting, Pokolbin, NSW.
15. **Jamsai, D.** (2008) *Mouse models of human male infertility*. Faculty of Allied Health Sciences, Chulalongkorn University, Bangkok Thailand..
16. **Jamsai, D.** (2008) *The role of Ggn in male fertility and embryogenesis*. Institute of Science and Technology for Research and Development, Mahidol University, Nakornpathom, Thailand.

17. **Koopman, P.A.** (2008) *Genetics of Intersex: Antagonism between male and female sex determining genes*. Association for Chinese Geneticists in America / Hong Kong Society for Medical Genetics International Conference on Genetic Medicine, Hong Kong, China.
18. **Koopman, P.A.** (2008) *Molecular pathways regulating gonadal development in mice* Australian Health and Medical Research Congress, Society for Reproductive Biology Symposium – Gamete Development: The Foundation of Life.
19. **Koopman, P.A.** (2008) *Dynamics and antagonism of the testis – and ovary – determining pathways during ootestis development* International Symposium of Sex Differentiation, Fukuoka, Japan.
20. **Koopman, P.A.** (2008) *Closing remarks: Germ cells Past, Present and Future* International Workshop on Germ Cell-Soma Interactions in Gonadal Development and Germ Cell Tumours, Baeza, Spain.
21. **Koopman, P.A.** (2008) *Molecular interactions between the male and female gonadal sex determining pathways* **Neena B Schwartz Lecture in Reproductive Science**, 29th Annual Symposium on Reproductive Biology, Center for Reproductive Science, Northwestern University, Chicago, Illinois, USA.
22. **Koopman, P.A.** (2008) *Mechanisms of sexual differentiation: Markers and regulators of gonadal development* NIH Genito-Urinary Development Murine Atlas Project Annual Meeting, Potomac, Maryland, USA.
23. **Koopman, P.A.** (2008) *Understanding sexual development: Genes that define maleness and femaleness*. University of Otago Research Forum, Dunedin, New Zealand.
24. **Koopman, P.A.** (2008) *Sex-determining cascades in gonadal development: Insights from ovotestes* World Congress in Reproductive Biology, Hawaii.
25. **Loveland, K.L.** (2008) *The impacts of Activin signalling on testis development and spermatogenesis*. Germ cell-soma interactions in gonadal development and germ cell tumours Workshop sponsored by the Universidad Internacional de Andalucia the “Sede Antonio Machado”, Baeza, Andalusia, Spain.
26. **Loveland, K.L.**, Mithraprabhu, S., Barakat, B., Matzuk, M.M. & Brown, C.W. (2008) *Activin regulates KIT mRNA and protein in the postnatal mouse testis*. Society for the Study of Reproduction Annual Scientific Conference. Kona, Hawaii, USA. Abstract 321.
27. **McLaughlin, E.A.**, Gunter, K., Woollett, K. & **Roman, S.D.** (2008) *Chemokines: Role in germ cell migration and survival*. XIVth International Workshop on the Development and Function of the Reproductive Organs, Villa Mondragone, Monte Porzio Catone Rome, Italy.
28. **McLaughlin, E.A.**, Gunter, K., Woollett, K. & **Roman, S.D.** (2008) *Chemokines: Role in germ cell migration and survival*. Germ cell-soma interactions in gonadal development and germ cell tumours, International University of Andalusia Workshops Current trends in Biomedicine Baeza, Spain.
29. **McLaughlin, E.A.**, Gunter, K., Woollett, K. & **Roman, S.D.** (2008) *Chemokines: Role in germ cell migration and survival*. 41st Annual Meeting of the Society-for-the-Study-of-Reproduction, Kona, Hawaii USA Biology of Reproduction. Meeting Abstract: 11.
30. **Miles, D., van den Bergen, J., Sinclair, A. & Western, P.** (2008) *Regulation of the mouse fetal germ cell cycle*. Cold Spring Harbour Laboratory Germ Cell Meeting, New York City, USA.
31. **Monk, A.C., Siddall, N.A. & Hime, G.R.** (2008) *The RNA-binding protein HOW is required for stem cell maintenance in the Drosophila testis*. 20th International Congress of Genetics, Berlin, Germany
32. **Nixon B.** (2008) Invited Symposium Speaker. *Molecular basis for sperm-egg interaction; prospects and problems for contraceptive development*. Proceedings of the 39th Annual Conference of the Society for Reproductive Biology, Melbourne, Australia. Abstract 55.
33. **Nixon, B., Bielaniowicz, A., McLaughlin, E.A., Tanphaichitr, N., Ensslin, M. & Aitken, R.J.** (2008) *The composition and significance of lipid rafts in mouse spermatozoa*. 41st Annual Meeting of the Society-for-the-Study-of-Reproduction, Kona, Hawaii USA Biology of Reproduction Meeting Abstract: 569.

34. **O'Bryan, M.K.** (2008) *The identification of critical male meiosis genes in mice and men*. The first World Congress of Reproductive Biology (WCRB), Hawaii, USA.
35. **O'Bryan, M.K.** (2008) *CRISP protein networks and male fertility*. The Ritchie Centre of Baby Health Research, Monash University. August.
36. Sarraj, M.A., Chua, H.K., Umbers, A., Escalona, R., **Loveland, K.L.**, Findlay, J. & Stenvers, K.L. (2008) *Betaglycan is required for normal Leydig cell development in mouse*. Society for the Study of Reproduction Annual Scientific Conference. Kona, Hawaii, USA. Abstract 573.
37. **Siddall, N.A.**, Johnston, N.L., Been, R.P., Kalcina, M., **Monk, A.C.**, **McLaughlin, E.A.** and **Hime, G.R.** (2008) *Musashi family proteins are functionally required in both somatic and germline stem cell populations in the *Drosophila testis**. 73rd Cold Spring Harbor Symposium on Stem Cells, New York, U.S.A.
38. **Sinclair, A.** (2008) *Analysis of patients with disorders of sexual development*. International Congress of Zoology, Paris, France 26-29 Aug.
39. **Sinclair, A.** (2008) *Analysis of patients with disorders of sexual development*. Australasian Paediatric Endocrine Group Conference, Canberra, ACT.
40. **Sinclair, A.** (2008) *Patients with disorders of sexual development provide new clues to sex determining genes*. Australian Health and Medical Research Conference, Brisbane, QLD.
41. **Van den Bergen, J.**, **Miles, D.**, Li, R, Ralli, R, **Sinclair, AH.** & **Western, P** (2008) *Dynamic regulation of cell cycle and pluripotency in the foetal male germ line*. Germ Cell – Soma Interactions in Gonadal Development and Germ Cell Tumors, Baeza, Spain.
42. **Western, P.**, **van den Bergen, J.**, Miles, D., Ralli, R., Li, R. & **Sinclair A** (2008) *Regulation of pluripotency in mouse fetal germ cells*. Cold Spring Harbour Germ Cell Meeting, New York City, USA.
43. White, S., Daggag, H., Gustin, S., Forrest, S., Bahlo, M., Bengtsson, H., Gordon, L., Vilain, E., Speed, T., **Sinclair, A.** (2008) *Copy number analysis of patients with gonadal dysfunction using high-density microarrays and MLPA*. XX International Congress of Genetics, Berlin, Germany, 12-17 July .
44. **Young, J.C.**, Dias, V.L. & **Loveland, K.L.** (2008) *BMP signaling in the induction of germline precursors from mouse embryonic stem cells in vitro*. Germ cell-soma interactions in gonadal development and germ cell tumours Workshop sponsored by the Universidad Internacional de Andalucia the "Sede Antonio Machado", Baeza, Andalusia, Spain

## 1.4 Invitations to visit leading international laboratories

### Aitken, R.J.

- Malaghan Institute of Medical Research, Victoria University, Wellington New Zealand.

### Hime, G.R.

- Dr. Barbara Jennings, University College London, U.K.
- Dr. Alex Gould, National Institute of Medical Research, Mill Hill, U.K.

### Holland, M.K.

- John Hopkins University (Baltimore) re Embryonic Stem Cells to gametes
- Viagen Austin – Meeting with Executive at Viagen re Horse Embryonic Stem Cell current contract and possibility of expansion in other species.

### Koopman, P.A.

- Dr Kevin Gaido, Hamner Institutes for Health Sciences, Research Triangle Park, North Carolina, USA, 13 February 2008.
- Dr Blanche Capel, Duke University, Chapel Hill, North Carolina, USA, 14 February 2008
- Dr Mitch Eddy, National Institute of Environmental Health Sciences, NIH, Research Triangle Park, North Carolina, USA, 13 February 2008.
- Dr Kathryn Cheah, Department of Biochemistry, University of Hong Kong, Pokfulam, Hong Kong, China, 10 June 2008
- Dr Peter Dearden, University of Otago, Dunedin, New Zealand, 25 August 2008
- Dr Kelly Mayo, Dr Neena Schwartz, Centre for Reproductive Science, Northwestern University, Evanston, Illinois, USA, 7 October 2008
- Dr Larry Jameson, Feinberg School of Medicine, Chicago, Illinois, USA, 8 October 2008
- Dr Teresa Woodruff, Obstetrics and Gynecology, Northwestern University, Chicago, Illinois, USA, 8 October 2008

### Loveland, K.A.

- Department of Molecular Biosciences and Center for Reproductive Sciences, University of Washington, Pullman WA USA; April 2008.

### McLaughlin, EA.

- Dr Norah Spears Centre for Integrative Physiology, University of Edinburgh UK,
- Dr Evelyn Telfer Institute of Cell Biology, University of Edinburgh UK,
- Dr Allan Pacey Diabetes, Endocrinology and Metabolism., University of Sheffield, UK
- Dr Jennifer Williams Bristol Centre for Reproductive Medicine, Bristol UK

### Sinclair, A.H.

- Dr Leendert Looijenga, Erasmus Medical Center – University Medical Center, NL
- Daniel Den Hoed Cancer Center, Josephine Nefkens Institute, Rotterdam, NL
- Dr Ieuan Hughes, School of Clinical Medicine, University of Cambridge, Addenbrooke's Hospital, Cambridge, UK
- Dr John Achermann, Clinical and Molecular Genetics Unit, UCL Institute of Child Health, London, UK

## 1.5 Number and nature of commentaries about the Centre's achievements

### Aitken, R.J.

- **NBN News.** Commentary on LIEF grant success for triple quadrupole mass spectrometer. 20/11/08
- **Weekend Australian.** Commentary by Dr Karl (Karl Kruszelnicki) about work of the Centre on male infertility under the title: 'Myth conceptions. Do men have fewer fertility problems than women.' 27/9/08
- **Obesity Fitness and Wellness Week.** Commentary about the Centre's work on male contraception under title: *Research Australia: New Concepts in Contraception.* 5/9/08
- **SBS News item in response to** Prince Henry's Hospital press release to coincide with SRB Symposium on 'New Concepts in Contraceptive Development' featuring the Centre's work on dual purpose formulations that attack both fertility and the spread of sexually transmitted disease. 28/8/08
- **Hunter Medical Research Institute** media release entitled 'Hunter Researchers Share the Love' featuring Centre research on male reproduction. This release resulted in interviews with **ABC Radio Newcastle** and **Radio 2HD** 2/7/08
- **Australian Life Scientist** news item on Centre research focusing on the development of a biotechnology device designed in conjunction with the Australian biotech company, NuSep, to improve the efficiency and effectiveness of sperm preparation in an assisted conception context. 20/6/08
- **Science Alert.** Press coverage following Professor Aitken's presentation to the ARC Graeme Clark Research Outcomes Forum at Parliament House in Canberra on the sperm-separation device being developed in conjunction with NuSep. 19/6/08
- **SBS radio** Coverage of the above presentation to the Graeme Clark Outcomes Forum in Canberra on development of a sperm separation device for the assisted conception industry. 24/6/08
- **ABC Science.** Interview with Anna Salleh about collaborative work with NuSep on the development of a sperm separation device and the future of Assisted Conception. 14/6/08
- **Sunday Telegraph.** Interview about the impact of paternal age on the health and wellbeing of children. 5/6/08
- **NHK film,** a Japanese film company preparing a documentary on Assisted Conception interviewed Professor Aitken about the work conducted by the Centre in the area of male infertility and filmed sequences in the laboratory. 21/5/078-22/5/08
- **Hunter Business Review. Innovation.** An article on innovation in the Hunter covering the Centre's work in the area of biotechnology.
- **ABC radio Newcastle.** Interview with Garth Russell on the Centre's work with the biotechnology company, NuSep. 13/3/08
- **Channel 7 Sunrise.** An interview Howard Smith, a clinician leading a team evaluating the clinical significance of the sperm separation device developed by the Centre in collaboration with NuSep. 18/3/08.
- **HMRI Publication** celebrating 10 years of innovation in biomedical research in the Hunter, cites Centre's work in the assisted conception area under the title, 'Infertility Solutions – creating a healthy baby'. 1/6/08
- **Newcastle Herald.** Centre's work on male contraception cited in an article entitled 'Seeing Value in Research' celebrating HMRI research accomplishments. 1/5/08
- **Sydney Morning Herald.** Research conducted by the Centre on the damaging effects of mobile phone radiation on male reproduction cited under the title 'Mobile phone radiation fries sperm'. 5/9/08
- **Searcher.** Article citing the centre's work on male infertility under the title 'World Class Research for our Future'. 1/6/08
- **University of Newcastle Press release** entitled 'Male infertility' concerning funding successes for the reproductive science group including an upgrade to our Advanced Mass Spectrometry facility to facilitate the development of our metabolomics platform. 16/12/08
- **Australasian Science.** Centre biotechnology research on the development of a sperm separation device featured in an article entitled, 'Sperm separator improves IVF Prospects'. 1/5/08

- **Life Scientist.** Article featuring Centre research with NuSep on the development of a commercial sperm separation device under the title, 'New Step for NuSep'. 1/9/08
- **Daily Telegraph.** One-page Article discussing the Centre's research on sperm separation technology reported under the headline, 'Aussie Breakthrough has parents ecstatic'. 14/3/08.

**Hime, G.R., Sinclair, A.H., Loveland, K.L., O'Bryan, M.K., Roman, S.D. & Aitken, R.J.**

- 'Avoid retrograde step' Letter to the The Australian Higher Education Supplement. 30/4/08

**Holland, M.K.**

- ABC Radio (syndicated to 37 metropolitan and regional ABC radio stations). 'Cloned cow meat'. 18/1/08

**Koopman, P.A.**

- **UQ News Online.** Press release 'For they are jolly good fellows: UQ scientists elected to top national body' relating to the election to the Australian Academy of Science. 25/3/08
- **The Courier Mail** News story 'Three aces' relating to the election to the Australian Academy of Science. 27/3/08
- **B magazine, Brisbane, issue 136.** 'Swimming upstream', relating to work on male germ cell development and fertility. 1/4/08
- **UQNews.** issue 573. 'Science success', relating to the election to the Australian Academy of Science. 1/4/08
- **UQ Press release** 'Top Australian showcased at peak science event' relating to the new fellow's talk titled: "The genetics and biology of sex development", Australian Academy of Science, 6/5/08
- **IMBoutput,** No. 1. 'IMB scientists elected to top national body', relating to the election to the Australian Academy of Science, 1/9/08
- **Westside News** 'UQ scientists elected to top national body', relating to the election to the Australian Academy of Science. 4/6/08

**McLaughlin, E.A.**

- NBN Television news with Natasha Beyersdorf, "Aging and fertility" 5/01/09.
- ABCV News on line News story focusing on NHMRC funding to study environmental impacts on fertility <http://www.abc.net.au/news/stories/2009/02/04/2481688.htm>. 5/01/09.
- University of Newcastle Press release entitled 'Aging and infertility' concerning funding successes for the reproductive science group 04/02/08

**O'Bryan, M.K.**

- **Biotechnology Victoria e-Bulletin** Victoria and Manitoba team up on new research projects. 1/03/08
- **www.presszoom.com** – Victoria and Manitoba team up on new research projects. 4/03/08
- **Sydney Morning Herald.** *The Case of the Disappearing Dads.* 10/04/08
- **Canberra Times.** Launch of Australian Phenomics Network. 21/08/08
- **Australian Financial Review.** Launch of Australian Phenomics Network. 25/08/08
- **The Australian** Launch of Australian Phenomics Network. 27/08/08

## 1.6 Additional competitive grant income

**Aitken, R.J.**

- NHMRC Program Grant \$350,000 pa
- CONRAD \$136,000 pa
- Invasive Animal CRC \$65,000 pa
- ARC Linkage grant joint with Eileen McLaughlin \$465,000 2007-2009

**Hime G**

- NHMRC Project Grant \$402,000 (CIB) with Eileen McLaughlin 2009-11
- NHMRC Project Grant \$565,500 (CIA) with Helen Abud and W.G. Somers 2008-2010

**Holland MK**

- Dairy Australia with Paul Verma \$250,000

**Jans, DA**

- NHMRC Project Grant (CIA) \$481,500 2006-2008
- NHMRC Senior Fellowship \$692,500 2006-2010
- NHMRC Project Grant (CIA) with P.Bardin \$476,500 2007-2009
- NHMRC Project Grant (CIA) \$447,000 2007-2009
- NHMRC Project Grant (CIA) with G. Zambetti \$473,000 2008-2010
- NHMRC Project Grant (CIA) \$508,500 2009/2011

**Loveland KL**

- NHMRC Research Fellowship \$618,750 2009-2013
- NHMRC Project Grant (CIA) with Sarah Meachem and Mark Hedger \$491,250 2009-2011
- ARC Discovery Grant \$885,000 with Yoichi Miyamoto (ARC Fellow), Norman Hecht and Yoshihiro Yoneda 2008-2012

**McLaughlin EA**

- ARC Linkage grant (CIA) with RJ Aitken \$465,000 2007-2009
- ARC Discovery Grant (CIA) \$270,000 with Darryl Russell and Rebecca Robker 2008-10
- NHMRC Project Grant \$362,000 (CIA) with Brett Nixon and Shaun Roman 2008-10
- NHMRC Project Grant \$402,750 (CIA) with Gary Hime 2009-11
- NHMRC Project Grant \$506,000 (CIB) with Keith Jones 2009-11
- NHMRC Project Grant \$474,000 (CIB) with Brett Nixon & Moira O'Bryan 2009-11
- NHMRC \$402,000 (CIC) with Ken Beagley, Peter Timms and Charles Wira 2009-11
- Hunter Medical Research Institute \$80,000 (CIB) with Keith Jones 2008-09

**Roman SD**

- NHMRC Project Grant \$362,000 (CIC) with Eileen McLaughlin and Brett Nixon 2008-10

**O'Bryan MK**

- NHMRC Research Fellowship \$618,750 2009-2013
- NHMRC Project Grant \$474,000 (CIC) with Brett Nixon & Eileen McLaughlin 2009-11
- NHMRC Project Grant \$580,500 (CIA) 2008-2010
- NHMRC Project Grant \$516,000 (CIA) 2008-2010
- Victorian State Government and the provincial Government of Manitoba (Canada) \$700,000 with G Hicks, P Hertzog, B Jenkins 2008-2009
- Monash IVF \$80,000 (CIB) with Rob McLachlan 2008

**Sinclair AH**

- ARC Discovery Grant \$86,000pa (CIB) 2005-2008
- NHMRC Program Grant \$300,000pa (CIA) 2005-2009
- NHMRC Fellowship \$136,000pa 2009-2013

**Koopman P**

- NHMRC Program Grant \$300,000 pa
- NHMRC Program Grant \$526,500 (CIA) 2007-2010
- ARC Federation Fellowship \$322,625 pa 2007-2012
- ARC Discovery Grant \$1,547,727 2007-2011
- Queensland Cancer fund \$160,000 2008-2009
- Invasive Animal CRC \$20,000pa

**Equipment**

- Jones et al ARC LIEF \$275,000 (CIB) Live cell imaging LSCM 2009
- Aitken et al ARC LIEF \$495,000 (CIG) Mass spectrometer
- McLaughlin et al Cancer Institute NSW \$233,000 (CIB) Live animal imaging

## 2. Research Training and Professional Education

### 2.1 Number of postgraduates recruited

Twelve postgraduate students recruited in 2008, 3 of whom have received Faculty Medals and one a VC's award for Outstanding Research Candidature:

Student: **Arash Arjomand**  
 Affiliation: Monash University  
 Thesis title: The spermatogenic Importome  
 Supervisors: Kate Loveland, Yoichi Miyamoto (Monash)  
 Funding: Monash University APA/ ARC Centre of Excellence

Student: **Matt Dun**  
 Affiliation: University of Newcastle  
 Project: Molecular mechanisms regulating sperm-zona interaction  
 Supervisors: R. John Aitken and Brett Nixon (Newcastle)  
 Funding: NSW State Fellowship/ARC Centre of Excellence

Student: **Kara Gunter**  
 Affiliation: University of Newcastle  
 Thesis title: Role of Chemokines in spermatogenesis  
 Supervisors: Eileen McLaughlin (Newcastle)  
 Funding: Newcastle University APA/ ARC Centre of Excellence

*This student was the recipient of a Faculty medal*

Student: **Jacky Hewitt**  
 Affiliation: University of Melbourne  
 Project: Analysis of patients with disorders of sex development using high-density whole genome microarray  
 Supervisors: Andrew Sinclair and Stefan White (Melbourne)  
 Funding: Melbourne Research Scholarship

Student: **Rose Keightley**  
 Affiliation: University of Newcastle  
 Thesis title: SOCS proteins in germ cell development  
 Supervisors: Eileen McLaughlin, Brett Nixon and Shaun Roman (Newcastle)  
 Funding: Newcastle University APA/ ARC Centre of Excellence

*This student was the recipient of a Faculty medal*

Student: **Savvas Koundouros**  
 Affiliation: Monash University  
 Thesis title: Improvement of the current culture in human embryonic stem cells  
 Supervisors: Michael Holland, Dr P Verma (Monash)  
 Funding: Personal funding/ ARC Centre of Excellence

Student: **Skye McIver**  
 Affiliation: University of Newcastle  
 Thesis title: MiRNA and testicular germ cell tumours  
 Supervisors: Eileen McLaughlin, Brett Nixon and Shaun Roman (Newcastle)  
 Funding: Newcastle University APA/ ARC Centre of Excellence

*This student was awarded the VC's award for Outstanding Research Candidature and was the recipient of a University medal*

Student: **Tegan Smith**  
 Affiliation: University of Newcastle  
 Project: DNA damage in the male germ line and embryonic development  
 Supervisors: R. John Aitken (Newcastle)  
 Funding: University of Newcastle APA/ ARC Centre of Excellence

Student: **Alexander Sobinoff**  
 Affiliation: University of Newcastle  
 Thesis title: Role of Musashi in germ cell development  
 Supervisors: Eileen McLaughlin (Newcastle)  
 Funding: Newcastle University APA/ ARC Centre of Excellence

Student: **Fangyuan Yang (Wendy) – Master of Biomedical Science (Part 1)**  
 Affiliation: Monash University  
 Thesis title: Identification of Adult Stem Cells during the Mouse Epididymal Development  
 Supervisor: Michael Holland (Monash)  
 Funding: Personal funding / ARC Centre of Excellence

Student: **Jaleh Barzideh (MSc)**  
 Affiliation: University of Newcastle  
 Thesis title: DNA damage in spermatozoa  
 Supervisors: R. John Aitken  
 Funding: Newcastle University APA/  
 ARC Centre of Excellence

Student: **Belinda Nixon**  
 Affiliation: University of Newcastle  
 Thesis title: Toxicology in the Male  
 Mouse Germline.  
 Supervisors: Shaun Roman and Brett Nixon  
 Funding: Newcastle University APA/  
 ARC Centre of Excellence

Student: **Kate Redgrove**  
 Affiliation: University of Newcastle  
 Thesis title: The molecular basis of human  
 sperm-egg interaction.  
 Supervisors: Brett Nixon, Eileen McLaughlin  
 and Moira O'Bryan  
 Funding: Personal funding / ARC Centre  
 of Excellence

## 2.2 Number of postgraduate completions

Eight Centre PhD completions including:

Student: **Daniel Campbell**  
 Affiliation: The University of Newcastle  
 Project: The Nature of Chromatin Packaging  
 in Human Spermatozoa  
 Supervisors: Shaun Roman and David Jans  
 (Newcastle & Monash)  
 Funding: ARC Centre of Excellence

Student: **Catherine Itman**  
 Affiliation: Monash Institute for Medical Research  
 Project: Developmentally regulated SMAD  
 signalling in the testis  
 Supervisors: Kate Loveland and David Jans  
 (Monash)  
 Funding: APA/ARC Centre of Excellence

Student: **Yun Hwa Lee**  
 Affiliation: The University of Newcastle  
 Project: Functional Maturation of Mouse  
 Epididymal Spermatozoa  
 Supervisors: R. John Aitken and Minjie Lin  
 (Newcastle)  
 Funding: Fee paying overseas student/ARC  
 Centre of Excellence

Student: **Camden Lo**  
 Affiliation: Monash Institute for Medical Research  
 Project: Microtubule-associated protein-2  
 in spermatogenesis  
 Supervisors: Kate Loveland and David Jans  
 (Monash)  
 Funding: ARC Centre of Excellence

Student: **Lisa Mitchell**  
 Affiliation: The University of Newcastle  
 Project: The Molecular Basis of Sperm-zona  
 Pellucida Interaction  
 Supervisors: R. John Aitken and Brett Nixon  
 (Newcastle)  
 Funding: NSW State Fellowship/APA  
 scholarship

Student: **Sridurga Mithraprabhu**  
 Affiliation: Monash Institute for Medical Research  
 Project: Regulation of KIT in spermatogenesis  
 and hematopoiesis  
 Supervisors: Kate Loveland (Monash)  
 Funding: ARC Centre of Excellence

Student: **Jonathan Paul**  
 Affiliation: The University of Newcastle  
 Project: Oolemmal proteomics: sperm  
 egg interaction  
 Supervisors: Eileen McLaughlin (Newcastle)  
 Funding: APA/ARC Centre of Excellence

Student: **Anette Szczepny**  
 Affiliation: Monash Institute for Medical Research  
 Project: Function and Regulation of Hedgehog  
 signalling in the adult mouse testis  
 Supervisors: Kate Loveland and David Jans  
 (Monash)  
 Funding: APA/ARC Centre of Excellence

### 2.3 Number of Honours students

A total 20 honours students were admitted to the Centre last year of which 9 received 1st Class Honours degrees and 3 were awarded University Faculty medals.

Student: **Amanda Anderson – 1st Class Honours**  
 Affiliation: The University of Newcastle  
 Project: Role of retinoids in the male germ line  
 Supervisor: Shaun Roman  
 Funding: ARC Centre of Excellence

Student: **Reeva Been – 1st Class Honours**  
 Affiliation: The University of Melbourne  
 Project: Investigating the role of Real Musashi in regulating germline stem cell behaviour in the *Drosophila melanogaster* testis  
 Supervisors: Gary Hime and Nicole Siddall  
 Funding: ARC Centre of Excellence

Student: **Trent Butler**  
 Affiliation: The University of Newcastle  
 Project: Investigations into Neddylation during Spermatogenesis  
 Supervisors: R. John Aitken and Mark Baker  
 Funding: ARC Centre of Excellence

Student: **Matthew Dun – 1st Class Honours**  
 Affiliation: The University of Newcastle  
 Project: Identification of sperm-egg receptor proteins  
 Supervisors: R. John Aitken and Brett Nixon  
 Funding: ARC Centre of Excellence

Student: **Rachel Gentles**  
 Affiliation: The University of Newcastle  
 Project: Cytokines and primordial follicle activation  
 Supervisors: Eileen McLaughlin  
 Funding: ARC Centre of Excellence

Student: **Kara Gunter – 1st Class Honours**  
 Affiliation: The University of Newcastle  
 Project: Chemokines and Spermatogenesis: the role of SDF-1/CXCL12 and CXCR 4/-7 in gonocyte survival and maintenance of the germ line stem cell niche  
 Supervisor: Eileen McLaughlin  
 Funding: ARC Centre of Excellence

***This student was the recipient of a Faculty medal***

Student: **Tamica Humby**  
 Affiliation: The University of Newcastle  
 Project: DNA damage reactive oxygen species in the male germ line  
 Supervisors: R. John Aitken and Geoffrey Delullis  
 Funding: ARC Centre of Excellence

Student: **Vanessa Hsu – 1st Class Honours**  
 Affiliation: University of Melbourne  
 Project: Identification of novel genes involved in disorders of sex development  
 Supervisors: Stefan White and Andrew Sinclair (Melbourne)  
 Funding: Department of Paediatrics, University of Melbourne

Student: **Rose Anna Keightley – 1st Class Honours**  
 Affiliation: The University of Newcastle  
 Project: Cytokine Signalling in the Murine Ovary  
 Supervisor: Eileen McLaughlin  
 Funding: ARC Centre of Excellence

***This student was the recipient of a Faculty medal***

Student: **Cherise Mooy**  
 Affiliation: The University of Newcastle  
 Project: DNA damage by Reactive Oxygen Species Induction in the Male Germ Line  
 Supervisors: R. John Aitken and Geoffrey Delullis  
 Funding: ARC Centre of Excellence

Student: **Rhiannon Newey – 1st Class Honours**  
 Affiliation: The University of Newcastle  
 Project: DNA damage in the male germ line  
 Supervisors: R. John Aitken and Geoffry Delullis  
 Funding: ARC Centre of Excellence

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Student: **Belinda Nixon – 1st Class Honours**  
 Affiliation: The University of Newcastle  
 Project: Toxicology in the Male Mouse Germline  
 Supervisor: Shaun Roman  
 Funding: University of Newcastle/ARC Centre of Excellence

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Student: **Rachael Ralli – 1st Class Honours**  
 Affiliation: University of Melbourne  
 Project: Germ cell differentiation and mitotic arrest in a mouse testis cancer model  
 Supervisors: Patrick Western and Andrew Sinclair  
 Funding: ARC Centre of Excellence

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Student: **Kate Redgrove – 1st Class Honours**  
 Affiliation: The University of Newcastle  
 Project: Molecular basis of sperm-egg interaction: identification of surface protein complexes in human spermatozoa  
 Supervisor: Brett Nixon  
 Funding: ARC Centre of Excellence

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Student: **Sewa Rijal**  
 Affiliation: Monash University  
 Project: Characterisation of the mouse sperm acrosome and tail associated protein (SATAP)  
 Supervisors: Duangporn Jamsai and Moira O'Bryan  
 Funding: ARC Centre of Excellence

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Student: **Amanda Ross**  
 Affiliation: Monash Institute of Medical Research  
 Project: Prenatal and postnatal development of the mouse epididymis  
 Supervisor: Michael Holland  
 Funding: ARC Centre of Excellence

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Student: **Alexander Sobinoff – 1st Class Honours**  
 Affiliation: The University of Newcastle  
 Project: Role of Msi 1 & 2 in Spermatogenesis  
 Supervisor: Eileen McLaughlin  
 Funding: ARC Centre of Excellence

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Student: **Padmini Sugamarin**  
 Affiliation: Monash University  
 Project: Cell biology of male germ cells  
 Supervisors: Claire Borg and Moira O'Bryan  
 Funding: ARC Centre of Excellence

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Student: **Emma Towney**  
 Affiliation: The University of Newcastle  
 Project: MTT reduction in the male germ line  
 Supervisors: Minjie Lin and R. John Aitken  
 Funding: ARC Centre of Excellence

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Student: **Nathan Watson**  
 Affiliation: The University of Newcastle  
 Project: BMP4 Signalling in the male germ line  
 Supervisor: Shaun Roman  
 Funding: ARC Centre of Excellence

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## 2.4 Number of professional courses

The Centre instigated a workshop for all CIs and post docs on the *Preparation of competitive grants* at the 6th Scientific Conference, Sofitel Spa and Mansion, Melbourne.

### Julia Young

- Australian Society for Medical Research Career Development Day

### Camden Lo

- Monash MicroImaging, Live Cell Imaging Course

### Koopman, P.A.

- Joint convener: International Workshop "Germ Cell-Soma Interactions in Gonadal Development and Germ Cell Tumours", Baeza, Spain, 20-22 October 2008.
- Invited speaker, Howard Florey Institute / WEHI Postdoctoral Fellows Society careers workshop, March 2008
- Centre of Excellence in Biotechnology and Development, Careers workshop, Werribee Park, Victoria, 7 September, 2008: "Attracting ARC Grants"

### McLaughlin, E.A.

- Speaker and Mentor at Women@UoN Promotion workshops
- Speaker and Mentor at Faculty Grant writing workshops

### Sinclair, A. H.

- Centre of Excellence in Biotechnology and Development, Careers workshop, Werribee Park, Victoria, 7 September, 2008: "How to get an NHMRC Grant"
- Invited speaker: Murdoch Children's Research Institute & Dept. of Paediatrics, University of Melbourne joint workshop on Grantsmanship. RCH, 6 July, 2008 : "What you need to do to get an NHMRC grant"

## 2.5 Participation in professional courses

- **Julia Young, Andrew Major, Catherine Itman**  
Monash MicroImaging, Live Cell Imaging Course
- **McLaughlin, E.A.**  
Was awarded a Career Enhancement Fellowship for Academic Women at Newcastle incorporating one-one mentoring programme with professional career advisor

## 2.6 Number and level of undergraduate and high school courses in the priority area(s)

### Aitken, R.J.

#### Teaching

- BIOL1001 1st year Biology course. Fundamentals of Development (360 students) 8 h of lectures.
- BIOL3200 Cellular Biotechnology (35 students) Third year course for B. Biotech students 6 lectures

#### Third Year B. Biotechnology Placement (10 week placements)

- Student: Sarah Whiting  
Role of PI3 kinase in sperm apoptosis

### Koopman, P.A.

#### Teaching

- BIOL1017 Advanced Study Program (1 lecture)

### Hime, G.R.

#### Course co-ordination

- 516302 3rd Year, Developmental Biology (108 students)
- 606309 3rd Year, Frontiers in Cell Biology (105 students)
- 516307 3rd Year, Research Project (42 students)
- Honours 25 students

#### Teaching

- 516201 2nd Year, Cell Biology: Tissues and Organs (129 students) (7 lectures, 18 hours practical, assignment and exam marking)
- 516302 3rd Year, Developmental Biology (6 lectures, 30 hours practical, assignment and exam marking)

**McLaughlin, E.A.****Course Coordination**

- Coordinator for First Year Biology BIOL1001 (500 students), BIOL1002 (250 students) & BIOL1003 (150 students) B. Biotech. B.Sc., B.Eng. B.Psych.

**Teaching**

- BIOL1001 Molecules, Cells & Organisms (500 students) (18 lectures + assignment marking + exam marking)
- BIOL2001 Molecular and Cell Biology lab skills (80 students) (8 lectures + 16 hours tutorials + assignment marking)
- BIOL3200 Cellular Biotechnology (42 students) (8 lectures + 16 hours tutorials + assignment marking + exam marking)

**Third Year B. Biotechnology Placement (10 week placements)**

- Student: Rebecca Watkins  
Xenobiotics and Fertility

**Summer Scholarship students**

- Student: Mark Bigland B.Biotech  
Msi2 in spermatogenesis
- Jessie Sutherland B.Biotech  
Phage display and germ cell surface mapping (Jessie was awarded a Faculty Research Summer scholarship)
- Alissa Wight B.Sci. – University of Technology Sydney  
Fertility and smoking  
(Alissa was awarded a Faculty Visiting Research Summer scholarship)

**Holland, M.K.****Teaching**

- GRS1001 and 1002 Education Program in Reproductive Development (12 students) (3 lectures + lab practicals + exam marking)
- DEV BIOL3011 and 3021 Developmental Biology (50 students) (5 lectures + exam marking)

- MCE 1011 Masters in Clinical Embryology (30 students) (3 lecture and exam marking)
- DEV 3032 Stem cells and regeneration (39 students)  
3rd .year course for BSc students  
Stem Cells and Animal Biotechnology 1  
Stem Cells and Animal Biotechnology 2
- Graduate diploma reproductive science (6 students)  
Biotechnology and Pest Animal Control  
One year course for GRS 2001 students

**Course Co-Ordination**

- DEV 3032: Practical 8  
Moderated Discussion: Stem Cells from Bench to Bedside  
3rd year course for BSc students

**Jans, DA****Teaching**

- Undergraduate course BCH3031 "Advanced molecular biology: Genomic structure" (4h lectures, 4h tutorials, plus essay and exam marking) at Monash University (Biochem. and Mol. Biol. Dept.)
- Undergraduate course BCH3021 "Cellular organisation: Organelle structure and function in health and disease" (4h lectures, 4h tutorials, plus essay topic and exam marking) at Monash University (Biochem. and Mol. Biol. Dept.)

**Sinclair, AH****Teaching**

- Bachelor of Biomedical Science – 3rd year (150 students)  
536-350 Genes to Phenotype: Control and Integration (1 lecture)
- Department of Genetics – Honours (20 students) (4 lectures + essay marking)

### **Sinclair, AH**

#### **Teaching**

- Bachelor of Biomedical Science – 3rd year (150 students)  
536-350 Genes to Phenotype: Control and Integration (1 lecture)
- Department of Genetics – Honours (20 students) (4 lectures + essay marking)

### **Roman, SD**

#### **Program Convenor**

- B. Biotechnology University of Newcastle (100 students).

#### **Course Coordinator**

- BIOL2001 Molecular Lab Skills (60 students)
- BIOL3200 Cellular Biotechnology (40 students)
- BIOL3250 Biotechnology Placement (30 students)

#### **Teaching**

- BIOL3090 Molecular Biology (60 students) (13 lectures + exam marking + assignment marking)
- BIOL2001 Molecular Lab Skills (60 students) (8 lectures + 12 hours practical + assignment marking)
- BIOL3200 Cellular Biotechnology (40 students) (8 lectures + 40 tutorials + report marking + assignment marking)
- BIOL3250 Biotechnology Placement (30 students) (Interviews, report marking, seminar marking)

### **Third Year B. Biotechnology Placement (10 week placements)**

Mark Bigland B. Biotech  
Mouse toxicology models.  
Paul Huthnance B. Biotech  
Elucidating the mechanisms of germ cell differentiation

#### **Summer Scholarship students**

Andrew Reid B. Sc.  
Chromatin Packaging in Human Spermatozoa  
(*Andrew was awarded a Faculty Research Summer scholarship*)

Selina Chan B.For.Sci. – University of Technology  
Sydney

DNA damage in mouse toxicology models.

(Selina was awarded a Faculty Visiting Research Summer scholarship)

### **Loveland, K.L.**

#### **Teaching**

- Monash University Education Program in Reproduction and Development (~12 students), 2 lectures on Growth factors (GRS1001) and 2 lectures on Gonadal growth and development (GRS1002). Additional lectures (3) and lab practical supervision supplied by Centre postdoctoral fellows, Catherine Itman and Julia Young.
- Monash University, Department of Anatomy and Developmental Biology, 3rd year courses 3011 and 3021 lectures (~60 students). 2 lectures on Growth factors in Development and 1 lecture on Reproductive Stem Cells. Additional lectures (3) supplied by Centre postdoctoral fellow, Julia Young.

### 3. International, National and Regional Links and Networks

#### 3.1 Number of papers published with international co-authors/reports for international bodies

**A total of 16 papers published with international authorships**

**Aitken, R.J.**

1. **Aitken, R.J.** et al (2008) Editors. *World Health Organization Laboratory Manual for the Examination of Human Semen and Semen-cervical mucus interaction*. Cambridge University Press, Cambridge (in press)
2. **Aitken, R.J., Baker, M.A.,** Doncel, G.F., Matzuk, M.M., Mauck, C.K. & Harper, M.J.K. (2008) As the world grows: contraception in the 21st century. *Journal of Clinical Investigation* **118**, 1330-1343. [Impact factor = 16.4]
3. **Baker, M.A., Hetherington, L., Reeves, G.H.,** Muller, J. & **Aitken, R.J.** (2008) The rat sperm proteome characterised via IPG strip pre-fractionation and LC-MS/MS identification. *Proteomics* **8**, 2312-2321. [Impact factor = 5.8]
4. Bennetts, L.E., **Deluliis, G.N., Nixon, B.,** Kime, M., Zelski, K., Mc Vicar, S.M., Lewis, S.E. & **Aitken, R.J.** (2008) Analysis of the impact of estrogenic compounds on DNA integrity in the male germ line. *Mutation Research* **641**, 1-11. [Impact factor = 4.0]
5. McLachlan, R.I., **Aitken, R.J.,** Cram, D., Krausz, C. & **O'Bryan, M.K.** (2008) Y chromosome mutations in assisted reproductive technology. Letter. *Fertility and Sterility* **90**, 463-4. [Impact factor = 3.2]
6. **Nixon, B, Bielanowicz, A., McLaughlin, E.A.,** Tanphaichitr, N., Ensslin, M.A. & **Aitken, R.J.** (2008) Composition and significance of detergent resistant membranes in mouse spermatozoa. *Journal of Cell Physiology* **218**: 122-134. [Impact factor = 4.0]

**Hime, G**

7. **Siddall, N.A.,** Lin, J.I., **Hime, G.R.** and Quinn, L.M. (2009) Myc – what we have learned from flies. *Current Drug Targets*, in press [IF=4.0]
8. Eid, J.P., Martinez Arias, A., Robertson, H. **Hime, G.R.** and Dziadek, M. (2008) The Drosophila STIM1 orthologue, dSTIM, has roles in cell fate specification and tissue patterning. *BMC Developmental Biology*. 8:104 [IF=3.3]

**Loveland, K. L.**

9. Dias, V., Meachem, S., Rajpert-deMeyts, E., McLachlan, R., Manuelpillai, U., & **Loveland, K.L.** (2008) Activin receptor subunits in normal and dysfunctional adult human testis. *Human Reproduction* **23**, 412-20. [Impact factor = 3.6]
10. **\*Loveland, K.L.** Rajpert-deMeyts, E. & Veeramachaneni, D.N.R. (2008) Testicular Cancer. *Comprehensive Toxicology*, 2nd Edition, Ed. Professor Charlene A. McQueen. Accepted 23 Sept 08.
11. Maatouk, D.M., **Loveland, K.L.,** McManus, M.T., Moore, K. and Harfe, B.D. (2008) Dicer is required for differentiation of the murine male germline. *Biology of Reproduction* **79**, 696-703. [Impact factor = 3.7]

**Koopman, P.A.**

12. **O'Bryan, M.K.,** Takada, S., Kennedy, C.L., Scott, G., Harada, S., Ray, M.K., Dai, Q., Wilhelm, D., **de Kretser, D.M.,** Eddy, E.M., **Koopman, P.A.** & Mishina, Y. (2008) Sox8 is a critical regulator of adult Sertoli cell function and male fertility. *Developmental Biology* **316**, 359-370. [Impact factor = 5.1]
13. Beverdam, A., Svingen, T., Bagheri-Fam, S., Bernard, P., McClive, P., Robson, M., Banan Khojasteh, M., Salehi Banan, M., **Sinclair, A.H.,** Harley, V.R. & **Koopman, P.A.** (2008) Sox9-dependent expression of *Gstm6* in Sertoli cells during testis development in mice. *Reproduction* (in press) [Impact factor = 3.0]

**McLaughlin E.A**

14. **McLaughlin, E.A.,** & Pacey, A.A. (2008) Cryopreservation & Storage of Sperm. In: *Textbook of Assisted Reproductive Technologies*, 3rd edition Eds. Gardener DK, Weissman A, Howles CM, Shoham Z, Informa Healthcare Press, London UK pp567-579.

**Sinclair A.H.**

15. Craig A Smith, Christina M Shoemaker, Kelly N Roeszler, Joanna Queen, David Crews, and **Andrew H Sinclair** (2008) Cloning and expression of R-Spondin1 in different vertebrates suggests a conserved role in ovarian development *BMC Dev Biol* 8:72

16. Beverdam, A., Svingen, T., Bagheri-Fam, S., Bernard, P., McClive, P., Robson, M., Banan Khojasteh, M., Salehi Banan, M., **Sinclair, A.H.**, Harley, V.R. & **Koopman, P.A.** (2008) Sox9-dependent expression of *Gstm6* in Sertoli cells during testis development in mice. *Reproduction* (in press) [Impact factor = 3.0]

### 3.2 Number of international visitors

#### Aitken, R. J.

- Professor Susan Suarez, – Department of Biomedical Sciences, Cornell University, Ithica, NY, USA
- Associate Professor David Elliott, Institute of Human Genetics, University of Newcastle, United Kingdom

#### Loveland, K.L.

- Dr. David Mottershead, Haartman Institute, Department of Bacteriology and Immunology, University of Helsinki, Finland.

#### Koopman, P.A.

- Dr Elisabetta Dejana, IFOM, FIRC Institute of Molecular Oncology, Via Adamello 16, 20139 Milan, Italy, 31 March 2008
- Dr Leigh Coultas, Mouse Imaging Centre, Hospital for Sick Children, Toronto, Canada
- Professor Susan Suarez, – Department of Biomedical Sciences, Cornell University, Ithica, NY, USA
- Associate Professor David Elliott, Institute of Human Genetics, University of Newcastle, United Kingdom

#### Loveland, K.L.

- Dr. David Mottershead, Haartman Institute, Department of Bacteriology and Immunology, University of Helsinki, Finland

#### Koopman, P.A.

- Dr Elisabetta Dejana, IFOM, FIRC Institute of Molecular Oncology, Via Adamello 16, 20139 Milan, Italy, 31 March 2008
- Dr Leigh Coultas, Mouse Imaging Centre, Hospital for Sick Children, Toronto Centre for Phenogenomics, Toronto, Ontario, Canada, 22 April 2008

- Dr Stefan Schulte-Merker, Hubrecht Institute, University Medical Centre Utrecht, Utrecht, The Netherlands, 6 June 2008
- Dr Ben Hogan, Hubrecht Institute, University Medical Centre Utrecht, Utrecht, The Netherlands, 30 October 2008
- Dr Serge Nef, Department of Genetic Medicine and Development, University of Geneva Medical School, Geneva, Switzerland, 18 November 2008

#### O'Bryan, M.K.

- Assoc. Prof. Geoff Hicks, The Manitoba Institute of Cell Biology, Winnipeg, Canada
- Dr Julie Merriman-Jones, University of Newcastle, United Kingdom

#### McLaughlin, E.A.

- Associate Professor David Elliott, The Institute for Cell and Molecular Biosciences (ICaMB) University of Newcastle, UK.

#### Holland, M.K.

- Dr Justin St John – Professor of Reproductive Biology – Clinical Sciences Research Institute, Warwick Medical School, University of Warwick, United Kingdom
- Assoc Prof Bernhard H Breier, MSc, PhD – Liggins Institute, University of Auckland, New Zealand
- Dr Stephen A Back – Hatfield Research Centre, Oregon Health & Science University USA
- Dr Wei Cheng – Associate Professor, Pediatric Surgery – Prince of Wales Hospital, The Chinese University of Hong Kong, Hong Kong
- Xiangli Zhang & Rongqu Li – Beijing Glorious Land and Agricultural Co, China
- Dr David Mottershead-Haartman Institute, Department of Bacteriology & Immunology; University of Helsinki, Finland
- Prof David Hume – Director & CEO, The Roslin Institute, Research Director, Royal School of Veterinary Studies, University of Edinburgh, United Kingdom
- Prof Dula Panda – Professor and Head School of Biosciences and Bioengineering, IIT, Mumbai, India

- Prof Hardyanto Soebono (Dean of Medicine), Prof Sofia Mubarika (Vice-Dean), Dr shandono (Head of the Department of Surgery), Dr Tri Wibawa – Faculty of Medicine, Gadjah Mada University, Indonesia
- Prof John E Parks – Department of Animal Science, Cornell University USA
- Academics from the Indian Institute of Technology, Bombay (IITB) India
  - Proff D Panda: Biotechnology, Stem Cells, Drug Delivery, Protein Folding
  - Prof S. Patankar: Biotechnology, Malaria, Bioinformatics, Stem Cells
  - Prof SK Maiti: Fracture Mechanics, Elastic plastic fracture mechanics, Fracture of composites, Finite element methods, Crack detection, Micro-Nano-fracture mechanics
  - Prof Arup Bhattacharya: Polymer Blends, Polymer Composites
  - Prof D. Manjunath: Communication network protocols, Systems & algorithms, Performance modeling, Queueing, Stochastic systems
  - Prof Chetan Solanki: Solar Photovoltaics, Thin film silicon solar cells, PV solar concentrators, Porous silicon, Carbon nano tubes

#### Sinclair, AH

- Prof Denis Daneman: Chair, Department of Pediatrics – University of Toronto  
Pediatrician-in-Chief –  
The Hospital for Sick Children  
RS McLaughlin Foundation Chair in Paediatrics  
Toronto, Canada
- Prof Sten Drop: Pediatric Endocrinologist  
Sophia Children's Hospital / Erasmus MC  
Rotterdam, The Netherlands
- Prof Robert Nicholls: Director,  
Birth Defects Laboratories  
Pittsburgh Childrens Hospital  
University of Pittsburgh, USA

#### Roman, SD

- Associate Professor David Elliott, The Institute for Cell and Molecular Biosciences (ICaMB)  
University of Newcastle, United Kingdom

### 3.3 Number of collaborative national and international workshops and exchanges

#### Aitken R.J.

- Workshop on technological advances in proteomics research. Prince Henry's hospital, Melbourne
- Men's Health Australia Longitudinal Study, Stakeholder Forum. Andrology Australia. Hosted by the Governor of Victoria, Professor David de Kretser AC. Government House, Melbourne

#### Hime, G.

- Germ cell-soma interactions in gonadal development and germ cell tumours, International University of Andalusia Workshops "Current trends in Biomedicine" Baeza, Spain October 20-22, 2008

#### Koopman, P.A.

- Joint convener: International Workshop "Germ Cell-Soma Interactions in Gonadal Development and Germ Cell Tumours", Baeza, Spain, 20-22 October 2008

#### Loveland, K.L.

- International Workshop "Germ Cell-Soma Interactions in Gonadal Development and Germ Cell Tumours", Baeza, Spain, 20-22 October 2008

#### McLaughlin, E.A.

- XIVth International Workshop on the Development and Function of the Reproductive Organs, Villa Mondragone, Monte Porzio Catone Rome, Italy September 15-17
- Germ cell-soma interactions in gonadal development and germ cell tumours, International University of Andalusia Workshops "Current trends in Biomedicine" Baeza, Spain October 20-22, 2008

#### Holland, M.K.

- Mon-Man workshop on genetically modified models of human disease, Melbourne
- VCE Biology Teachers Professional Development Workshops (Units 3 & 4), Melbourne
- National Dairy Alliance Forum, Melbourne
- CSIRO Stem Cell Workshop – Geelong Victoria

#### Hime, G.R.

- Siddall, N.A., Johnston, N.L., Been, R.P., Kalcina, M., Monk, A.C., McLaughlin, E.A. & Hime, G.R. "Musashi family proteins are functionally required in both somatic and germline stem cell populations in the *Drosophila* testis". 73rd Cold Spring Harbor Symposium on Stem Cells, New York, USA
- Hime, G.R. Australian Insect Molecular Biology Meeting, Yarra Valley, Victoria
- Monk, A.C., Siddall, N.A. & Hime, G.R. 'The RNA-binding protein HOW is required for stem cell maintenance in the *Drosophila* testis'. 2nd International Congress on Stem Cells and Tissue Formation, Dresden, Germany
- Monk, A.C., Siddall, N.A. & Hime, G.R. 'The RNA-binding protein HOW is required for stem cell maintenance in the *Drosophila* testis'. 20th International Congress of Genetics, Berlin, Germany
- Monk, A.C., Siddall, N.A. & Hime, G.R. 'The RNA-binding protein HOW is required for stem cell maintenance in the *Drosophila* testis' Melbourne Cell and Developmental Biology Meeting

### 3.4 Number of visits to overseas laboratories

#### Aitken, R.J.

- Professor Mike Berridge, Malaghan Institute of Medical Research, Victoria University, Wellington New Zealand
- Professor Rob Millar, MRC Human Reproductive Sciences Unit, University of Edinburgh, Scotland, United Kingdom

#### Hime, G.

- Dr. Alex Gould, National Institutes of Medical Research, Mill Hill, U.K., Oct 2008
- Dr. Barbara Jennings, University College London, Oct 2008

#### Loveland, K.L.

- Washington State University, Centre for Reproductive Biology, April 2008

#### Koopman, P.A.

- Dr Kevin Gaido, Hamner Institutes for Health Sciences, Research Triangle Park, North Carolina, USA, 13 February 2008

- Dr Blanche Capel, Duke University, Chapel Hill, North Carolina, USA, 14 February 2008
- Dr Mitch Eddy, National Institute of Environmental Health Sciences, NIH, Research Triangle Park, North Carolina, USA, 13 February 2008.
- Dr Kathryn Cheah, Department of Biochemistry, University of Hong Kong, Pokfulam, Hong Kong, China, 10 June 2008
- Dr Peter Dearden, University of Otago, Dunedin, New Zealand, 25 August 2008
- Dr Kelly Mayo, Dr Neena Schwartz, Centre for Reproductive Science, Northwestern University, Evanston, Illinois, USA, 7 October 2008
- Dr Larry Jameson, Feinberg School of Medicine, Chicago, Illinois, USA, 8 October 2008
- Dr Teresa Woodruff, Obstetrics and Gynecology, Northwestern University, Chicago, Illinois, USA, 8 October 2008

#### McLaughlin, E.A.

- Dr Norah Spears Centre for Integrative Physiology, University of Edinburgh, United Kingdom
- Dr Evelyn Telfer Institute of Cell Biology, University of Edinburgh, United Kingdom
- Dr Allan Pacey Diabetes, Endocrinology and Metabolism., University of Sheffield, UK
- Dr Jennifer Williams Bristol Centre for Reproductive Medicine, Bristol UK

#### Sinclair, A.H. and Lab members

##### Miles, D

- Azim Surani, Gurdon Institute, University of Cambridge, Cambridge, United Kingdom
- Robin Lovell-Badge, Molecular Genetics of Sex Determination, National Institute for Medical Research, London, United Kingdom

##### Daggag, H

- Prof Stylianos E. Antonarakis, University of Geneva, Geneva, Switzerland
- Prof H. Hilger Ropers, Max Planck Institute for Molecular Genetics, Berlin, Germany
- Prof J.T. den Dunnen, Leiden University Medical Centre, Leiden, Netherlands

##### Hewitt, J

- Prof Marc Nicolino, Hopital Debrousse, University of Lyon, Lyon, France

### 3.5 Number of memberships of national and international professional committees

#### Aitken, R. J.

- Member of the Andrology Australia Affiliate Program (2008-2009)
- Member of the Program Organizing Committee. North American Testes Workshop, USA
- Member of the Scientific organizing committee, Third Asia-Pacific Forum on Andrology, Nanjing, China
- Editorial Board member for the following journals:
  - Journal of Andrology, American Society of Andrology.
  - Asian Journal of Andrology, Blackwell Publishing
  - Reproduction, Society for Reproduction and Fertility
  - The Open Reproductive Science Journal
  - Recent Patents in Biotechnology

#### Hime, G.

- Member of the External User Reference Group for the NHMRC RIMES (online grant application) programme.
- Member of the Editorial Board "The Open Tissue Engineering and Regenerative Medicine Journal"

#### Holland, MK.

- President Society for Reproductive Biology (2007-09)
- Executive Committee American Society of Andrology
- Editorial Board Small Ruminant Research
- International Embryo Transfer Society. Co-chair of Health and Safety Committee
- Board member and (Acting) Vice President of FASTS

#### Jans, D.A.

- Editorial Board Member for Biochem. Journal. (2006-present)
- Member Committee International Photodynamic Association (2005-)

#### Koopman, P.

- Editorial board membership for the following journals:
  - Developmental Dynamics (American Association of Anatomists, USA), 2004 –
  - Sexual Development (Karger, Switzerland) – Section editor, 2006 –
  - Mechanisms of Development (Elsevier) 2007–
  - Gene Expression Patterns (Elsevier) 2007–
  - Biology of the Cell (Portland Press, UK) 2007–
- Conference organization:
  - Organizer, Current Trends in Biomedicine International Workshop Germ Cell-Soma interactions in Gonadal Development and Germ Cell Tumours, Baeza, Spain.
  - Joint convenor, research/clinical workshop Disorders of sexual development: the underlying molecular causes, clinical management and future strategies, Melbourne, July.
  - Joint convenor, Second Australian Sex Summit, Flowerdale, Vic, November.
- Session chair, ACGA-HKSMG International Conference on Genetics & Medicine, Hong Kong.
- Stream co-ordinator, ComBio, New Zealand.
- International committee service:
  - Specialist Advisor, HUGO Gene Nomenclature Committee, UK, 1998 –
  - Advisory Committee, Alexander Kowalevsky Medal, St. Petersburg Society of Naturalists, Russia, 2002 –
  - Annotation Terms Group, GUD-MAP, National Institutes of Health, USA, 2005 –
- National committee service:
  - Steering Committee, ARC Network in Genes and Environment in Development, 2005 –
  - Outreach Committee, ARC Network in Genes and Environment in Development, 2006 –
  - Management Group, Australian Phenomics Network, 2007–
  - NHMRC GRP Member Selector Panel, Grant Advisory Group Committee 2008

#### Loveland, K.L.

- Chair of the Society for Reproductive Biology SRB-RCRH Awards Panel
- Member of the Awards, Nomination and Awards Committees of the American Society for Andrology
- Co-Chair of the 2010 Program Organising Committee of the American Society for Andrology
- Member of the Hunter Cell Biology Meeting Program Organising Committee (2005-2009)
- Editorial Board member for *Reproduction* and *Journal of Andrology*
- Editorial Advisory Board member for *Reproduction, Fertility and Development* Member of *Faculty of 1000*

#### McLaughlin, E.A.

- Member of the Council of the Society for Reproductive Biology (2008-2010)
- Member of Program Organizing Committee Society for Reproductive Biology

#### Sinclair A.H.

- Editorial Board PathoGenetics
- Editorial Board Sexual Development
- Chair: NHMRC Project Grant Review Panel – Genetics
- Australian Biotechnology Advisory Council

OzBio 2010 Program Committee

### 3.6 Research projects with international partners

#### Aitken, R.J.

- Aetiology of DNA damage in the male germ line. Professor Sheena Lewis, Queen's University, Belfast.
- Cryostorage of primate spermatozoa. Professor Stuart Myers, University of California, Davis, USA.
- Proteomic search for biomarkers of testicular cancer in human seminal plasma. Professor Neils Skakkebaek, University of Copenhagen, Denmark.
- Role of lipid rafts in orchestrating the assembly of multimeric zona recognition complexes in mammalian spermatozoa. Professor Nongnuj Tanphaichitr, Professor in Obstetrics and Gynecology, University of Ottawa, Canada.
- Phenotype of the SP<sup>trx</sup> knock out mouse. Professor Mitch Eddy, NIH, Bethesda, USA.
- Proteomic analyses of epididymal plasma. Professor Barry Hinton, Department of Cell Biology, University of Virginia, USA.

#### Hime, G.

- p53 family proteins in stem cells, Prof. Thorstein Stiewe, Philipps-University in Marburg, Germany
- Role of Snail family proteins in germ cell development, Dr. Leanne Jones, Salk Institute, San Diego, U.S.A

#### Holland, MK

- Mitochondrial DNA transmission during assisted reproduction technologies – Justin St John University of Warwick
- Diagnostic markers for mastitis in cows – Dr R Wall UDA Beltsville Laboratories

**Loveland, K.L.**

- Testicular cancer aetiology. Ewa Rajpert-deMeyts, University of Copenhagen, Denmark
- Activins and inhibins in testis growth and disease. Martin M. Matzuk and Chester Brown, Baylor College of Medicine, Houston, Texas USA
- Growth factor regulation of testis growth. Michael Griswold and Chris Small, Washington State University, Pullman, Washington USA
- Hedgehog signaling in spermatogonial stem cells. Marvin Meistrich, M.D. Anderson, Houston, Texas
- Novel nuclear roles for importin alpha proteins. Norman Hecht, University of Pennsylvania, Pennsylvania USA, and Yoshirio Yoneda, University of Osaka, Osaka, Japan.

**McLaughlin, E.A.**

- Developing a Chlamydia vaccine for males. Professor Charles Wira, Dartmouth College, USA.

**Sinclair, A.H.**

- Male germ cell tumours. Professor Leendert Looijenga, Erasmus University, Rotterdam, Netherlands.
- Whole genome analysis of patients with Disorders of Sex Development. Professor Sten Drop, Erasmus University, Rotterdam, Netherlands.
- Whole genome analysis of patients with Disorders of Sex Development. Professor Eric Vilain, University of California Los Angeles, LA, USA.
- Whole genome analysis of patients with Disorders of Sex Development. Professor Harry Ostrer, University of New York, NY, USA.

**O'Bryan, M. K.**

- Mouse models of infertility. Jacqui White and Karen Steel, Wellcome Trust Sanger Institute, UK
- Genetic causes of cilia dysfunction Drs Lucia Bartolini and Jean-Louis Blouin, University Hospitals of Geneva and University of Geneva-School of Medicine, Switzerland
- Genome instability and human male infertility. Ewa Rajpert de Meytes, The University of Copenhagen, Denmark
- Sox8 function in adult male fertility. E. Mitch Eddy and Yuji Mishina, National Institutes of Health, USA:
- Mouse models. Nadia Rosenthal, European Molecular Biology Laboratories, Italy

**Koopman, P.**

- Sox8 in Sertoli cell function and male fertility. Dr Shuji Takada, National Institute of Environmental Health Science, Research Triangle Park, NC, USA
- Analysis of ovotestis development in mice. Dr Eva Eicher, The Jackson Laboratory, Bar Harbour, ME, USA

## 4. End-User Links

### 4.1 Number and nature of commercialization activities

#### Aitken, R.J.

- The Centre has negotiated formal Heads-of-Agreements with end-users including CSIRO, the Invasive Animal CRC and industry (Schering AG, Life Therapeutics and Pestat) to share technologies and work together towards our commercial aims.
- One of the Centre's biotechnology partner, **Life Therapeutics**, has created a spin-off company, **Nusep**, a major component of which is the gamete separation technology patented by the Centre. In association with Life Therapeutics we have successfully applied for an AusIndustry grant to commercialize this cell separation device in the context of Assisted Conception Therapy. Clinical trials have been successfully completed at Westmead Hospital. The parent company has been sufficiently encouraged by these results to establish a spin off company '**Spermgen**' housing the Centre's IP in relation to sperm isolation. The preparation of the sperm preparation device will now be up-scaled in preparation for additional large scale clinical trials in 2008-9.
- Another biotechnology partner, **Pestat**, is working with Centre CIs on the development of non-surgical methods of sterilizing domestic and feral animals. Even if we were only successful in developing an injectable method for domestic animals, it would capture a global market worth billions of dollars a year. We have established a novel protocol for the non-surgical sterilization of mammals that has been patented, with Pestat being granted an exclusive license to exploit this technology in the context of fertility regulation. The method is currently being assessed in animal trials, using the mouse as an animal model.
- The exploitation of our technology for the control of feral animal populations will be undertaken in collaboration with the Invasive Animal CRC (IACRC). Additional funding has just been committed by IACRC to fund an additional technical position in support of this research program.
- Two of the Centre's CIs (RJA and MOB) are key members the Australian Reproductive Healthcare Network established in 2006 with over \$2 million funding from the German pharmaceutical company, **Schering AG**. This partnership between Australian Science and a major pharmaceutical company won a Monash Industry Engagement Award in 2005-2006. The purpose of the program supported by

Schering is to conduct strategic research with the ultimate aim of providing a rational, scientific basis for the development of novel approaches to male contraception.

- A patent was filed in 2008 for a topical contraceptive agent that not only instantly immobilizes hundreds of millions of spermatozoa but also attacks pathogenic microbes that are present in semen such as Chlamydia. In vitro tests suggest that the compound is safe and therefore has considerable potential as a dual-purpose topical contraceptive agent that will not only provide protection against pregnancy but will also protect the user from sexually transmitted disease. Funding agencies in the USA have agreed to assess our lead compounds for anti-HIV activity. We are currently negotiating with potential commercial partners to exploit this IP.

#### Holland, M.K.

- Under review: ARC Linkage application – "Gamete quality and management in Australian native fish" – A\$950,000. Commercial Partner: Fisheries Victoria
- Under review: International Science Linkage Program – "Could the regulation of mitochondrial DNA transmission and the selection of appropriate haplotypes result in new founders of super-breeds of cattle being generated" – A\$243,555.00. Commercial Partner: University of Warwick Medical School, UK

#### Jans, D.A.

- Dr Helmut Thissen, CSIRO Molecular and Health Technologies

#### Loveland, K.L.

- CSIRO Food Futures Flagship Spermatogenesis program
- Dr Helmut Thissen, CSIRO Molecular and Health Technologies

#### O'Bryan, M.K.

- **Bayer-Schering research contract, 2006-2008**. "The Australian network" to identify novel contraceptive targets, and endometrial regulators. CIs: Rob McLachlan, **Moira O'Bryan**, **John Aitken**, Lois Salmonson, Luk Romberts, Evan Simpson.

#### Sinclair, A.H.

- Member of the Australian Biotechnology Advisory Committee – provides high-level independent advice to the Australian Government Biotechnology Ministerial Council

## 4.2 Number of government, industry and business briefings

Aitken, R.J.

- **Bayer-Schering-Pharma.** Briefings in the area of contraceptive research and development
- **Roche Consumer Health.** Briefings in the area of male infertility and potential antioxidant therapies.
- **Assisted Conception Centres** (Hunter IVF, Fertility First, IVF Australia, Westmead Fertility Centre) advice on sperm preparation and cryopreservation technologies.
- **Starpharma.** Briefing about the development of dual purpose contraceptive reagents that simultaneously protect against fertility and the spread of sexually transmitted disease.
- **CONRAD.** Briefings in the area of contraception-on-demand and the development of spermicide microbicide formulations

McLaughlin, E.A.

- **Australian Education International and Australian High Commission in Kuala Lumpur** on the work of the Centre and the impact of environmental toxicants on human fertility.

Jans, D.A.

- **Merk,** Darmstadt, Germany

Holland, M.K.

- **Government:** 4 (Fisheries Victoria, DPIV, DIIRD, DOHA)
- **Industry and Business:** 4 (Genetics Australia, ABS Global, Australian Agriculture Company, Oswald Group (India))

O'Bryan M. K.

- International Advisor to the CREATE program – Seventh Framework Program (Health Theme) FP7-HEALTH-2007-B. This is an international initiative to develop a Cre-Zoo i.e. mouse deleter strains for use in the generation of tissue specific knock-out mouse strains.
- NHMRC Project Grant Review Panel 3b – Perinatology / Paediatrics / Obstetrics / Reproduction
- Convenor of the management group of the Australian Phenomics Network.
- International Liaison Committee, The American Society of Andrology

## 4.3 Number of Centre associates trained/ing in technology transfer and commercialization

- The entire complement of PhD students and Postdoctoral Fellows supported by the Centre attended a workshop at the 6th Annual Scientific meeting in September 2008 on:
  - ARC Discovery Grant Applications
  - NJMRC Project Grants
  - NHMRC Fellowships/Career Options
- CI's and Advisory Board attended IP Audit Feedback Session with Christian Touli of BioLink

Sinclair, A.H. & O'Bryan, M.K.

- New technologies for genome wide analysis of gene copy number in relation to male infertility and testicular cancer

Aitken R.J.

- Technology transfer activities in the areas of electrophoretic sperm isolation, male infertility diagnosis and treatment, feral animal control and contraceptive development, as listed above.
- Al Mark Baker has been instrumental in establishing new proteomic and metabolomic technologies within the centre in order to allow us to develop a 'systems biology' approach to the cell biology of germ cells where emphasis is placed on the plurality of signaling networks and metabolic processes that drive cell function individual pathways. The development of such capabilities has drawn interest from international companies such as Bayer-Schering-Pharma wishing to access our technology platform.

#### 4.4 Number and nature of Public Awareness programs

##### Hime, G.R.

- Guest presenter, 3RRR “Einstein-a-go-go” science programme

##### Holland, M.K.

- Boronia Probus Club
- Kyneton Lions Club
- Victoria Science Teachers Association

##### Aitken, R.J.

- *A local success story. From Patent to Product.* Department of State and Regional Development, Newcastle, NSW. A public lecture delivered as part of the Hunter Means Innovation Festival, 2008.
- *Reproductive technologies and their role in our future.* Newcastle City Hall. A public lecture delivered as part of the Hunter Means Innovation Festival. HMRI e-book, p 35. 2008

##### Sinclair, AH

- Everything you wanted to know about Sex determination. Public Lecture for ANZAAS Science Week 24 July 2008 RMIT “”

##### Roman, SD

- Presentation to Careers Advisors in the Hunter Region.

#### 4.5 Cash contributions from end-users to the Centre including research contracts.

##### Aitken, R.J.

- **ARC Linkage grant** (McLaughlin and Aitken) with Pestat to develop novel approaches to fertility regulation in domestic animals (\$140, 000/annum)
- **Invasive Animal CRC** (McLaughlin and Aitken) funding to extend the above research on novel approaches to fertility regulation to include pest animal species (\$62,000/annum)
- **NuSep** Funding in support of the further development of an electrophoretic sperm isolation device (\$50,000/annum).
- **HMRI.** Funding to support Centre research with Hunter IVF (\$50,000 /annum).

#### 4.6 In-kind contributions from end-users to the Centre

##### Aitken, R.J.

- Pestat. \$25,000 per annum
- NuSep. \$200,000 per annum
- IVF collaborators (IVF Australia, Fertility First, Hunter IVF and Westmead Fertility Centre) \$1,500,000 per annum.
- Bayer-Schering Pharma. This company has developed Sptrx KO mice (sperm specific thioredoxins) which have now been forwarded to the Centre to determine if they have a reproductive Phenotype. The company produced and provided the mice while the phenotypic analysis is being funded by CONRAD, a branch of the US Agency for International Development. The in-kind support offered by the company would amount to around \$100,000.

##### Holland, M.K.

- Genetics Australia. \$20,000

## 5. Organisational Support

### 5.1 Annual cash contributions from Collaborating Organisations

▪ The University of Melbourne	\$135,000
▪ Queensland University	\$150,000
▪ Monash University	\$200,000
▪ University of Newcastle	\$200,000

### 5.2 Annual in-kind contributions from Collaborating Organisations

▪ University of Newcastle:	\$520,000 p.a.
▪ University of Queensland:	\$514,000 p.a.
▪ Monash University:	\$608,000 p.a.
▪ Melbourne University:	\$475,000 p.a.

### 5.3 Number of new Organisations recruited to or involved in the Centre

**Prince Henry's Institute** A collaborative arrangement between members of the Centre (Aitken, Baker) and Prince Henry's Institute of Medical Research to support the application of Centre know-how and IP in a reproductive medicine context. This interaction has been funded with the aid of an NHMRC program grant and specific areas of collaboration are (i) to use the Centre's expertise in the diagnosis of oxidative stress in human spermatozoa (oxidative DNA base adduct formation, lipid peroxidation and mitochondrial ROS generation) to conduct a clinical assessment of the efficacy of antioxidant therapy for infertile males using a randomized, double-blind cross-over design (ii) use the Centre's expertise in phosphoproteomics to search for biomarkers of reproductive tract cancers and spermiation (iii) use the Centre's expertise in metabolomics to develop protocols to monitor changes in specific reproductive tract metabolites, particularly steroids.

### 5.4 Level and quality of infrastructure provided to the Centre

The host institutions have fully supported the Centre with the provision of laboratory infrastructure and in the case of the University of Newcastle, with PA and laboratory manager support to the Director.

### 5.5 Annual cash contributions from other organizations

▪ NHMRC project and program grants	\$2,675,165
▪ NHMRC Fellowships	\$512,000
▪ ARC Federation fellowship	\$322,625
▪ CONRAD	\$135,000
▪ Invasive Animal CRC	\$85,000
▪ ARC Linkage	\$155,000
▪ ARC Discovery Grants	\$675,000
▪ Dairy Australia	\$250,000
▪ NSW Cancer Institute	\$233,000
▪ NSW Dept of State and Regional Planning	\$148,637
▪ Queensland Cancer Fund	\$80,000
<b>Total additional income for 2008</b>	<b><u>\$5,271,427</u></b>

### 5.6 Annual in kind contributions from other institutions

- Pestat. \$25,000 per annum
- NuSep. \$200,000 per annum
- IVF collaborators (IVF Australia, Fertility First, Hunter IVF and Westmead Fertility Centre) \$1,500,000 per annum
- Bayer-Schering Pharma. This company has developed Sptrx KO mice (sperm specific thioredoxins) which have now been forwarded to the Centre to determine if they have a reproductive Phenotype. The company produced and provided the mice while the phenotypic analysis is being funded by CONRAD, a branch of the US Agency for International Development. The in-kind support offered by the company would amount to around \$100,000.

## 6. Governance

### 6.1 Breadth and experience of the members of the Advisory Board

The Advisory Board has served the Centre extremely well, providing an independent perspective on the quality and direction of our research programs, constantly reinforcing the need to link our research goals and our research activities and providing fresh ideas and wise counsel at critical stages in the Centre's development.

### 6.2 Frequency and effectiveness of Advisory Board meetings

The Scientific Advisory Board formally meet with Centre Staff once a year at our Annual Scientific Meeting. The Director and senior CIs are also in regular contact with individual members of the Advisory Board throughout the year; they have been extremely generous with their time on behalf of the ARC Centre of Excellence.

### 6.3 Quality of the Centre strategic plan

Our strategic plan has been approved by the Scientific Advisory Board and external ARC assessors and is delivering world-class outcomes.

### 6.4 Effectiveness of arrangements to manage Centre nodes

These arrangements have worked seamlessly over the past 7 years.

### 6.5 The adequacy of the Centre's Key Performance Measures

The key performance measures provide us with an objective benchmark of our performance; they are challenging but help to keep the activities of the Centre focused on outcomes that are valued by the ARC.

## 7. National Benefit

### 7.1 Measures of expansion of Australia's capability in the priority area(s)

The Centre's contribution to the designated priority area of genome to phenome can be seen in the following performance measures:

- More than 400 research publications published between 2003 and 2009, including 62 articles published or in press in 2008.
- More than 30% of these papers published in journals with an impact factor of more than 5 including recent publications in the world's most prestigious journals.
- More than 350 national and international research presentations given in 2003-2008 including another 47 invited presentations in the past year
- 9 patents generated over this period of time including one that has now progressed to international PCT filing in N America and Europe. A new provisional patent filed in 2008 for monitoring gamete and embryo quality.
- International and national research linkages established with the private sector that generated more than \$750,000 worth of additional investment in the Centre in 2008.
- Contribution of enabling facilities to the national science base including establishment of the Australian Centre for Vertebrate Mutation Detection (ACVMD) with a grant of \$1.6M from the NHMRC to a consortium including Associate Professor Moira O'Bryan.
- Undergraduate and post-graduate education programs in subject areas (biotechnology, developmental biology and biomedical science) related to the designated priority area of 'genome-to-phenome'.
- Establishment of world-class technology platforms at each of the Centre nodes. Development of a systems biology approach to the study of cellular mechanisms through the integration of proteomic and metabolomic data, places the Centre at the forefront of a rapidly expanding field.
- Centre representation by Associate Professor Moira O'Bryan on the Board of the Australian Phenomics Facility
- Centre representation by Associate Professor Moira O'Bryan on the International Liaison Committee of the American Society of Andrology.
- Presence of two CIs (Professor Aitken and Associate Professor O'Bryan) on the Infertility Advisory Group of 'Andrology Australia'
- Heads-of-agreements and research collaborations established with the Invasive Animal CRC in the area of pest animal control. In this context, Centre technology is being used to develop reagents to control the feral rabbit population.
- The Centre has developed a device for the isolation of spermatozoa possessing low levels of DNA damage for the assisted conception industry. This research has been conducted in collaboration with the Australian biotechnology company NuSep and in 2008 our prototype device successfully completed clinical trials at Westmead Fertility Centre. We are now seeking funding to permit a full commercial roll out of the device in 2009-2010.
- Our Centre graduates are now making a significant contribution to the national and international workforce in the areas of Biotechnology and Development. For example:

#### Honours Students

Rowena Jones – Research Pharmacist at Mater Hospital Newcastle

Lucan Baillie – Research officer Therapeutic Goods Administration Canberra

Joel Chick – PhD Student Department of Chemistry & Biomolecular Sciences, Macquarie University now post doc Massachusetts Institute of Technology USA

Rebecca Dorey – Embryologist Sydney IVF Sydney

Brendan Goswell – Staff Scientist Virology Hunter New England Health Newcastle

Alex Wilding – PhD student, Monash Institute of Medical Research, Melbourne Victoria

#### M Sc Students

Hua Su – PhD student Centre for Diabetes and Endocrine Research, University of Queensland, Princess Alexandra Hospital, Brisbane, Queensland, Australia.

Melanie Gibson – Research Co-ordinator, Jean Hailes Foundation, Melbourne, Victoria

### PhD Student

Daniel Blackmore – Post Doc at Queensland Brain Institute, now Staff Scientist with Pfizer Cambridge

Janet Holt – Post Doc at Biochemistry Monash and now Post doc in Biomedical Sciences Newcastle

Kelly Asquith – Post Doc Vaccines, Immunity, Viruses & Asthma Research Program (VIVA) University of Newcastle (NHMRC post doc fellow)

Jonathan Paul – Post Doc Mothers and Babies Research Group Medicine and Public Health, University of Newcastle

Cathryn Hogarth – Postdoctoral Researcher, Washington State University, Pullman, WA, USA

Catherine Iman – Postdoctoral Research Fellow, Monash Institute of Medical Research, Melbourne Victoria

Anette Szczepny – Postdoctoral Researcher, Monash Institute of Medical Research, Melbourne Victoria

Camden Lo – Postdoctoral Research Fellow, Monash Microimaging, Monash University, Melbourne Victoria

Sridurga Mithraprabhu, – Postdoctoral Researcher, Monash Institute of Medical Research, Melbourne Victoria

## 7.2 Case studies of economic, social, cultural or environmental benefits

The Centre of Excellence has negotiated strong strategic alliances with external bodies such as CSIRO, the Invasive Animal CRC and a variety of commercial companies, including both small national biotechnology companies (NuSep and Pestat) and major international players (Bayer Schering Pharma AG, and CONRAD) in areas that are of direct commercial, social and environmental benefit to Australia.

- Public awareness of the importance of intellectual property in the development of the Australian biotechnology industry has been promoted by a public lecture given by John Aitken on ‘From patent to product – a local success story’ as part of the Hunter-Means-Innovation Festival.
- In the same Hunter-Means-Innovation Festival John Aitken also gave a public lecture on the role played by assisted conception in the reproductive future of Australia. This is an important topic because currently 1 in every 35 children born in Australia is the product of the assisted conception industry. It is important for the public to understand why this is, the risks involved in such a trend and how this situation can be addressed.
- The Invasive Animal CRC. This Co-operative Research Centre has recently been funded by the Australian Federal Government to focus research on the development of technologies for the eradication of pest animal species such as cane toads, carp, mice, rats, rabbits, feral dogs and foxes that are doing irreparable damage to the Australian environment. The Centre possesses know-how, technologies and patents that are germane to this area of research. Indeed, this CRC represents a natural end-user for certain types of expertise bound up within the Centre of Excellence. In light of this, the Director has signed a Heads-of-Agreement with the CRC that will facilitate the establishment of collaborative linkages between the two organizations. This link has been further reinforced by the presence of the CEO of the Invasive Animal CRC (Professor Tony Peacock) on the Centre’s Steering Committee (see section 1.7.2) and the presence of Professor Peter Koopman on the Carp Reference Group of the CRC. The CRC invested further resources in the Centre of Excellence in 2008 in order to promote technology transfer in this area of research and develop a wing of the program specifically targeting pest animal species.

- CSIRO. A potential area of collaboration between the Centre and CSIRO concerns the development of novel technologies for the generation of transgenic animals. Several lines of research being conducted within the Centre are potentially relevant to the development of technologies for delivering commercially important transgenes into large domestic animals. In order to facilitate the exchange of information and technologies in this area, the Centre has negotiated a Heads-of-Agreement with CSIRO that will enable this co-operative research venture to proceed.
- Andrology Australia. The Director and Moira O'Bryan serve on the Male Infertility Advisory Group to Andrology Australia which has as its mission 'to undertake those measures that will enhance the reproductive health of males including community and professional education strategies and support of national research programs'. It was launched in 1999 and has since developed into a major advocate for male health issues, receiving in excess of 400,000 hits a month on its website – [www.andrologyaustralia.org](http://www.andrologyaustralia.org). The many programs developed by Andrology Australia include a GP education workshop for indigenous GPs, a national telephone survey of male reproductive health issues, a 'What Every Man Needs to Know' national community education program, pharmacy fact cards on male health as part of the Pharmacy Self Care program, the APCC Prostate Cancer Bio-resource and a research program into the relationship between male sex hormones and ageing.
- NuSep. The Director has established a collaborative research program with a Sydney-based biotechnology company, Nusep, in order to commercialize a device patented by the Centre for the rapid, selective isolation of high quality spermatozoa for assisted conception purposes. Successful Phase 1 clinical trials were conducted in 2007 that have led the parent company to create a spin-off entity, SpermGen, to bring this product into full commercial production and establish a pipeline of project development around the Centre's IP.
- CONRAD. The Contraceptive Research and Development wing of the US Agency for international Development has given the Centre a grant of \$136,000 to study the phenotype of knock out mice lacking the Sptx 1 and 2 genes. These genes encode for sperm specific thioredoxins that, by virtue of their cellular specificity, functionality and drugability are deemed suitable targets for contraceptive development.



## ▶ 8. Quality Indicators

The quality of the CI's and students that comprise the CBD is clearly indicated by the national and international awards they have received over the past year. Some examples of the distinctions conferred upon Centre members are indicated below.

- **Moirá O'Bryan** elected Young Andrologist of the Year by the American Society of Andrology and NHMRC senior Research Fellowship B.
- **Peter Koopman** elected a Fellow of the Australian Academy
- **Peter Koopman** won the Lemberg Medal from the Australian Society of Biochemistry and Molecular Biology
- **R. John Aitken** presented the Inaugural Anne McLaren Memorial lecture at the Fertility 2009 conference in Edinburgh.
- **Yun Hua Lee** a Centre PhD student supervised by John Aitken won the **Merck-Serono ART Young Investigator award for 2008** at the Combined ASPIRE2008 conference (Asia Pacific Initiative in Reproduction) and PRSFS2008 (6th Biennial Meeting of the Pacific Rim Society for Fertility and Sterility), Suntec, Singapore.
- **Yun Hua Lee** also won the Third year PhD student Research Excellence Award at University of Newcastle Post Graduate Conference.
- **Adam Koppers** a Centre PhD student supervised by John Aitken won the Second year PhD student Research Excellence Award at University of Newcastle Post Graduate Conference.
- **Adam Koppers** was also presented with a Faculty of Science and IT award for Outstanding Postgraduate Research Student Achievement for 2008 by the University of Newcastle.
- **Matt Dun** a PhD student supervised by Brett Nixon and John Aitken won the 2008 Oozoa Student Award at the annual meeting of the Australasian Society for Reproductive biology.
- **Kate Ewan** a PhD student supervised by Peter Koopman and John Aitken was awarded a travel grant by the 15th European Workshop on Molecular and Cellular Endocrinology.
- **Natasha Zamudio.** 2008 American Society of Andrology Lalor Foundation Travel Award
- **Duangporn Jamsai** 2008 International Society of Andrology Travel Award
- **Duangporn Jamsai** 2008 The CASS (Contributing to Australian Scholarship & Science) Foundation Travel Grant
- **Duangporn Jamsai** 2008 Outstanding Trainee Investigator Award from the American Society of Andrology
- **Sewa Rijal** ED Daniels Honours Scholarship from the Australian Federation of University Women Victoria (AFUW-Vic)
- **Rose Keightley** University of Newcastle Faculty Medal
- **Kara Gunter** University of Newcastle Faculty Medal
- **Skye McIver** VC's award for Outstanding Research Candidature and recipient of a University medal

## 2008 Financial Statement

### ARC Centre of Excellence in Biotechnology and Development (CBD)

Research Project: Centre of Excellence in Biotechnology & Development  
 Project ID: CE0348239  
 Director: Professor R John Aitken  
 Administering Institution: University of Newcastle

#### Income:

ARC Income 2008	\$ 2,227,000.00	
ARC Contribution Carry Over from 2007	\$ -	\$ 2,227,000.00
Cash Contributions 2008:		
The University of Newcastle	\$ 217,589.00	
The University of Queensland	\$ 150,000.00	
The University of Melbourne (for G. Hime)	\$ 67,500.00	
The University of Melbourne (for A. Sinclair)	\$ 67,500.00	
Monash University	\$ 200,000.00	\$ 702,589.00
University of Newcastle Expenses Re-imbursed	\$ 8,168.97	
<b>Total CBD Income:</b>		<b>\$ 2,937,757.97</b>

#### Expenditure:

##### Salaries:

University of Newcastle	\$ 439,580.75	
University of Queensland	\$ 280,861.93	
University of Melbourne (Hime)	\$ 171,778.09	
University of Melbourne (Sinclair)	\$ 245,235.42	
Monash University	\$ 516,523.00	\$ 1,653,979.19

##### Equipment:

University of Newcastle	\$ 72,265.85	
University of Queensland	\$ 2,180.90	
University of Melbourne (Hime)	\$ 28,242.91	
University of Melbourne (Sinclair)	\$ -	
Monash University	\$ 13,964.00	\$ 116,653.66

##### Accommodation:

University of Newcastle	\$ 77,664.30	
University of Queensland	\$ 29,692.94	
University of Melbourne (Hime)	\$ -	
University of Melbourne (Sinclair)	\$ -	
Monash University	\$ 3,660.00	\$ 111,017.24

##### Travel:

University of Newcastle	\$ 24,690.22	
University of Queensland	\$ 13,026.03	
University of Melbourne (Hime)	\$ 5,851.74	
University of Melbourne (Sinclair)	\$ 3,218.18	
Monash University	\$ 45,288.00	\$ 92,074.17

**Expenditure (continued):****Maintenance/Consumables:**

University of Newcastle	\$	219,994.65	
University of Queensland	\$	213,500.78	
University of Melbourne (Hime)	\$	36,965.51	
University of Melbourne (Sinclair)	\$	47,200.00	
Monash University	\$	338,306.00	\$ 855,966.94

**Other:**

University of Newcastle	\$	46,506.90	
University of Queensland	\$	–	
University of Melbourne (Hime)	\$	14,017.19	
University of Melbourne (Sinclair)	\$	–	
Monash University	\$	13,047.00	\$ 73,571.09
<b>Total of All Institution's Expenditure 2008:</b>			<b>\$ 2,903,262.29</b>

**SUMMARY AND RECONCILIATION:**

Total ARC Funds Provided to CBD 2008 (Nil Carried Forward from 2007)	\$	2,227,000.00
CBD Expenditure applied to ARC funds 2008	\$	2,227,000.00

<b>ARC FUNDS TO BE CARRIED FORWARD</b>	\$	–
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Non ARC Contribution Balance B/Fwd from 2007	\$	532,244.31	
Non ARC Contributions 2008	\$	710,757.97	\$ 1,243,002.28
CBD Expenditure 2008 Charged to Non ARC Funds	\$	676,262.29	

<b>CBD POSITION TO CARRY FORWARD INTO 2009</b>	<b>\$</b>	<b>566,739.99</b>
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In my opinion this financial statement fairly presents the transactions for the period ended 31 December 2008. The information contained in this statement has been extracted from the accounting records of the University of Newcastle which has been prepared on the basis of accounting policies consistent with applicable Australian Accounting Standards.

Damien Ryan  
Senior Research Accountant  
The University of Newcastle  
Date: 29/03/09

## Contact Details



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