

*Western Australian
Reproductive Technology Council*

*Proceedings from Seminar
Cloning, stem cell research and transgenics*

24 May 2002

(Back of cover)

Published by and available from:
The Western Australian Reproductive Technology Council
Department of Health
189 Royal Street
EAST PERTH WA 6004

Telephone: (08) 9222 4307
Fax: (08) 9222 4236

© The Western Australian Reproductive Technology Council 2002

All rights reserved. No part of this book may be reproduced or transmitted in any form without the written permission of the publisher.

ISBN – 0 7307 0099 2

2002: Perth Western Australia

(Back Cover)

**Produced by
The Western Australian Reproductive Technology Council
2002**

*Proceedings from Seminar
Cloning, stem cell research and transgenics*

Convened by the

*Western Australian
Reproductive Technology Council in
conjunction with Murdoch University*

*Seminar organised by A/Professor Jim Cummins, Murdoch
University and Ms Patrice Wringe, Reproductive Technology
Council*

*Held at the Kim Beasley Lecture Theatre, Murdoch University
24 May 2002*

Acknowledgements

The Reproductive Technology Council (Council) wishes to thank many people for their work in organising, preparing presentations and participating in this seminar. It had the greatest number of participants of all Council seminars to date.

Special thanks are extended to A/Professor Jim Cummins, Murdoch University, for organising the speakers and the venue. His knowledge of the area and his enthusiasm for the project ensured a very successful outcome.

Dr Anne Jequeir has worked tirelessly in the area of andrology and assisted reproductive technology for many years. She is the current chairman of the Fertility Society of Australia. Sincere thanks to Dr Jequier for being chairman for the seminar.

The high calibre of the speakers and their balanced presentation was appreciated by all participants. The Council is grateful to them for their time and energy in preparing and delivering excellent presentations.

The venue for the seminar was very suitable and the Council is grateful to Murdoch University for making the Kim Beasley Lecture Theatre available for the seminar. The Council also thanks the Murdoch University staff who prepared an audio and video presentation of proceedings.

The Council also thanks all participants who gave up their Friday afternoon to attend and for the quality and thoughtfulness of their questions and comments.

Table of Contents

ACKNOWLEDGEMENTS	1
FOREWORD	3
THE SPEAKERS.....	4
EMBRYONIC STEM CELLS AND THE FOUNTAIN OF YOUTH – FACT OR FANTASY	6
STEM CELLS AND CENTRAL NERVOUS TISSUE REPAIR.....	11
THE POTENTIAL OF STEM CELLS FOR REPAIR OF SKELETAL AND CARDIAC MUSCLE.....	16
TRANSGENIC ANIMALS.....	20
TOWARDS THE NATIONAL REGULATION OF HUMAN CLONING AND EMBRYO RESEARCH	23
CLONING ETHICS: EVOLUTION OF MORAL PHILOSOPHY	29

Foreword

It was an enormous privilege for me to chair this seminar. I am, and have been for many years, involved in assisting people to form their families through assisted reproductive technology treatment. So the seminar topic is very relevant to my work.

One of the ‘hottest’ topics in the last twelve months has been human cloning, and the potential medical breakthroughs in the use of stem cells to heal a range of disease. The birth of ‘Dolly’ the cloned sheep, in 1997 was heralded as a marvellous event by some and with horror by others. Dolly was created by somatic cell nuclear transfer from the fusing of a complete udder cell with an enucleated oocyte by an electrical pulse.

Scientists have claimed that embryonic stem cells have the potential to cure disease. They need access to embryonic stem lines in order to further their research. There are ‘excess embryos’ from assisted reproductive technology treatment, when people who have completed their IVF treatment choose not to donate them to another couple or allow them simply to die. These people may donate them for stem cell research.

The Commonwealth Government considered cloning and stem cell research over the past few years. The Council of Australian Governments agreed on 5 April 2002 to legislate to ban human cloning but to allow embryonic stem cell research, using ‘spare’ embryos, created for *in vitro* fertilisation treatments, when donated by couples who had no further use for them. The *Research Involving Embryos and Prohibition of Human Cloning Bill 2002* was introduced into Federal Parliament on 27 June 2002 and is expected to pass through both Houses of Parliament by the end of 2002.

This seminar was born out of the perceived confusion in the community about the complex issues of cloning and embryonic stem cell research. Its main aim was to try to present a balanced picture of the technology to date, look at the ethical issues involved, as well as updating participants about the present status of legislation.

A/Professor Jim Cummins, Division of Veterinary and Biomedical Sciences, Murdoch University, was the major organiser of this seminar. He recognised its potential to interest and inform students, academic staff, clinicians and members of the public. He chose an excellent group of speakers, who spoke with great clarity and authority from their fields of study and experience.

It is hoped that the proceedings of the seminar as presented in this booklet will contribute to increasing the knowledge and understanding of the complex issues involved.

Comments or feedback on the seminar are welcomed. Please contact Ms Patrice Wringe on (08) 9222 4307 or on e-mail patrice.wringe@health.wa.gov.au.

Dr Anne Jequier
Seminar Chairman
September 2002

The Speakers

Dr Sue Fletcher

Dr Fletcher completed a PhD at UWA in 1988. She has worked at the Centre for Neuromuscular and Neurological Disorders (formerly ANRI) at UWA since completing her studies. Her Centre is evaluating various genetic therapies for the treatment of muscular dystrophy. Dr Fletcher's interest in stem cells lies in their potential to contribute to the repair of muscle in inherited muscle disease and the use of these cells to unravel the mysteries of myogenic commitment and differentiation.

Professor Alan R Harvey

Professor Harvey has a BA (Hons), M.A. University of Cambridge, England (1975) and PhD Australian National University, ACT, Australia (1978). His major fields of academic and research interest include: Neurotrauma; Gene Therapy; Neural Transplantation; Cell Death; Biopolymers; Development, Plasticity and Regeneration in the Brain and Spinal Cord. Since 1979, Professor Harvey has held a number of positions in universities in the USA, South Australia and is currently a Professor at the Department of Anatomy and Human Biology, at the University of Western Australia, Perth, WA. He has had continuous research funding support from the Australian National Health and Medical Research Council (NH&MRC) since 1983 and has published about 100 full-length research papers in international refereed journals. This includes invited authorship of a number of chapters in books published by overseas publishers.

Professor Miranda Grounds

Professor Grounds has BSc Hons (WA) (1969) and a PhD (London) (1978).

In 1994 Professor Grounds was appointed as Professor in the School of Anatomy and Human Biology, at the University of Western Australia. From 1994-1998, she was a Senior Research Fellow funded by the NH&MRC. For over 20 years, since 1980, she was funded continuously by NH&MRC as an independent researcher.

Professor Grounds' research has included: factors controlling the repair of skeletal muscle (for almost 30 years); cell based therapies (using conventional muscle precursor cells as well as stem cells) for the lethal childhood muscle disease Duchenne muscular dystrophy. Tissue Engineering of skeletal muscle is an extension of these research areas. In 1999, Professor Grounds established (and is the coordinator of) a new Tissue Engineering Research Centre (TERC) at the University of Western Australia.

These research achievements are widely recognised internationally as evidenced by overseas funding and many invitations to conferences. Between 1989-1999 Professor Grounds received 27 invitations to speak at international and local meetings, and 17 for 2000-2003. Almost 100 papers and reviews have been published in peer reviewed journals.

Dr Frank Koentgen

Dr Koentgen was awarded a PhD from Freiburg University & Max-Planck-Institute, Freiburg, Germany, in 1992. He worked in the United States at the Roche Institute for Molecular Biology (RIMB), Nutley, NJ, & Roche Pharmaceuticals, Nutley, NJ. In 1993 he worked at the Walter and Eliza Hall Institute (WEHI) with Prof Gustav Nossal, in Melbourne, Victoria, and was Head of "Genetically-Modified Mouse Laboratory" (GMML) and Principal Investigator at the Catalytic Antibody Laboratory. In 1999 he founded Ozgene Pty Ltd, in Perth, of which he is the Director and CEO. Dr Koentgen has publications in a

number of scientific journals, including Nature, Science, EMBO, Nature Medicine, Immunity, J Exp Medicine, PNAS, Blood, and Methods in Enzymology.

Dr Koentgen is a member of the following associations: the Australian Institute of Company Directors, AICD; the Australian Biotechnology Association, ABA; and the Australasian Society for Immunology, ASI. He is an adviser to the Transgenic Advisory Committee, National Health Research Institute (NHRI), Taiwan.

Dr Sandra Webb

Dr Sandy Webb is the Senior Policy Officer Reproductive Technology in the Department of Health (WA), and Executive Officer of the WA Reproductive Technology Council. Her roles are varied, relating predominantly to coordinating the implementation of the *WA Human Reproductive Technology Act 1991*, and range from legal to ethical, scientific, medical and social matters. Dr Webb is currently a member of the Australian Health Ethics Committee, bringing to that Committee experience in public health research, and a member representing AHEC on the new Gene Technology Ethics Committee. She has participated in a number of national and international committees that have developed guidelines relating to assisted reproductive technology, and is currently working with officials from all States and Territories to develop nationally consistent regulation of the assisted reproductive technologies and stem cell research.

Mr Philip Matthews

Mr Philip Matthews is a lecturer in Philosophy and Ethics at Curtin University and the University of Notre Dame (WA). He was editor of a social ethics journal, Faith and Freedom for six years, and is currently engaged in research on pragmatism and moral theory, particularly as it relates to bioethics.

Embryonic stem cells and the fountain of youth – fact or fantasy by Dr Sue Fletcher

The isolation and culturing of human embryonic stem cells has generated enormous public interest and controversy. I will begin with definitions and explanations of key concepts regarding stem cells and end with an assessment of the potential uses of stem cells. In between are important details that summarise what researchers have learnt about development and how ES cells are being used.

You may have seen dramatic headlines in the public press, such as

'Capturing the promise of youth....'

'Humans are not to be farmed...'

'Let the doctor clone humans-if he can...'

'Cell lines lift transplant hopes'

'Cloning puts the gelding back in the genetic race...' -

many of them promising that we will ultimately be able to replace body organs and tissues that are past their use-by date.

I am going to mostly talk about the facts as they stand at this point in time. The facts relate to the anticipated abilities and projected benefits of stem cells, particularly embryonic stem cells, to cure disease and alleviate suffering. However, there are constraints regarding ethical and legal issues, funding and the current practical limitations. It is the latter that is hampering the scientific progress.

So, what is a stem cell? A stem cell has the unique ability for long term self-renewal, that is the ability to give rise to more stem cells. These cells have the capacity to generate differentiated progeny when directed to do so, when placed in the environment that will allow them to differentiate. The stem cell probably exists (as a function if not as an entity) in most tissues. It is present at all stages in development.

I am going to define a number of terms:

- *Multi-potent cells* can develop into many different cell types.
- *Pluripotent cells*, to which I will be referring much of the time, have the ability to give rise to a wide range of somatic tissues; and into this category fall embryonic stem cells, embryonic germ cells and embryonal carcinoma cells.
- *Totipotent cells* have the ability to give rise to a new individual, given adequate maternal support, and really this term refers to the fertilised egg and its immediate progeny. The fertilised egg is totipotent. After the initial few divisions, all cells have the potential to form a new individual. This is how identical twins occur.
- *Plasticity* refers to the ability of tissue stem cells to differentiate into other types of cells. The jury is still out as to how plastic adult stem cells are, but there is some evidence that there is reasonable amount of plasticity in some adult stem cells.
- *Clone*: This is the 'C' word which I will not be using much during this talk. The Oxford English Dictionary definition of a clone is 'a group of organisms produced asexually from one stock or ancestor'.

Development begins at fertilisation, and, as I said earlier, after the initial few divisions, all cells have the potential to form a new individual. Twins, and in some rare cases, quads (I think it is the South American Armadillo that has four identical babies) illustrate this. After fertilisation, four days in mouse and five days in humans, the cells specialise to form the blastocyst. The outer cells have the ability to form the fetal component of the placenta and associated tissues, and the inner cell mass (ICM) which is a little blob, has the potential to form the embryo. The cells of the ICM are *pluripotent*.

The human ICM consists of about 25 to 35 cells out of a total of 250 cells in the blastocyst. The fertilised egg is *totipotent* and the ICM is *pluripotent*. The ICM on its own cannot form all the tissues to make an embryo. The ICM differentiates into three embryonic layers, endoderm, mesoderm and ectoderm and from these arise all the tissues of the body. The trophoectoderm, the outer layer of the blastocyst, forms the embryonic side of the placenta.

This little being is known as an embryo from the onset of development, that is, from fertilisation until it becomes a foetus, which in humans is eight weeks after fertilisation. It is then known as a foetus until birth. The ICM can probably form all the cells of the body. This has been demonstrated in mice, in which they have done cross-species transplantation of cells into blastocysts and shown that virtually all the tissues examined are chimeric. In other words, they contain cells from both strains (four parents). The ICM on its own cannot form an organism. If they are removed from the embryo they are not able to generate an axis and form a body plan, although these cells remain pluripotent and developmentally plastic at least until eight days.

Embryonic stem cells lines grown *in vitro* are derived from pluripotent cells of the ICM. Embryonic cell lines can give rise to all the somatic cell types and the germ line cells when placed in the correct environment. Again, these cells are harvested from the human and mouse embryos at five days and four days after fertilisation, respectively.

The stages of stem cell derivation are; firstly the culture of the cleavage stage embryo, then the blastocyst. The trophoblast cells are actually inhibitory to the development of embryonic stem cell lines in culture and they are removed by immuno surgery. The ICM is then isolated, and plated on to a layer of feeder cells. These are usually embryonic mouse mesodermal cells. The ICM can then be dissociated and replated onto new feeder cells and eventually used to establish embryonic stem cell cultures.

Those who work on embryonic stem cells (ES cells) have had to establish a list of properties that define these cells. ES cells originate from the pluripotent cell populations, the inner cell mass of the blastocyst, and are capable of unlimited, undifferentiated proliferation without transformation, in-vitro (long-term self-renewal). They maintain a normal, stable, diploid complement of chromosomes (normal karyotype) and differentiate into somatic cells representative of all three embryonic germ layers. They are also capable of colonising the germ line and giving rise to sperm and egg cells. This has not been tested for human embryonic stem cells. They are clonogenic, that is, a single stem cell can give rise to a colony of genetically identical cells which have the same properties as the original cell. They express the transcription factor Oct 4, a factor which maintains them in a proliferative non-differentiated state. They can be induced to continue proliferating or differentiate, depending upon the environment. They lack the G1 checkpoint in the cell cycle and they do not require an external stimulus to initiate DNA replication. They do not show X chromosome inactivation. In every somatic cell of a female mammal, one of the two X chromosomes

becomes permanently inactivated but X inactivation does not occur in undifferentiated embryonic stem cells.

Of the 200 plus cell types in the body, the following is a list of cells that have been derived from mouse and/or human embryonic stem cells lines. This list is growing all the time as researchers develop the techniques and find which factors cause the cells to differentiate into different cell types. The list includes: adipocyte, astrocyte, cardiomyocyte, chondrocyte, definitive hematopoietic cell, dendritic cell, endothelial cell, keratinocyte, lymphoid precursor, neuron, oligodendrocyte, mast cell, osteoblast, pancreatic islets, smooth muscle, striated muscle, yolk sac mesoderm, and yolk sac endoderm.

I'll briefly mention the time-line for this work. It actually had its beginnings in 1878 when there was a first attempt at *in vitro* fertilisation. There was no success until 1959 using a rabbit; although much of the early work was carried out on mice. It was around 1988 that human embryonic stem cell lines that proliferated and differentiated were developed. In 1994 human embryonic stem cells were isolated and went through two to three cycles of division and then died.

Mice have often been used as a model for human disease and the mouse embryonic stem cells have provided an enormous amount of information which has led to the development of human embryonic stem cell lines. But mice and men are different.

Mouse and human ES cell express some different surface markers. However, both express the transcription factor Oct 4 and telomerase, which are essential characteristics of embryonic stem cells. It is not certain whether human embryonic germ cells express telomerase. Mouse cells can be maintained on a feeder layer of embryonic mesodermic cells, and this can be substituted by Leukaemia Inhibitory Factor (LIF). Human ES cells so far have been dependant on feeder cells, which cannot be replaced by LIF alone. Human embryonic stem cells also respond to the basic Fibroblast Growth Factor (bFGF). The embryonic germ cells do respond to LIF but still require feeder cells. Morphologically, mouse and human ES cells appear very different in tissue culture. Mouse and primate cells can be maintained on a feeder layer.

The potential application of embryonic stem cells is:

- embryological and developmental research - they are particularly useful when studying birth defects;
- functional genomics;
- growth factor, drug discovery and testing (for example, the testing of treatments for Alzheimer's Disease in modified mouse ES cells);
- toxicology;
- gene therapy and cell transplantation.

It is gene therapy and cell transplantation that has caused such excitement in medicine. This interest is based on the assumption that it is going to be possible to grow ES cells on a large scale; introduce genetic modifications (gene therapy); and direct their differentiation.

There is a series of events that have to be gone through before cells can be used for transplantation. The ES cells that do differentiate into embryoid bodies are little disorganised arrays of differentiated cells of all types which must be isolated and placed in selective medium.

Many diseases result from the disruption of cellular function or destruction of tissue, for example, muscular dystrophy (which is my main interest), Alzheimer's Disease, Parkinson's, diabetes, heart disease, the list is extensive. Some of these conditions can be treated by organ transplantation, however there is a chronic shortage of organs for transplantation.

Similar to organ transplantation, the use of embryonic stem cells will be subject to immunological surveillance by the host. One way of overcoming this difficulty is to create tissue banks of cells of different HLA types or to modify cell surface markers so that they are not seen by the immune system.

Another option, which has been very controversial, is a somatic cell nuclear transfer (SCNT). The nucleus from a patient's somatic cell can be transferred to an enucleated donor egg. This can develop into a blastocyst, the ICM will then yield ES cells almost identical to the patient's cells. If these cells could then be induced to differentiate - (eg muscle, pancreatic islets, neural cells etc) they could be used to help repair damaged tissues.

How many embryos will this take? SCNT is a very inefficient process, and as you can see, we do not get many full term animals arising by this technique. It is estimated that 280 human oocytes will be required for one successful human somatic cell nuclear transfer. This is also the process by which it is proposed to recreate a thylacine. If you watched the ABC a few weeks ago, you may have seen a program about an 84 year old sterile man who had lots of money and was hoping for a son that he proposed to create by this process. Dolly, copycat, mice, calves and others that have been produced by somatic cell nuclear transfer.

Animals produced by SCNT often have abnormalities. For example, many suffer perinatal death, are born oversized, have genetic defects, immunological deficiencies and some of them have liver defects. Epigenetic effects, those which are not coded for on the chromosomes, are crucial during development. By disrupting the epigenetic effects, we disrupt the imprinting system. The imprinting of genes is extremely important. There have been lethal interspecies crosses between very close species of American deer mice because of uniparental imprinting. Nuclear transfer between two mice of the same species, but different strains (C57/Bl6 and CBA/2) resulted in altered gene expression and methylation patterns. Another problem is that somatic cells have shortened telomeres. As our chromosomes divide the telomeres get progressively shorter. Embryonic cells have long telomeres and express an enzyme, telomerase, which adds repeating DNA sequences to the ends of the chromosomes so that telomere length is maintained. Dolly, who I am sure you will know about, appears to have shortened telomeres, although accurate measurement of telomere length is difficult to achieve. It is possible that certain of her cells may undergo premature senescence.

Individuals created by somatic cell nuclear transfer are not identical to the nucleus donor, because mitochondrial DNA is maternally inherited and incompatibilities exist between nuclear and mitochondrial encoded proteins. In addition, even mitochondrial proteins can cause an immune response. The steps which will be required, prior to development of any human treatments, is to establish pure cultures of differentiated cells or partially differentiated cells, demonstrate that they do the job, eg, that they produce insulin or which ever specialised protein they are supposed to make.

To demonstrate safety, we need animal models. We must show absence of tumour formation and an absence of transmission of infectious agents. The major concern is with regard to transmission of retroviruses between species. It will also be necessary to prevent rejection -

some of these issues I have already mentioned. Multi drug immuno suppression is also a possibility and establishing tolerance is another.

There are important practical limitations to work on embryonic stem cells. ES cells from any species can only give rise to a new individual when combined with the immediate progeny of a fertilised egg. We cannot use ES cells to make large numbers of identical individuals because ES cells alone are not able to generate all the extra embryonic tissues and they have an inability to generate a body plan and undergo axis formation.

Little cells, big issues - at present no one has reported large scale growth, efficient generation or genetic manipulation of human embryonic stem cells. They have done well with mouse ES cells, but, at this time, human embryonic stem cells do not seem as easy to manipulate as the mouse cells. It is going to be very important to identify the factors that facilitate growth and inhibit differentiation until it is required. Ideally, we would want the cells to continue proliferating until we have a large number, then induce them to differentiate to form the tissues needed for a particular transplant. Efficient liberation from feeder cell requirement is going to be very important for transplantation, although the feeder cells are irradiated and should not be dividing. There is going to be a lot of opposition to using cells which require feeder layers.

Finally, for many this work is moving far too fast, as there are enormous concerns about ethical and safety issues. But I would also like to point out that for many other people, this work is progressing far too slowly - their needs are great. Twenty five years ago recombinant DNA technology was virtually banned. Many people today are very dependent upon recombinant molecules for their quality of life.

Thank you

Stem cells and central nervous tissue repair **by Professor Alan R Harvey**

In this presentation I will briefly summarize a number of important nervous system issues; how the nervous system develops, how it ages, the effects of injury and various degenerative diseases and so on. At the end you will, I hope, agree that there are plenty of repair options other than using embryonic stem cells that we have yet to explore properly.

The brain, as you may appreciate, is currently the most complex organ that we know of in the universe, and if it turns out that we are the only life form in the entire universe, then the human brain is probably the most complex living thing that exists anywhere. One important point to get across at the outset is that the brain is not designed to go on for a limitless period of time. It has billions and billions of cells in it, the vast majority of which do not divide or replicate after birth. So you are essentially born with almost all the neurons that you are ever going to have throughout life. The lack of an intrinsic pool of back-up, reparative cells is obviously of critical importance if you suffer a trauma, whether it is a stroke or a physical injury, that damages fibre tracts or parts of the grey matter where the neurons are situated. Alternatively you may suffer some sort of degenerative disease in which the neurons start to die, such as in Alzheimer's disease or Parkinson's disease. Whatever the insult/disease, the pressure is on to find ways of fixing these problems because as the baby boomers get older we will become an enormous burden on all the youngsters out there who will have to support us. We want to live as long as we can, we want to be cognitive as long as we can and it is going to cost all you remaining taxpayers a lot of money. The proportion of older people being treated for neurological problems is already high and is going to increase with time, so there is understandably a big push to solve these problems and to reduce the need for long term hospital and/or remedial care.

In early development, the nervous system of the embryo is specified between the second and third week after fertilization. The embryo and associated nervous system is tiny at 21-28 days and grows at a great rate throughout pregnancy. It is important to appreciate the enormous amount of brain growth and the enormous amount of differentiation that goes on during fetal development. By birth we have about 10^{12} nerve cells and we have achieved the development and specification of a whole range of different neurons and their supporting cells. After their final cell division, neurons have to migrate to their final destination, they have to differentiate into the appropriate cell type with the appropriate cellular processes. These developing neurons have to integrate into the appropriate circuitry, whether it is local or distant circuitry. Neurons have to develop the appropriate molecular characteristics and neurochemistry, which can be very complicated, and then on top of that they have to be adaptable, because all of the synaptic connections in your brain, all of the interconnections between nerve cells, are plastic and subject to change. That is why we learn, why we can remember and that is why experience alters the way the brain functions.

What we are asking is, if we lose a large number of these cells, is it really possible to transplant a few more in and replace them? Will newly grafted cells go through all of those developmental processes, find where they are supposed to be in a particular part of the brain, turn into the appropriate cell, hook up to the right circuitry, make the right neurotransmitters - essentially integrate into the community of the other 10^{12} or so neurons. Is that possible, is it credible? Well we have been trying. Neurons normally express complex but highly patterned processes and developing a rich network of connections. So if we are asking for cell replacement strategies in adults using, for example, embryonic stem cells we really have

to understand everything about the way nerve cells grow, the way they mature, the way they know who they are supposed to be, who they are supposed to be next to and how they are supposed to integrate into the circuitry. And that is obviously not a trivial exercise.

There is an enormous diversity of neuronal types in the central nervous system – both in terms of morphology and neurochemistry. The types of cell found in the spinal cord differ from those found in cerebral cortex or hippocampus (which is associated with learning and memory). Other unique classes of neuron are found in the cerebellum, for example the remarkable Purkinje cell – more of this neuron later. These various cell types differentiate, they develop different morphologies, but one thing you must understand is that in the developing brain neural cells are not driven only by their own genetic code. The development of the nervous system requires unbelievably complex spatial and temporal interactions so that cells acquire their mature phenotype as a result of either the spatial relationships they had with cells they talked to yesterday or who they are talking to today or who they want to talk to tomorrow - not only in time but in terms of space, who they are next to, who they interact with and when. All these absolutely fundamental features of development are essential in order to build the central nervous system (CNS) properly, and if you try to put totally undifferentiated cells into an adult nervous system you have missed out on all those subtle spatial and temporal interactions. So to put new, immature cells into this ‘foreign’ environment and to get them to turn into what you want is an extraordinarily difficult process.

Probably half of you in the audience will need or seek some sort of neurological treatment at some stage in your lives. Perhaps the simplest way of thinking about repair strategies is to divide them into two basic approaches; damage to the pathways interconnecting nerve cells, or loss of the neurons themselves. Think of a set of nerve cells in one location and another set somewhere else. They communicate with each other via large numbers of interconnecting nerve fibres (axons). One set of neurons is the transmitter, the other the set of cells is the receiver. They may actually talk reciprocally to each other so both may be senders and both may be receivers - but either way they communicate via this axonal cable. If you damage the cable the actual cells themselves might be unaffected but the cable between them has been damaged. The classic incidence of this is in spinal cord injury where ascending and descending motor and sensory fibres are disconnected due to trauma. The nerve cells by and large may be unaffected in the brain or further down in the spinal cord but you have damaged the linkages between the senders and the receivers.

Since his tragic accident, Christopher Reeve has been very vocal in pushing for embryonic stem cell research because, for reasons which I admit I do not fully understand, he thinks these cells are going to be able to solve his problem. By transplanting embryonic stem cells into the injury site there will be repair of the damaged cable. In fact, after fibre pathway damage in the spinal cord, there is a range of other approaches that are currently under investigation that may help to repair the injury without having to resort to the use of these controversial stem cells. For example, it may be possible to use grafts of peripheral nerves, either from the injured person (autograft) or from donors. It may prove possible to construct different types of nerve bridges using cells that we already have available, such as olfactory ensheathing glia, adult-derived stem cells or perhaps immature spinal cord or other cells from fetuses. It will also be possible to genetically manipulate those bridges before implantation, and work is underway to develop polymer/cell hybrid structures that may support the regeneration of injured fibres. A range of new molecular/pharmacological approaches are also in the research and development stage. We are really only one or two faltering steps

down the path of knowing what these various strategies will, or can, achieve. There is a great deal of intensive research still to be done and in my view it is premature, and there is no obvious need right now, to explore with any urgency the use of embryonic stem cells in spinal cord repair. We have plenty of problems as it is, basic issues still to be resolved, without adding the complex and difficult stem cell ingredient into the mix.

The second repair issue relates to the replacement of neurons that have degenerated and have died - classic examples of course are in Alzheimer's disease or in Parkinson's disease. In an Alzheimer's brain there are all sorts of pathological changes. We don't really understand what causes those changes; we have all sorts of ideas and know that certain genes are involved but the pathology is probably triggered by a range of different problems or insults that give a similar sort of degenerative outcome. There are thought to be many risk factors but one of the things that does happen is that neurons are lost - they die. In Parkinsonism there is also a catastrophic loss of neurons, so an obvious, perhaps simplistic approach is to replace the lost cells with new, appropriate neurons. Now replacing these lost neurons, you have to realise, is the last port of call. What we would really like to do is to have an approach that recognises there is a problem early on and then stop it from getting any worse. You might want to protect vulnerable neurons either with growth factors or something that inhibits cell death or even genetically engineer the cells using vectors to give them a better capacity to survive.

In Parkinsonism, when the overt symptoms first arise it is estimated that as many as 85% of the dopaminergic neurons in the midbrain have already disappeared. Thus if we could pick up the degenerative changes earlier it is possible we would never need to replace anything - because we could stop the disease from progressing any further and thus help to sort out or at least minimize the problem. If, however, we continue to miss these changes and we have to replace the lost cells, then we need to think about what cells would be best used as replacements. It isn't only neurons that we have to be concerned about in degenerative diseases. In the CNS, glial cells called oligodendrocytes make the insulating myelin sheaths that surround axons in white matter. This insulation allows axons to conduct nerve impulses more quickly - so most of the nerve fibres in our brains contain this myelin coat. These glial cells disappear and are damaged in multiple sclerosis. So another major push is to develop techniques that might allow us to effectively replace these cells or put new cells in to remyelinate or reinsulate compromised nerve fibres in the sclerotic CNS.

In Parkinsonism, the region that has been targeted in clinical studies and in drug therapies is a region in the midbrain called the substantia nigra. We know that cells in this region that express the neurotransmitter dopamine die, affecting the pathway from the substantia nigra to a forebrain structure called the basal ganglia; loss of these neurones gives rise to a number of the characteristic symptoms of Parkinson's disease. Note however that other types of neuron are also lost in this degenerative disease, a fact often overlooked by researchers and clinicians. Much of the research is aimed at trying to replace the lost dopaminergic neurons and there is much discussion about using embryonic stem cells to address the problem. However we can already do something to fix it. What happens is that immature dopamine cells are obtained from aborted fetuses and are transplanted directly into the basal ganglia. The first of these trials was carried out in the UK as long ago as 1987. Careful follow-up studies using modern scanning and brain imaging techniques have shown that in some patients the grafted cells have survived within the host basal ganglia and they continue to make and express dopamine. There are a number of clinical trials currently under way

around the world using human fetal material to try to treat Parkinsonism – something I will return to in a moment.

One issue I would like to get across to you in this short discussion is this. Almost any article that you read about embryonic stem cells will say or imply that we have to be quick, we have to understand these cells and develop protocols for their use as quickly as possible so that we can cure this and the other including Parkinsonism, Alzheimer's disease, diabetes, make the lame walk etc etc. By next year, is often the way it sounds. But we already use fetal tissue in Parkinson's patients - an approach that has been underway for 15 years. I have been involved in fetal transplantation for 24 years. I can remember the first exciting study that came out showing that grafted fetal dopaminergic tissue had a functional impact in host animals and I can remember the first patient who received a graft back in 1987 in Birmingham, UK. Now in 15 years that field has progressed very slowly – probably more slowly than we would have hoped. There was an initial explosion of operations, but some clinical trials had problems because patients died or had significant negative outcomes from this sort of approach. It was quickly realised that we had gone too far, too fast, and the field then became more cautious. In order to really know whether the procedure was effective a number of long-term, carefully controlled trials were established in which the patients were monitored for a year or two before the operation and followed for many years afterwards. We are still trying to get the procedure to work more effectively.

These pioneering transplant trials were performed using fetal tissue, tissue that many of us believe is potentially much more effective than any other tissue. This is because if you use fetal material that is already committed to becoming neural tissue, there is good evidence to suggest that immature neurons in this tissue have the greatest chance of integrating into the host brain and its circuitry. Yet in 15 years we are still taking only faltering steps forward in the use of this material in Parkinsonism. Thus even using what I think is optimal material for transplantation, it is noteworthy how slow a field such as this has progressed, particularly if the trials are carried out properly. There are a whole host of other alternatives that may be available that we are yet to explore, and I think we ought to explore, before we say that the only cells that should be used are embryonic stem cells.

Earlier I referred to a highly specialized and unique type of neuron called the Purkinje cell in the cerebellum. In a recent study, non-differentiated cells transplanted into the cerebellum developed morphologically and neurochemically into neurons that closely resembled host Purkinje neurons. Remarkably, the donor cells that differentiated into these highly specialized neurons were derived from adult bone marrow. These stromal cells from the bone marrow have many stem cell-like characteristics. We also know that stromal cells from the bone marrow can make oligodendrocytes, they can make neurons, and they may also differentiate into astrocytes after transplantation into the CNS.

In later discussions in this symposium we will talk about the ethics of using embryonic stem cells and where such studies may lead. My message is this: there is considerable confusion in the neural field - whether precursor/progenitor cells are pluripotent, multipotent, tissue specific etc. There is much debate, some of it heated, over claims and counter claims made by different laboratories. There are many potential sources of cells and I have described a couple of examples of grafted cells obtained from these different sources. There are stem cell-like cells within your adult brains. We can perhaps get those undifferentiated cells out and use them in cell replacement protocols, although there is still some debate as to how useful these cells will really be. There is the potential of using cells from bone marrow, from

umbilical cord; we have a 20 year history or more of using fetal neural tissue, we can also get fetal neural stem cells or germ cells.

And then there are embryonic stem cells. Proponents of their use argue that it is important to obtain embryonic stem cells at all costs in order to begin intensive and rapid research - because these magical cells can supposedly do things that no other cell can do. My view is that this is not proven, and may turn out not to be true. While I accept that we need to understand more about human embryonic stem cells and that limited, carefully controlled research must be done on them, I do not accept the suggestion that we need billions of them quickly because it is the only way to fix this, that and the other disease. Such an attitude could quickly take us to the dangerous moment when there are no spare embryos and pressure is brought to bear to create human embryos purely as a resource for others – an anathema to many, including myself. There are many other multipotent cells we can access, plenty of other approaches we can try, and many reasons why in fact other cells might be even more effective than embryonic stem cells, especially in the CNS. So it is vitally important not to let the hype get too far ahead of the hope in this emotionally charged debate.

The potential of stem cells for repair of skeletal and cardiac muscle by Professor Miranda Grounds

Thank you very much for the opportunity to speak here. I am going to follow on from the theme of Alan Harvey whereby I am also talking about stem cells, but not necessarily embryonic stem cells.

The tissue that I am interested in is skeletal muscle. This constitutes a great deal of the mass of your body, it is a fairly simple tissue with specialised contractile proteins that are designed to contract and move parts of the skeleton. Each skeletal muscle is attached to different parts of the skeleton (bones) at either end and the shortening of these very long cells move the parts of the body. Skeletal muscles have a terrific capacity for repair, unlike the brain and unlike the heart that I will be talking about. If you damage skeletal muscle, it will form perfectly good new skeletal muscle; this makes sense because in day-to-day business when you get bashed around, cut or hurt on a sports field, you can make new skeletal muscle.

Skeletal muscles are enormously long multi-nucleated cells so they are very different from most cells in the body that only contain a single nucleus. They are many centimetres long, stuffed full of hundreds of nuclei and highly organised contractile proteins occupy most of the cell. When muscle is damaged, there is activation of cells lying outside on the surface of the muscle fibre that are defined geographically and so they are called 'satellite cells'. These are the reserve muscle cells. When activated they are called myoblasts. The myoblasts can divide many times and then they fuse together to form little baby (multinucleated) muscle cells called myotubes which subsequently mature into the muscle fibre. The nuclei lying within the old muscle fibre are considered to never contribute to new muscle formation.

It has been dogma for a very long time that skeletal muscle has a terrific capacity to repair and that satellite cells are the source of the precursors that form new muscle in adults. However, in some disorders where there is a genetic defect in the muscle, for example in boys who have Duchenne Muscular Dystrophy (that Sue Fletcher mentioned), there is a weakness in the muscle fibres. The muscle keeps falling apart and it keeps trying to regenerate but, in the end, this fails and instead the muscle is replaced by fat and connective tissue. These children by the age of ten are wheelchair bound, they have terrific muscle wasting due to this apparent lack of muscle regeneration and they usually die by their early 20s. One of the major interests in our laboratory is trying to actually replace the defective genes in these muscle cells. The way we do this is to take normal muscle precursor cells (myoblasts) and inject them into the dystrophic muscle fibre: the idea is that these normal myoblasts after fusion will coexist within the one great big multi-nucleated cell and the correct genetic information in these normal nuclei can then make the correct protein.

It is interesting that Alan Harvey mentioned that neural cell therapy has been actively investigated for about 15 years, because the first big conference on myoblast transplantation therapy was held almost 14 years ago in New York. This conference resulted in clinical trials but, unfortunately, they have had no real success. The major problem with the clinical trials seems to be that the myoblasts die. It now appears that cells that have been through tissue culture seem to be a problem when subsequently transplanted *in vivo* into humans or into animal models.

People have become very interested in the muscle precursor cells. In the clinical situation the normal myoblasts are taken from someone who is closely matched immunologically with the dystrophic boy to avoid problems with immune rejection. Now people are asking whether there are stem cells for these myoblasts. Perhaps we could find these stem cells in the dystrophic boys, take out these stem cells, genetically correct them and then put them back into the same boy and this would avoid problems with immunological rejection. Such autologous (or self) transplantation is very attractive and there has been a great focus on stem cells recently.

It was widely considered that when you extracted cells out of skeletal muscle and grew them in tissue culture, that all of the myoblasts would be derived from satellite cells. What is important to realise (and this applies to any tissue) is that there are lots of other cells in muscle tissue located in-between the muscle fibres. This area outside the muscle fibres is called the interstitial connective tissue and it contains many different cells; for example there are blood vessels lined with endothelial cells and smooth muscle cells, there are fibroblasts, macrophages and nerves. (Similar cells are present in all organs of the body i.e in the kidney, liver, pancreas and the brain.) So, when you extract cells from skeletal muscle and grow them in tissue culture you are also extracting a whole range of these other cells. Now it has become apparent that some cells in this interstitial connective tissue can also contribute to new skeletal muscle: it seems that the precise environment that such cells find themselves in will determine whether they become skeletal muscle or some other cell type. So the environment becomes of extreme importance in determining the fate of cells that perhaps have multi-potential ability. Therefore we can expand our scheme in skeletal muscle so that, apart from satellite cells that can give rise to myoblasts, there are also other cells arising outside the muscle fibre that can give rise to myoblasts.

There is no doubt that other cells can give rise to myoblasts but there is a great deal of doubt as to whether these cells are indeed stem cells or whether they are merely cells who just have a capacity to change their kind of cell lineage. This is a very important distinction that has, I think, been greatly overlooked in the current debate. A recent review [1] summarises the kinds of cells that have now been shown to be able to give rise to skeletal muscle. It is important to note that most of these studies are conducted in tissue culture and they demonstrate that if you take cells and put them into particular conditions they can change into skeletal muscle like cells. It does not necessarily mean to say that they do this *in vivo* but it raises the possibility. We are particularly interested in cells of the skin. If fibroblasts from the skin are grown in tissue culture and exposed to factors derived from either injured muscle or from an isolated factor called Galactin-1, these dermal fibroblasts will be converted into myoblasts with a very high efficiency. This raises interesting possibilities. For example with a boy with Duchenne Muscular Dystrophy, perhaps you could take fibroblasts from the skin (which is a relatively easy procedure), convert them into myoblasts, genetically correct these cells and then use them as a source of 'normal' myoblasts to reimplant into the same boy. These studies show that many cells have plasticity, but we do not know if they have the important stem cell property of cell replacement and can divide many times as would be required for such a therapy.

The two really important properties of stem cells are the same for adult and embryonic stem cells. One is plasticity, which is the ability to go down more than one lineage i.e to change the cell pathway or fate. The second property is the capacity for continual renewal by cell division. I consider that a great deal of the recent literature actually relates just to plasticity: it says, 'yes, cells can do all sorts of extraordinary things and can turn into other unexpected

cells under particular conditions'. But, most of the papers have not tested whether that is a 'one-off' event, or whether these multi-potential precursors do indeed represent a magic stem cell that can continue to divide and produce more precursors. I think that this is an extremely important distinction with respect to stem cells and potential therapies.

One source of stem cells that has attracted a great deal of attention is bone-marrow derived stem cells and I will now discuss the issue of circulating muscle precursor cells. With the identification of very good cell markers, it has been possible to demonstrate that bone-marrow derived cells can give rise to a huge range of tissues. In essence, what was done was to take bone-marrow cells from a donor mouse that you can easily identify, in simple terms let us say a green mouse. You then take a white mouse, get rid of its (white) bone-marrow and replace it with the bone marrow out of the green mouse, so all cells that come from that donor bone marrow will be green. Then by looking in various tissues e.g the brain, the heart, the skeletal muscle, you can show that there are indeed green cells that give rise to nervous tissue, heart tissue or skeletal muscle. What is demonstrated is that (green) bone-marrow derived cells can give rise to a whole range of cell types that were previously not anticipated. This does not tell you whether it actually was a stem cell that was converted, but it is nonetheless a very interesting observation.

The analogy has been made (by Helen Blau) of a super highway with stem cells (like cars) whirling all around the body. Occasionally, these cells will encounter a particular signal that makes them take an off-ramp and exit this freeway and go down into the brain and form neural tissue, or they will encounter an off-ramp with particular signals that make them go down into the heart and turn into cardiac tissue. Now this is a powerful and compelling image and it is one that a lot of people have been very excited by. However, it appears that the exiting down 'off-ramps' may be rather infrequent. In fact, in skeletal muscle it has been said that, rather than it being a super highway, it is more like a very narrow pavement where the occasional stem cell stumbles into the myogenic lineage (T Partridge), as such conversion into skeletal muscles is not impressive.

People would like to be able to take bone marrow cells (which is a very standard procedure) from a boy with Duchenne Muscular Dystrophy, isolate out the muscle stem cells, genetically correct them and then put them back into the same boy where these (genetically corrected 'normal') cells would go through the circulation to all parts of the body and be taken up by the skeletal muscle tissue (where they would effectively replace the defective gene). This is an ideal scenario, but is far from reality at this stage. There was one boy with Duchenne Muscular Dystrophy who also had leukaemia and fortuitously had a bone marrow transplantation to fix the leukaemia. This presents a clinical experiment (similar to the situation described with the green bone-marrow mouse) to test if these normal donor bone-marrow cells implanted into this boy, did indeed influence and correct or help the muscular dystrophy. This boy was examined thirteen years after he had the bone marrow transplant, so that is a mighty long time for those normal cells to really help out the muscle if they going to. Unfortunately there was very little effect on the correction of the skeletal muscle defect (L Kunkel and E Gussoni). The bottom line, unfortunately, is that all experiments to date using such bone marrow cross transplantation show extremely low efficiency, and less than about one per cent of the bone marrow cells contribute to skeletal muscle. So, while it can happen, it is rare. The challenge is how to increase the efficiency of conversion into muscle.

I would now like to just mention cardiac muscle. Cardiac muscle has some similarities with skeletal muscle as it is contractile tissue with the same kinds of specialised contractile

proteins. But the heart is very different to skeletal muscle, because the heart is a big round three dimensional object that has to beat all the time (twenty-four hours a day and can never take time off) and it is composed of many mononucleated cells linked together.

When skeletal muscle is damaged, we know that it can be repaired from the satellite cells (and possibly others precursors) but, in contrast, when cardiac muscle is damaged it cannot form new heart muscle. This has been the dogma. It now appears that some heart cells may divide to a very small extent, but the inability to effectively form new heart muscle presents a major problem. When the heart is damaged as in a heart attack, there is restricted blood supply to some of the heart tissue, and this will die and be replaced by scar tissue. Attempts have been made to inject into these damaged areas various cell types, such as myoblasts, foetal cardiac myocytes, and embryonic cardiac myocytes. Varying success has been reported (1).

Studies that do look promising have used bone-marrow stem cells and also endothelial cells derived from very young blood vessels (foetal, embryonic or neonatal), whereas endothelial cells from adult blood vessels are not effective. Embryonic stem cells potentially have a role for heart repair as the adult heart cells normally will not divide (or only rarely), whereas the embryonic heart cells will. I would like to emphasise that the mouse embryonic stem cell seems to behave in a very different way to that of the human. For example, there is a very low efficiency of conversion of human embryonic stem cells to the heart and less than ten per cent of the cells will convert, whereas in the mouse it is about eighty per cent, so this is a very big difference. Further research may well improve the behaviour of human embryonic stem cell cultures. These are still early days and many rigorous studies need to be done. There is certainly more hope now for this kind of replacement of damaged heart tissue, either by stimulating cell replication (which I have not discussed today), or by taking some of these cell types (endothelial cells, embryonic stem cells, bone marrow derived cells) and turning them into heart tissue.

Finally, the key issue that I think is important is, what is the local environment that causes these stem cells (adult or embryonic) to turn into a particular lineage? Why would a bone marrow cell become a nerve or a skeletal muscle? It is the local conditions that the stem cells encounter that most likely determine their fate. Very little attention to date has focused on what are the inducers in mature skeletal muscle that are going to recruit cells into the myogenic lineage. This is an area that we are interested in. The topic that has received a great deal of attention to date is trying to identify, isolate and expand these myogenic stem cells, since finding particular markers on these cells would allow you to say, "yes we have obtained the magic stem cell".

The results that have emerged over the last five to ten years have been extremely exciting, but I feel that some of the literature has distorted and misrepresented the significance of the findings. I think that researchers have to be more honest and really look much harder at the efficiency and significance of what it is they are observing rather than just focusing on the novel observation itself. Thank you.

Reference

Grounds MD, White J, Rosenthal N, Bogoyevitch M. (2002) The role of stem cells in Skeletal and Cardiac Muscle repair. *J Histochem. Cytochem.* 50: 589-610.

Transgenic Animals **by Dr Frank Koentgen**

I am not really sure why I am here today because I am talking about something very different in many ways, which has some relation to basic research, but mainly as you see here a commercial venture that was established here at Murdoch, and not at UWA, just to keep the balance! So a transgenics talk is about advances in functional genomics, which is a big title. What it basically describes are the techniques we use to study the gain or loss of function of genes in vivo. Now about two years ago we had a big announcement that the entire human genome had been sequenced, by an academic consortium and by Celera.

I guess one of the big surprises at the time was that there were only about 30,000 to 40,000 genes. Before this entire sequence was announced, everybody had anticipated that there would be at least 120,000 or even 150,000 genes. Another good thing that came out of the human genome sequence, was the small differences between humans – as low as 0.1 per cent. The difference between humans and animals was interesting also – 98.5 per cent similarities between humans and chimpanzees; and humans and mice had only a 15 per cent difference. There are only between 150 to 300 genes that are only either in human or mouse.

Now it took another year, in actual fact it took another 2 years, we only received notice in the last couple of months that finally the mouse genome had been sequenced. The initial announcement of the completion of the sequence of the mouse gene was disappointing, because the sequencing data was very inaccurate. A lot of academics paid a lot of money for it but did not think it was worth it.

The bottom line is that we now know all of the letters in the “genome”- book but we do not know what they actually mean. The long journey ahead of us is to decipher the function of these individual genes, hence functional genomics. Just to give you a bit of an idea of the comparison between mouse and human, up here (in the slide) you see the human chromosomes painted in a particular colour and down here you see the equivalent mouse chromosomes, so not only are the genes and sequences different, they are also differently organised.

Now how do you get from gene discovery to gene function? There are many ways and most of these ways have been done in tissue culture in the past 50 years. People have had all sorts of ideas of particular genes and factors and so forth. But only in the last 15 years people have been able to use functional genomics in the mouse, and this we see on the right hand side of the slide. Of course this has been achieved with embryonic stem cells and in the 10 years prior to this, by using transgenic animals. So this is still today the gold standard, either a knock out mouse ie delete a particular gene or change the particular gene or a transgenic mouse.

So what I am going to do now is use pictures to explain how each of these individual stages work in the mouse and along the way I'm also going to tell you a bit about embryonic stem cells. Transgenic mice' developmental biology: You get a fertilised egg, and it develops into a blastocyst, as you have seen in Sue's presentation already. Day 21 you have a mouse and, no, this is not a newborn mouse. Now to make a transgenic mouse you have the fertilised egg; on the left-hand side you have a holding pipette, which holds the mouse embryo in place, you have the zona pellucida which is simply a sugar coat which protects the early embryo. Just after fertilisation you have two pronuclei, one male and one female, and you basically take a glass needle that is loaded with a DNA solution and inject it into one of the

pronuclei. You can see if the injection has been successful if you get a little swelling and a little clearing around the injected pronucleus.

This technology has been around for about 25 years and has been successfully used in a mouse of course. It had less success in the rat, it has been used in pigs and cattle and so forth. This little movie that is running behind me is probably the new version of making transgenic mice and this is using retroviral transduction of the mouse embryo. What you have just seen there is, that if you inject a viral particle between the zona pellucida and the egg, the perivitelline space, the viral particle enters the cell and the retroviral RNA is transcribed into DNA, then the DNA is taken by internal proteins from the cell and from the virus and after replication is integrated into the chromosomes. Now this technology has also been extensively used for the past 10 years but without any success. It is only two months ago that David Baltimore has published a Science paper. The difference between a normal pronuclear transgenic system and this "TranzEmbryo" transgenic system are the efficiencies. The efficiencies in the pronuclear injection may be about 1 to 5 per cent and it is only in a few strains of a mouse and very difficult in a rat and with others it is even more difficult. With our new TranzEmbryo technology we can actually get 100 per cent transgenesis in a mouse, and a 100 per cent transgenesis in a rat, and this is strain independent.

We have already spoken about the green cells that you get from the green bone marrow from the green mice, this is actually the first result we have received from the TranzEmbryo system where we used GFP or green fluorescent protein as a model system to make transgenic animals. These are basically tips of the tail of the mouse and we put them under the microscope. This is a wildtype tail which is over exposed, just so you can visualise it, and these transgenic tails are bright green.

This next slide was done for a lecture about 5 or 6 years ago. I termed it the ultimate transgenic mouse, because it simply swapped the entire genetic information that was in there with new genetic information. The instrumentation and handling is almost identical to making a transgenic mouse and of course the press has called it cloning. The second cloned animal that we have seen was by Wakajama in Hawaii and of course the much more famous one is Dolly where a complete udder cell was fused with an enucleated oocyte by an electrical pulse.

In all of my other talks before I got to the question of whose next, and of course I have a human there, but I did not think this was appropriate for today but we all know that it is happening. Now the second stage we have seen after the zygote is the blastocyst stage, as stated by Sue earlier. The blastocyst has a couple of features, the big "blob" in the bottom is the inner cell mass (ICM); the cavity in the centre is called a blastocoel and the outer cells are called the trophectoderm. The trophectoderm forms part of the placenta, the ICM forms the embryo, and the cavity has a particular purpose if you make mice using embryonic stem cells, as we see there. Now you take these blasts and stick them on to a petri dish and you have these feeder cells called PMEF, the primary mouse embryonic fibroblasts. Then some of the cells from some of the blastocysts that we use will give rise to these little mouse embryonic stem cells. The human works almost identically in human PEF as well, but there has been a very recent publication that Sue may have overlooked. You can culture human embryonic stem cells on a petri dish in the presence of certain factors.

Now just a note on a comment that Anne said earlier about why we use embryonic stem cells when there are so many other cells available. To my knowledge the only cell that we have that we can grow in the dish without transforming it is an embryonic stem cell. All other

cultured tissue cells that live in a petri dish either die or are transformed. Embryonic stem cells will not do this. The stem cell set that you are looking at here is 15 years old picture that I did during my PhD. If anyone is interested, these cells are still growing and are still making genetically modified mice and many other laboratories around the world are also doing our cells. These are the only cells that I am aware of that will do this.

Now how do you make a mouse out of this, you can take these cells and this is where a blastocoel cavity comes into importance. You can take these cells and inject them back into this little cavity, we inject about 10 to 15 cells so if you look at the blastocysts here, there are probably 64. If you use *cells* and *hosts* in different colours, so if you take embryonic stem cells from a black mouse and inject them into a host blastocyst from a white mouse you get our little chequered chimera on the right hand side, it's black and white and brown. Now a chimera is an animal with four parents - two parents from the host blastocyst and two parents of the blastocyst that gave rise to the embryonic stem cells.

Why is this of importance in science and why has it revolutionised the way we look at gene function in the past 15 years? It is because we can genetically manipulate these embryonic stem cells in the dish and they don't die and don't transform. You can grow them, you can make the corrections or change them if you want, you can select them, expand them and you can put them back into the blastocyst. You then get the chimera. If you take this chimera and breed it with the appropriately coloured mouse you get what is termed as a germline offspring or germline transmission or in this case C57BL/6 ES cell derived Mouse Pups. What you are looking at is the white blast and you are looking at black ES cells. If you cross this with a black mouse then black by black is black, so these two black pups will contain the genetic modification that we introduced. The other brown pups are simply a hybrid between the white blasts and the black mouse.

So that basically brings me to the end about transgenic mice. We have three principal ways of generating genetically modified animals:

1. by DNA injection into the fertilised egg,
2. by viral transduction on fertilised eggs. If you go into the literature, as I said, you will see attempts in the last 15 to 20 years leading to the Science paper that came out by David in February 2002, and
3. by specific modification of ES cells.

The principal difference between one, two and three is that in one and two whatever you stick into the egg will go somewhere, we don't know where, we cannot direct it. In number three you cannot direct it either, but you can take the embryonic stem cells and select the ones that have the desired genetic modification.

And just before people think that we are trying to make any sort of weird things here, these genetically modified ES transgenics mice are used to identify the functions of genes. For example if you have identified a mutation in a protein that might be responsible for cystic fibrosis in a clinical sample, we can go back into a mouse and say, 'I would like to generate a mouse that has exactly that same mutation as I found in the clinic sample'. You can make this animal to your design specifications. If that mouse develops cystic fibrosis then you have a fairly good hint on which genes are involved in the disease in the human and potentially find medication for future treatment or cure.

Thank you.

Towards the national regulation of human cloning and embryo research by Dr Sandra M Webb

Introduction

At present there are three parallel policy processes in train that will continue the current prohibition of human cloning and review the way in which human embryo research is carried out in Western Australia (WA). All are totally and necessarily independent, but ultimately they will be inter-woven.

These three processes are-

- the currently fast moving development of consistent national legislation to ban human cloning and regulate some human embryo research;
- the current review of the National Health and Medical Research Council's (NHMRC's) 1996 *Ethical guidelines on assisted reproductive technology*; and
- the current review of WA's *Human Reproductive Technology Act 1991* (HRT Act)

All propose to continue the prohibition of human cloning, and all appear likely to be consistent in prohibiting the creation of an embryo other than for implantation into the body of a woman, but allowing some use of 'excess ART embryos' for research.

Before I start to give you some of the chronology of the overlapping developments towards consistent legislation dealing with cloning and embryo research around Australia, I would like to clarify some of the terms I will be using...Being clear about these terms is vital for discussing the policies and understanding the issues and current politics of cloning in Australia!

The use of cloning technology intending to create people who are copies of people is often referred to as human cloning, or the cloning of a whole human being, or it may be called 'reproductive cloning'.

It seems that the whole world is united in agreement that reproductive cloning should be prohibited, although the reasons why may differ.

The use of cloning technology (such as somatic cell nuclear transfer SCNT) to develop an embryo with the intention that the embryo will not be allowed to develop but will be used to derive embryonic stem cells, perhaps in the future for patient-specific tissues for transplantation is usually called 'therapeutic cloning'.

Stem cells may be derived from adults, the umbilical cords of newborn babies, or embryos. Embryonic stem cells are the ethically and politically sensitive stem cells – and the very significant distinction must be made as to whether the embryos from which they were derived were created solely for that purpose, or were 'excess ART embryos' donated by people who had completed their IVF treatment and chose not to donate them to another couple or allow them simply to die.

There are wide differences of opinion in the community with regard to the derivation of stem cells from embryos, and in particular about the creation of embryos specifically for 'therapeutic cloning'.

Early history of concern about cloning and genetic modification of embryos

Although the media frenzy and subsequent worldwide political flurry to ban human cloning all seemed to start with the birth of Dolly in early 1997, there was an awareness of this well before that time in the many eminent bodies that were set up. Bodies such as the Warnock Committee in the UK and in almost every state of Australia in the early 1980's discussed for the first time ethical, legal and social issues surrounding the then new reproductive technologies...this of course followed rather than preceded the birth of the world's first IVF baby in the UK in 1978, and Australia's first in Victoria in 1980.

Then not only was Australia at the forefront in the IVF world, it led the way in legislating in this area, with the first legislation in the world to deal specifically with IVF and related matters being passed in Victoria in 1984. By 1988 SA's *Reproductive Technology Act* and by 1991 WA's HRT Act were also in place, regulating human embryo research, banning human reproductive cloning and the genetic modification of embryos and a variety of other **then** science-fiction- sounding procedures.

In addition the NHMRC's 1996 *Ethical guidelines on assisted reproductive technology* also prohibit experimentation with the intent to clone a human individual, and again, various other manipulations of embryos and gametes. These guidelines became then, and are still, central to standards set for the voluntary accreditation of Australian IVF clinics in the states without specific legislation, that is in states other than Victoria, SA and WA, by the Reproductive Technology Accreditation Committee.

All these prohibitions are variously flawed, but the intent is clear...that human reproductive cloning is unacceptable and prohibited, that a variety of other procedures (such as the germ line genetic modification of humans) are unacceptable, and that human embryo research should be closely regulated.

Development of consistent national regulation

Following the birth of Dolly however, the Commonwealth Minister for Health requested advice from the Australian Health Ethics Committee (AHEC) which, in December 1998, presented to him its Report on *Scientific, ethical and regulatory considerations relevant to the cloning of human beings*...This report recognised existing bans on reproductive cloning in SA, WA and Victoria and recommended all States and Territories introduce such a ban. It also recommended that the Minister should promote public debate on therapeutic cloning, which research had advanced rapidly during the preparation of the report.

In August 1999, the Minister for Health commenced the process of consultation with all states and territories about the development of uniform national standards for ART and a ban on the cloning of human beings.

Then in September 1999 the Minister for Health also referred the AHEC report to the House of Representatives Standing Committee on Legal and Constitutional Affairs (the Andrews Committee) for review.

And in WA, by December 1999, the Select Committee that reviewed the HRT Act tabled its report in Parliament, recommending continuation of the ban on human cloning but also amending the WA Act to bring embryo research provisions in line with the NHMRC

guidelines. This would allow some research on excess ART embryos (now prohibited in practice under the HRT Act). It also recommended careful consideration being given to allowing beneficial developments into therapeutic cloning. The current ban in the HRT Act on the creation of embryos for research should remain however, making developments in therapeutic cloning problematic.

In the meantime the *Gene Technology Act 2000* (GT Act) passed in December 2000 and, although that Act was explicitly not meant to deal with this, as an apparently last minute addition section 192B of that Act does contain a ban on the cloning of a 'whole human being'. The GT Act would however regulate the development of transgenic animals...including those with human genes, such as the famous insulin producing cow.

In July 2001 the Council of Australian Governments (COAG) committed itself to achieving nationally consistent provisions in legislation to ban human cloning, and also asked jurisdictions to work towards nationally consistent approaches to regulate ART and related emerging technologies. The aim was that a nationally consistent approach should be in place by June 2002.

Officials from all jurisdictions began work immediately, and a report on technical matters produced by this group was considered by COAG on 5 April 2002, along with the Report of the Andrews Committee, which had finally been released in August 2001.

At this time COAG agreed that the Commonwealth, States and Territories would introduce nationally consistent legislation to ban human cloning and other unacceptable practices. COAG also agreed that research be allowed on excess ART embryos under a strict regulatory regime, to be administered by the NHMRC as national regulatory and licensing body.

It was stated that the agreement had been reached to be so that Australia could 'remain at the forefront of research that may lead to medical breakthroughs in the treatment of disease', and the NHMRC was also asked to report within 12 months on the adequacy of supply of embryos for research. However, there was also concern that embryos may be created specifically for research. An Ethics Committee was to be established to develop protocols to preclude this and, initially at least, no embryos created after the date of the agreement were to be used for such research.

Draft Commonwealth legislation to give effect to the COAG agreement is now being finalised in a period of intense consultation between the Commonwealth, the States and Territories, with advice from the NHMRC and the AHEC and other experts in ART, medical research, consumer issues, ethics and law.

It is intended that the Bill to give effect to this will be introduced into Parliament by the end of June this year.

What is the scope of the proposed legislation?

In line with the COAG agreement, there are three main elements to the draft Bill:

- a ban on human cloning;
- a ban on certain other unacceptable practices relating to reproductive technologies; and
- the establishment of a system of regulatory oversight for use of excess ART embryos that have been donated to research.

Prohibited practices

A range of practices associated with reproductive technology, including human cloning, are to be prohibited. The prohibitions have been drafted to focus on intent rather than relying on specific procedures that may be superseded as the technology advances.

As a result the prohibitions are technically broader in scope than corresponding prohibitions in WA's HRT Act, but cover currently available technologies that are already prohibited in WA. The HRT Act will need to be amended to reflect the wording of the prohibitions in this section.

The most fundamental of the prohibitions is on the creation or development of an embryo outside the body of a woman for a purpose other than to achieve pregnancy in a particular woman.

In summary the practices proposed to be prohibited relate to the creation, implantation in a woman and/or importation of:

- a human clone
 - embryos that have been created other than by the fertilisation of a human egg by human sperm;
 - embryos that contain genetic material from more than 2 persons;
 - embryos that have been maintained outside the body of a woman for a period of more than 14 days;
 - embryos that have been created or developed using precursor cells from an embryo or human fetus;
 - embryos that contain a human cell whose genome has been altered in such a way that the alteration is heritable;
 - embryos that are the result of a viable embryo having been removed from the body of a female person; and
 - hybrid and chimaeric embryos that have been created by combining human cells and animal cells.

Regulation of research involving embryos

Essentially the regulatory framework will require that:

- there is approval from an human research ethics committee constituted and operating in compliance with NHMRC Guidelines;
- the research is in accordance with the NHMRC Guidelines;
- for all uses of an excess ART embryo from an ART program, including research or therapy that damages or destroys the embryo, but not including storage or removal from

storage, a licence has been issued by the Licensing Committee. This Committee is to be established as a committee of the NHMRC.

- The Licensing Committee's approval is on the basis that various criteria, agreed by COAG, have been met, including that-
 - there is a likelihood of significant advance in knowledge or improvement in technologies for treatment as a result of the proposed procedure;
 - the significant advance in knowledge or improvement in technologies could not reasonably be achieved by other means;
 - the procedure involves a restricted number of embryos and a separate account of the use of each embryo is provided to the ethics committee and the national licensing body (NHMRC);
 - all tissue and gamete providers involved and their partners or domestic partners, if any, have consented to research for each embryo used including by specifying restrictions, if they wish, on the research uses of such embryos; and
 - the embryo had been created prior to 5 April 2002.
- The Bill also provides for offences for the use of an ART embryo that is not a use related to achieving a pregnancy in a particular woman, storage or removal from storage of an embryo, or a use licensed by the Licensing Committee.

In states such as NSW where such research has been permitted as there is currently no legislation dealing with ART, the new regime may impose novel restrictions on embryo research. For the states with current legislation, such as Western Australia however, the model proposed in the Bill does not represent an expansion of the scope to regulation of embryo use, but may in practice allow more embryo research to go ahead.

All research involving embryos in WA is currently subject to approval of the Reproductive Technology Council (RT Council) established under the HRT Act. The proposed regulatory model would mean that all uses of excess ART embryos would be licensed by the Licensing Committee, rather than the RT Council.

The RT Council would continue to regulate and license ART clinical practice, including storage of embryos.

State Commonwealth interactions

As the Commonwealth does not have the power to legislate comprehensively in relation to all the matters in the Bill it is intended that States and Territories will introduce corresponding laws.

The precise way in which the Commonwealth and State laws will interact is under consideration, but it is not intended that there be a dual licensing system and it is also considered desirable that there be a consistent approach to all Western Australians, that is not dependent on whether they fall within the Commonwealth constitutional powers or are subject only to State legislation.

Transitional arrangements also require further consideration.

Sunset clause and review provisions

The Bill will include a provision to repeal the requirement in relation to embryos created prior to 5 April 2002 and a provision to require that there be a review of the operation of the Act within 3 years. This is in accordance with the COAG agreement.

Consequential amendment

Provisions included in the *Gene Technology Act 2000 (Cth)* banning cloning are to be repealed as they are no longer required

Conclusion

The exact form of the legislation is still being finalised in wide consultation, and of course agreement has to be obtained from all Governments before this phase of the process is complete and the Bill makes its way to Parliament.

What happens to it subsequently in Parliament, and as each State and Territory develops its own complementary legislation and this in turn progresses through each Parliament remains to be seen, as politicians in many jurisdictions are being given a conscience vote.

The development of legislation in this fast moving area of science and medicine is in many ways as important and exciting as the developments in the science and the medicine, and similarly, many questions remain. Will we achieve consistent national legislation? If so, when, and will it be reasonable effective?

And finally, will the apparent great potential of the therapies that are to be progressed in part by the passage of this legislation, come to fruition?

Cloning Ethics: Evolution Of Moral Philosophy **by Philip Matthews**

When people talk about ethics they assume that something is basic about our understanding of right and wrong. Debates about complex ethical issues are often based on conclusions drawn from what is thought to be self-evident. All of us are familiar with the adversarial history of confrontation over conclusions reached on bioethical issues. The abortion debate, for instance, remains fixed, confrontational, and sometimes violent. The decision of a Queensland woman in the last week to end her life will again promote discussion about the “ethics” of euthanasia. Choose any complex ethical issue and you will find people who think they have the solution and that everyone else is wrong. What we need to appreciate about ethics in general and bioethics in particular is that any idea that “right” conclusions can be drawn from self-evident premises should be discarded. We can no longer take for granted those truths we used to take for granted. There are no “basic rights” or “fundamental principles” or “reason-based solutions” that are self-evident to all of us or even the majority of us. The impossibility of establishing a foundational starting point, that is self-evident, is enough to suggest that it is not going to be possible to solve many complex ethical issues. The evolution of moral philosophy in general and bioethics in particular is therefore going backwards to a time when ethics was less concerned with truth concerning specific issues.

What I propose this afternoon is a suggestion that we need to recognize the limitations of being human and, as a result, take a more pragmatic view of how we should live. The solutions to Socrates great question “How should we live?” is found in what philosophers and theologians have in common about the way ethics should be practiced rather than in their numerous interpretations of bioethical truth.

Using the genomic revolution as a case study we will see that ethical pragmatism (sometimes called practice-centred ethics) is far more useful than debates about truth for which there is no resolution. However, before I explore the benefits of pragmatism in ethics it is worth taking a look at two of the most common criticisms of recent scientific development in genetics.

Playing God and Interfering with Nature

A common criticism against the genomic revolution is that humans are starting to play God when they engage in reproductive techniques, or genetic therapy or cloning. However, when we examine what it means to play God we discover that we are not starting to play God, we always have been. There is nothing new in the way humans have approached issues of gene-based medicine. A millennia ago humans gave up the idea that God controls human health. Even people who take it for granted that God exists have adopted an evidence-based approach to the way God interacts in human affairs. The use of antibiotics to combat infection is now routine. Viral diseases that in the past killed millions of our ancestors are, at least for the moment, held in check by vaccination programs. Numerous examples like this can be found in the practice of medicine. We play God not because we want to but because we have no choice. God is not available to us in any sense that is workable in modern medicine. This realization is not restricted to medicine. Danish theologian Soren Kierkegaard recognized in the 19th Century that belief in God requires a leap of faith because of the distance that separates us from God. He describes the distance between humankind and God as both “infinite” and “qualitative.” Another 20th century theologian Bette Midler put it this way in one of her songs, “God is watching us, but from a distance.” This awareness has nothing to do with whether God exists or not. It is simply a recognition that human analytic tools, sense-experience and reason, have not helped us to discover God in any way that is

transparent for all of us. So we end up playing God not because we want to but because we must.

A second criticism of genomics is that humans are interfering with nature. Once again this fear is presented as if all of a sudden scientists are doing something different. Humans have been interfering with nature since we began using fire, or building houses, or using contraceptives, or helping sick people to live, or splitting the atom, or changing the shape of rivers, or altering the global temperature of the planet we call home. We have always interfered, sometimes positively sometimes negatively, because it is an inevitable by-product of our ability to reason. We are a curious species and the desire to interfere is sometimes referred to as a technical imperative. We are compelled both to invent and to utilize. Part of the fear associated with human interference is because we tend to over-emphasize human interference and under-emphasize the role of Mother Nature. The use of the term “Mother Nature” promotes romanticized images a compassionate and caring force that nurtures her biological inhabitants and always keeps things in balance. However, a cursory understanding of the biological history of life on earth shows that far from being a compassionate care-giver Mother Nature has at various times been a vicious bitch. Several mass extinctions diminished the biotic life on this planet with some estimates being as high as 90% of all life being extinguished. A well-known extinction event, though certainly not the only one, is the impact of comets or bits or comets. In 1994 when Comet Shoemaker-Levy 9 slammed into Jupiter we witnessed what has previously happened on earth several times, and what will happen again in the future. In astronomical terms it was a near-death experience and provides good evidence for not letting Mother Nature have her way. Given that there is nothing unnatural about comets slamming into planets there would be very few people who would object to whatever interfering with nature was possible if it ever becomes necessary. We could also talk about the necessity to interfere with naturally occurring bacteria because the antibiotic protection we took for granted in previous decades is no longer as effective as it used to be. Or we could talk about the natural occurrence of transgenic viruses that might make DNA based research a necessity not a luxury in the future. So we play God and we interfere with nature because sometimes we have to.

The problem of reason, universalization and practice-centred ethics

So what can we say about ethics and the new genomic revolution. I suggest three central ideas offer a way forward. Firstly, we need to appreciate that our ability to reason has not turned out to be as useful in solving ethical dilemmas as some philosophers advocated. Secondly, we need to be prepared to universalize any policy we do come up with so that the benefits are human not local. Thirdly, we need to appreciate the role professional practice plays in determining ethical behavior.

Because God is not as transparent as historically understood, philosophers initially attempted to find some way of doing ethics based on reason. It is still quite common to read bioethics texts that appeal to reason as a vehicle for “solving” moral problems. The two most popular bioethics text-books, Beauchamp and Childress’ four principle-ethics and Peter Singer’s preference utilitarian ethics, both attempt to convince the reader that reason works in the way they think it does.¹ This is a misleading caricature of what is possible in philosophy. Philosophers who appeal to reason turn out to be just as dependent on concepts they take for granted as the theologians they were trying to replace. Preference utilitarian philosophers

¹ Beauchamp, T. L. and J. Childress (1994). *Principles of Biomedical Ethics*. Oxford, Oxford University Press (A simplified version with Australian content is used by some health professionals in Australia). Singer, P. (1993). *Practical Ethics*. Cambridge, Cambridge University Press.

disagree with each other on how to calculate preferences and people who are taught to juggle the four principles don't form uniform opinions as to which principle trumps another in any given situation. So in short, REASON should be seen less as a means to solve ethical issues and more as a pragmatic tool that aids decision-making. The failure of reason to supply solutions has led to the rediscovery of a more ancient idea about human behavior. A premise that both moral philosophy and moral theology share turns out to be far more useful for applied ethics than any of the respective conclusions drawn. All of the major religious traditions and most of the major ethical theories share a common theme when it comes to understanding the starting point of ethics. Namely, that ethics begins when we are prepared to go beyond self-interest.

I note that student participants in this seminar are examining social justice from a global perspective. Global justice is an excellent place to begin our examination on the ethical implications of gene technology because it incorporates an idea that is as old as any idea about ethics can be. Whatever we say about ethics we should be prepared to take a global perspective. This type of thinking is known as the principle of universalisation (sometimes called maximization or generalization). Peter Singer is right when he says ethics begins with this principle because by using it we go from self-interest to the interest of others. Universalisation is, however, a modern word for an ancient idea. Colloquially called the "golden rule" the idea that ethical decision-making concerns thinking about others as you would yourself is older than the printed word by a several thousand years. It seems to have evolved in human communities all over the world with no apparent connection of language or culture. Catholic moral philosophers argue that this is because humans are tapping into "natural law," a legacy of God's creative will, even when they don't realize it. Singer disagrees, he sees the cooperative behavior exemplified in the golden rule, as a by-product of evolutionary altruism. In order for humans to survive we developed altruistic genes of cooperation. How humans inherited this altruistic tendency is not as significant for bioethics as the practice of it. Even amongst the most violent human societies altruistic behavior dominates. Put simply, we cooperate really well. So whether we call the starting point of ethical deliberation natural law or ethical altruism is not that important for applied ethics. What matters is far more practical. Any ethical policy that does not take seriously the principle of universalisation is a policy not worth having.

Medicare provides a good example of health policy that attempts to incorporate universalisation into the practice of health delivery. Whatever money is allocated to health-care is maximized to achieve the greatest benefit. Any comparison between universal health systems and other types of health delivery show that the health dollar goes much farther in utilitarian health care systems than in other types. The weakness of utilitarian health is that it ignores the health status of marginalized people, Australian indigenous health being a case in point. Utilitarian health care involves setting limits on what can be paid for and what can't. Most cosmetic surgery is not covered and major costs associated with reproductive medicine are not covered by Medicare. If you desire to have Pamela Anderson type boobs then you will have to pay for them yourself and even some treatment options that do benefit some patients will not be covered simply because they are too cost prohibitive.

Philosophers employed by the human genome project who recognized early that benefits derived from mapping the human genome should be available to all human beings provides another example of universalisation. The Human Genome Project (HGP) is one of the truly great international collaborative projects and the allocation of significant funding to examine ethical, legal and social issues is a marked improvement on the way humans dealt with other technical imperatives like those associated with nuclear technology.

My third and final suggestion as we face an interesting genomic future is that we take more seriously the role of practice-centred ethics. Once again this is an ancient idea that has modern application. Practice-centred ethics defines a “good act” as one that conforms to a traditional definition of what constitutes “good practice.” This type of moral pragmatism can therefore argue that moral practices (internal goods) provide guidance for rational actions without needing to claim that such actions are true or right. The focus of practice-centred ethics is that the actions of a moral agent should be viewed from an historical or tradition-guided perspective. The individual moral agent is therefore not an impartial decision-maker who decides right from wrong but a partial decision-maker whose decision-making is considered “good” by others if it conforms with historic practices in similar situations. So a good doctor is one who conforms to good practices and a bad doctor is one who does not (malpractice). This type of inquiry provides a methodology for evaluating the actions of an individual doctor or scientist and also a means for developing protocols of action that incorporate internal goods.

Human reproductive cloning, for example, is quite clearly an unsafe clinical practice and given the recent problems associated with medical indemnity an unwise financial risk as well. It cannot constitute an internal good and should be prohibited because it remains, at this stage, bad practice medicine. The issue of stem cells is more complicated because of the wide range of applications and because of the disagreement about access to embryonic stem cells. Even here, however, practice-centred ethics offers some guidance. Given that IVF techniques already involve established practices that lead to surplus embryos being destroyed the use of stem cells from those embryos does nothing more. People who object to stem cell harvesting will also object to embryo freezing and probably reproductive technology in general. A person who accepts the premise that an embryo is a potential person will necessarily find most issues associated with reproductive technology problematic. There is no surprise here because the same premise underlies many of the difficulties that some have with other ethical issues like abortion or euthanasia. Thus we find ourselves returning to disputes about complex issues that are never really solved because disparate starting points are used to justify disparate conclusions. Sometimes a pragmatic legal resolution is found as in the case of abortion. A line is drawn somewhere near the second trimester that allows access to safe abortion. A similar (14 day) pragmatic limitation is being suggested for embryonic experimentation. Obviously this will not please everyone but this is not the main point behind ethics. As long as genetic protocols attempt to universalize benefits and maintain professional practice standards this is about as good as ethics gets.

Both the NHMRC and the RTC protocols are typical of this type of pragmatism. Human research, animal research, reproductive technology and human cloning are all governed by guidelines designed to reflect professional practice standards. New techniques are considered innovative until evidence of the benefits associated with the practice become established. Obviously, not everyone likes being constrained by this type of public policy and some researchers object to the slowness of policy development but that is too bad for them. We are entering one of the most exciting periods of human history when major revolutions in health care are predicted. It takes time to think ethically because that means thinking about how we can live to benefit all of humankind. If we have to slow down the pace of genetic therapy, open up a bottle of wine and spend time slow-thinking in the Socratic style then so be it. The history of the technical imperative is warning enough for me to suggest that we need to spend time thinking about what we should do (ethics) before we get overexcited about what we can do (technique).