Submission to the Community Affairs Committee of the Australian Senate

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1. PREAMBLE

Somatic Cell Nuclear Transfer (SCNT, or "therapeutic cloning") is the term used to describe preparation of embryonic stem cells by taking the nucleus from an adult cell (e.g. skin, muscle, bone marrow etc), and inserting it into an ovum from which the nucleus has been removed. When it is successful, the adult nucleus is "reprogrammed" to behave as an ES cell, and an embryo results, albeit with a very low success rate. Use of this for "reproductive cloning" has been universally rejected, but "therapeutic cloning" aims to produce patient-specific embryonic stem cell lines, by using donor eggs to create patient-specific ES cells.

2. THERAPEUTIC CLONING AS SUBJECT MATTER IN DRAFT BILLS

There are many things that need to be achieved before the manufacture of embryos for the purpose of therapeutic cloning could be argued with any conviction. The following is a short list of essential requirements, all of which could be addressed under existing legislation.

(i) Proof in animal experiments of the concept that therapeutic cloning is effective. Any move towards the deliberate manufacture of human embryos for research purposes constitutes a major elevation in the ethical barrier, and the standard of proof required for a positive outcome of that research becomes all the higher. In the event, the Lockhart Committee's decision was based largely on the benefits that they think will accrue from ES cell research to sufferers of a number of chronic, serious diseases. There is no evidence from animal experimentation, in Australia or elsewhere, that animal ES cells can be used as treatment for any disease in a manner that is effective, and is safe in the long term. Of course there have been no trials of human ES cells in man. Animal models of several of the relevant diseases exist, which provide this as an open and obvious way to search for evidence to support the credibility of therapeutic cloning. There could be no possible purpose in therapeutic cloning unless it is established that ES cell therapy can be applied effectively and with long term safety.

The Lockhart Committee was briefed to review the advances in ES cells science since 2002, and in their report no advances were noted that provided evidence pointing to the need for therapeutic cloning by SCNT. This might be noted especially in Table 5, page 44, summarising preclinical and clinical data with ES and adult stem cells. No proof of concept of the success of ES cell transplantation has to the present time been achieved as an effective, prolonged, safe treatment of any disease in experimental animals. This is not a matter of opinion, but is very clear from the scientific literature. Among other aspects of therapeutic cloning, it is discussed in detail in a recent review by Cobbe (1). The conclusion of this section

of Lockhart (bottom page 42) was *.."since 2002, most of these trials have involved adult stem cells because, at this stage ES cell research has not reached the stage needed to start clinical trials*". In her speech to the Senate on the draft bill, Senator Stott Despoja noted that her bill "*enshrines the scientific recommendations of the Lockhart review*", and stated that it is "*misleading*" to argue that advances in ES cell research since 2002 are insufficient (pages 1 & 2 of speech). Indeed, one of the major deficiencies of the Lockhart Committee's report was that it failed to acknowledge the indisputable fact that necessary preclinical advances have not taken place. Relevant to this issue also is the fact that the Committee has before it a list of 8 references tabled before the Senate by Senator Patterson on Thursday September 13. This is a list of 7 scientific papers and a PhD student opinion, providing Senators with material that perhaps was intended to help them in their consideration of this issue. I have reviewed that material in detail from the scientific point of view, and have attached as an ***Appendix*** my analysis, which points out that none of these papers provides evidence supporting a need for SCNT/therapeutic cloning, some are quite irrelevant, and some argue against the need.

Finally on this point, proceeding through this major ethical barrier undermines defining principles of ethical behaviour in research and medicine, under which we all have to work. Both the Nuremberg code and the Declaration of Helsinki stipulate that any allowed experimentation involving human subjects should be capable of being supported by the relevant research literature and preceded by corresponding humane work in animals if necessary. The Declaration of Helsinki states (2) that *"research involving human subjects includes research on identifiable human material*" and "*medical research involving human subjects must conform to generally accepted scientific principles, be based on a thorough knowledge of the scientific literature, other relevant sources of information, and on adequate laboratory and, where appropriate, animal experimentation*".

(ii) Whatever the origin of ES cells, animal or human, whenever they are transplanted into an animal, they have up to a 25% incidence of growth of a particular type of cancer, a teratoma. No substantial progress has been made towards resolving this problem of cancer development with ES cells. This problem is sufficient by itself to exclude any possibility of using ES cells in therapy for human disease, even if there were strong indications of likely efficacy on other grounds. This problem is central to the issue of application of ES cells in human therapy, it is obviously of profound importance, but was glossed over in Lockhart and essentially ignored in the draft bills.

(iii) The methods for the growth and differentiation of human ES cells need to be refined, so that they are more efficient, and the cells can be more effectively maintained in their new, specialised state. This problem of "phenotypic instability" needs to be overcome, and it is clear that this does not require work with cells derived by SCNT. It can be addressed with animal ES cells as well as with human ES cells under existing legislation.

A major issue with any projected use of ES cells is their genetic instability also. This is also a matter which should have been given attention in the scientific considerations of the Lockhart Committee. The experience of cloning animals has

revealed that fetal death rate is extremely high, and even when animals have been born, the abnormality rate approaches 100%. In fact some years ago Wilmut (cloner of the sheep, Dolly) and Jaenisch doubted whether any normal mammals had ever been born as a result of SCNT pregnancies (3). Cloning by SCNT introduces many abnormalities in gene expression in both embryos and in tissues in later development (4). Wilmut has repeatedly pointed out the abnormalities in gene expression that are seen in cloned animals, and conceded that there is no way of predicting abnormalities in cloned embryos and in their gene expression (5,6,7). This even extends to data showing that cloned offspring can be more variable in gene expression than siblings (8). It is difficult to accept an argument that despite overwhelming evidence for altered gene expression in cloned embryos that are permitted and are able to proceed in development, nevertheless ES cells derived from these same embryos are normal. There is an onus of proof that lies with those who maintain this position. If genetic variation among clonal ES cell lines in any way resembles the situation in cloned embryos, this would clearly invalidate the use of ES cells to study subtle effects on drug metabolism that could be applied to therapeutics (5).

(iv) In the absence of any credible proposal that human ES cells could be used in any clinical trial with hope of a positive outcome, it is argued that therapeutic cloning could be used to generate ES cells by taking a nucleus from patients with certain diseases, transferring them to enucleated eggs, and developing ES cell lines. This proposal was accepted by the Lockhart Committee. This is open-ended, "blue sky" research which would be confronted by very many obstacles, but could readily be tested by using appropriate animal experimentation. First, the genetic instability discussed in the last paragraph is obviously relevant to any proposal to use ES cells to study disease, and its persistence would completely invalidate such an approach. Second, any research on embryos generated in this way for the study of disease would certainly require embryo development beyond 14 days, and it is difficult to imagine how anything meaningful could be done without actually implanting such an embryo into a uterus, such as can be done in an experimental animal. Third, if this approach is used with diseases of late onset and variable development among different individuals, how could results be interpreted - what would they be compared with? All of these are matters that should have been considered by the Lockhart Committee if they had set about a thorough scientific analysis.

(v) To the present time no-one has successfully developed human ES cell lines through therapeutic cloning. The Lockhart Committee regarded as very influential the claim by a South Korean research group which was shown to be fraudulent at about the time (December, 2005) that the Committee's report was submitted. It is a pity that the Lockhart Committee, in light of this dramatic development, did not submit an amendment to its Report. In one of those countries where SCNT is permitted, someone will most likely succeed in doing so, by modifying by trial and error the technical methods that have worked for other species. No great science is required for this, although it might be imagined that IVF technologists would see it as a goal to be aspired to.

Much higher quality science is directed at "reprogramming" adult cells, and this year has seen the single most significant advance for many years in this relevant

area of research. This recently published paper (9) reported that it is possible to "reprogram" an adult cell by providing it with a set of genes - 4 in number – and finish with cells that can behave virtually as ES cells. In a Commentary on the paper in the same issue of *Cell* by independent scientists from the Harvard Stem Cell Institute (10), they raised a number of points of detail, but concluded that this work *"represents a significant step toward a rational approach for generating patient-specific ES cell lines that could be used either as a source of autologous tissue for transplantation or for modelling different diseases*. *This method is encumbered by neither the logistical constraints nor the societal concerns presented by somatic cell nuclear transfer".* This work was carried out in mouse cells, it obviously needs to be confirmed, and there will be much more to be done to refine the method and establish whether the reprogramming is complete, and fully reproduces the ES cell. What can be said though, is that it is an exciting "proof of concept" that a pluripotent cell could be generated from an adult cell without cloning. It remains to be translated to human cells, and the approaches used for the mouse work will be invaluable in informing that work.

2. THE LOCKHART REPORT

The Lockhart Committee's Report is subject to serious criticism on a number of grounds. A number of those criticisms are expressed in the above sections of this submission. There are two other main points I wish to make.

(i) The unsatisfactoery nature of the Lockhart Committee's review of community attitudes. The Committee confessed to having great difficulty in assessing community standards. Their difficulty, as well as their unsatisfactory management of this difficulty, are discussed in depth by Professor Frank Brennan in his 2006 Thomas More Lecture (10). Rather than considering any overall change to community standards the Committee came to the view that there is no single Australian community, but "many communities' and that *"therefore any scientific exploration should be permitted, provided there were not strong arguments against it from all groups, including those who discounted the moral significance of the life of the human embryo"*.

A serious criticism of the Lockhart Committee is that they refer to a 2006 Morgan Poll as though it were the only community survey available. The Morgan Poll published on 21 June 2006 told respondents: "*Scientists can now make embryonic stem cells for medical research by merging an unfertilised egg with a skin cell. In this case, no fertilisation takes place and there is no merger of the egg and sperm"*
Respondents were then asked:
"Knowing this, do you favour or oppose embryonic stem cell research?"
Eighty percent responded that they favoured embryonic stem cell research.

The information given to respondents is false. No scientist has yet made a human embryonic stem cell. It also gives an entirely misleading description of cloning. Most lay people would not understand from this description that this process would still form a living human embryo which is then destroyed by the extraction of stem cells.

The Lockhart Review did not refer to either of the following surveys:

Earlier research conducted by two researchers from Swinburne University was published in 2004 (11) and was not referred to in the Lockhart Review. It had found that:
"Almost 30% of the sample was not at all comfortable with using cloned embryos, and the majority of the sample (63.4%) scored under the mid point (i.e. 5). Given this, and that the mean score for cloning was well below five and the modal response was zero, there was good evidence to conclude that the Australian public do not feel comfortable with scientists cloning human embryos for research purposes".

Recent research into public attitudes to human cloning was carried out by Sexton Marketing Group for the Southern Cross Bioethics Institute in January 2006. It found that only 29% of respondents support the cloning of human embryos as a source of stem cells while 51% opposed the cloning of human embryos for stem cells. This increased to 55% when it was clarified with respondents that in these embryos are destroyed in the process of obtaining stem cells from them. (43% of respondents were not previously aware of this fact.)

(ii) A further point of criticism of the Lockhart Review is that there is documented evidence that at least three of its members had firmly held views of the outcome before the Review Committee was formed**.**

Assoc Professor Ian Kerridge was quoted in ABC Online, June 12, 2001 (Anna Salleh), from a symposium sponsored by Embryonic Stem Cell International, a body that facilitates and finances ES cell research
" *Therapeutic cloning has massive potential. Animal work has shown promising insights into how it can be used to repair tissues that can't normally repair themselves, or for which there is a shortage. There are strong moral imperatives to do stem cell and cloning research*".

Professor Peter Schofield, when President-elect of the Australian Society for Medical research, wrote in October 9, 2001, to Ms Jillian Skinner, Shadow Minister, NSW. The letter was written without consulting the ASMR membership, and this was the subject of complaint by some members:
"Parts 4 and 5 of the (Human Reproductive Cloning and Trans-Species Fertilisation) Bill (NSW) will allow research on human stem cells, including embryonic stem cells and their use in cloning. This is to be commended as it provides a regulatory basis by which exciting and significant new developments in medical research can be progressed, while providing clarity and simplicity about lines of investigation that will not be permitted because of overwhelming ethical concerns".

Professor Loane Skene was long known to be supportive of human cloning for biomedical research. In her own submission to the House of Representatives Standing Committee on Legal and Constitutional Affairs in March 2000, she had written: "*Even if one regards reproductive cloning as contravening human dignity,*

surely the same is not true of therapeutic cloning. A person's 'dignity' is best respected by trying to save the person's health and life. Even if embryonic cells are used, I do not believe that any 'dignity' interest of the embryo outweighs the interests of a dying or diseased person." (12)

3. DRAFT BILLS.

Each of the draft bills put forward by Senators Patterson and Stott Despoja recommends approval of both SCNT for research purposes, and the manufacture of hybrid embryos, either by fertilisation of animal eggs with human sperm or by the transfer of human adult cell nuclei to enucleated animal eggs.

(i) Prohibition of human cloning for reproduction and the regulation of human embryo research amendment bill 2006. (*Senator Patterson*)

The title of this bill is entirely misleading. More than anything else, this bill is "permission for human cloning for experimental purposes". It is disingenuous to entitle it in the proposed format.

(ii) Generation of hybrid embryos.

Each of the draft bills proposes approval of the Lockhart recommendation that permission be given under licence to prepare animal/human hybrid embryos, either by fertilising animal ova with human sperm, or by transferring human adult cell nuclei to animal eggs. The only stated purpose of this, if it is in pursuit of the Lockhart proposal, is to provide the opportunity for laboratory training and the testing of sperm quality. Since each of these processes produces an embryo which is an animal/human hybrid, the question must be asked - is the purpose sufficient to justify the means?

On the other hand, if the purpose is also to develop ES cell lines from hybrid embryos generated by transfer of human adult cell nuclei to animal eggs, this approach is fatally flawed, in that such lines would inevitably carry with them the contribution to genetic material provided by mitochondrial DNA from the cytoplasmic compartment of the animal eggs. Reading of Senator Patterson's draft bill of page 9, "23B Offence – creating a hybrid embryo" suggests that that this could indeed be what is proposed. The Note at the end of this paragraph states :
"A licence to create or develop a hybrid embryo can only be issued under Section 21 of the Research Involving Human Embryos Act 2002.
(a) *for the purpose of testing human sperm quality in an accredited ART centre – up to, but not including, the first mitotic division.*
(b) *In the case of a hybrid embryo created by introducing the nucleus of a human cell into an animal egg – for not longer than 14 days*.

In proposing in this draft bill that licences can be issued for development of hybrid embryos by these two means - why is 14 days' development allowed for the human cells transferred to animal eggs, but only about 20 hours for human sperm fertilisation of human eggs? Perhaps this is an error in drafting the bill. Alternatively, it is indeed intentional, and indicates that scientists will be permitted to attempt to develop ES cell lines from these hybrid embryos, despite the

contribution that will be made by the animal DNA derived from mitochondria. Note also under *Schedule 1, Section 6(8):*
A reference in this Act to a human embryo does not include a reference to:
(a) *a hybrid embryo*
(b) *a human embryonic stem cell line*.
It is made clear in several places that it will be an offence to place a human embryo clone into the body of a woman, or to place an animal embryo into the body of a woman. No mention is made of restrictions on implanting a hybrid embryo into an animal. If this is not specifically prohibited, even the 14-day rule would allow a human-mouse hybrid embryo, for example, to be implanted in a mouse uterus, which if successful would provide for more than half the total embryonic/fetal development of the mouse.

These matters require much more explanation and clarification. No explanation is provided in the Explanatory Memorandum circulated by authority of Senator Patterson.

Senator Stott Despoja in her draft bill makes the same provision for hybrid embryo development to be licensed and in the case of human adult cell to animal egg embryos, to be used up to 14 days. No mention is made of the time allowed for human sperm fertilisation of animal eggs, but why is 14 days' development necessary if the procedure is being permitted only for technical laboratory practice? If that is so, simply allowing development to the first mitosis is all that is necessary. Again, no information on this point is contained in Senator Stott Despoja's Explanatory Memorandum.

(iii) I have commented earlier on the superficial nature of the Lockhart Committee's review of embryonic stem cell research progress since 2002. Senator Stott Despoja, in her explanatory memorandum, summarises the Lockhart discussion, adding her own quotes from Time magazine and unpublished plans for human ES trials of a US Biotech company in support. She argues that Parliament must "foster the dazzling promise of new technologies such as SCNT", in face of the fact that no-one has provided any evidence to support an argument to extend the existing legislation in Australia.

REFERENCES

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2. (Declaration of Helsinki (1964). Adopted by the 52nd World Medical Association General Assembly, Edinburgh, Scotland, 2000, http://www.wma.net/e/policy/b3.htm (accessed Feb 14, 2006).

3. Jaenish and Wilmut, Developmental biology. Don't clone humans! *Science* 291:2552, 2001).

4. Humphreys et al, Abnormal gene expression in cloned mice derived from embryonic stem cell and cumulus cell nuclei. *Proc Nat Acad Sci USA* 99:12889, 2002).

5. Rhind et al, Human cloning; can it be made safe? *Nat Rev Genetics* 4:855, 2003;

6. Wilmut et al, Somatic cell nuclear transfer. *Nature* 419: 583, 2002)

7. Lee et al, Cloned cattle fetuses with the same nuclear genetics are more variable than contemporary half-siblings resulting from artificial insemination and exhibit fetal and placental growth deregulation even in the first trimester. *Biol Reprod* 70: 1, 2004.

8. Takahashi & Yamanaka, Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. *Cell* 126: 652, 2006

9. Rodolfa & Eggan. A transcriptional logic for nuclear programming. *Cell* 126: 652, 2006.

10. Brennan F. Public Ethics in Bioethics – A Response to the Lockhart Review. The 2006 Thomas More Lecture, Canberra, 22 June 2006.

11. Critchley C, Turner L. Understanding Australians' perceptions of controversial research. Australian Journal of Emerging Technologies and Society. 2: 95, 2004.

12. Submission to the House of Representatives Standing Committee on Legal and Constitutional Affairs by Associate Professor Loane Skene, 1 March 2000, para 3,2,3

APPENDIX

Comments on the papers tabled by Senator Kay Patterson on Thursday, September 14. Not one of these provides scientific material that supports the need for therapeutic cloning, and in some cases the material is quite irrelevant to the issue.

1. Chang, J et al, Correction of the sickle cell mutation in embryonic stem cells. PNAS, vol.103(4) pp 1036-1040 January 24 2006

This is not a therapeutic cloning paper, and is not particularly relevant to the current debate, but rather to the application of gene therapy, a mode of teatment that has its own problems. It reports the use of genetic engineering to correct an abnormality that had been introduced into a mouse gene and leading to sickle cell anaemia. The authors made ES cells from the mice by SCNT, corrected the gene defect and showed that the blood-forming cells could now form normal haemoglobin. Such an approach to gene therapy could theoretically be used for many single-gene defects. There is no need for the starting point for such gene therapy to be ES cells however, rather than (adult) haemopoietic stem cells.

2. Stojkovic, M et al, Derivation of a human blastocyst after heterologous nuclear transfer to donated oocytes. Reproductive Biomedicine Online, 2005 Aug 11(2) pp 226-31

This provides no argument for the need for therapeutic cloning – rather, it describes unsuccessful attempts to do so. This is a technical paper from the Newcastle-Upon-Tyne group published rapidly in this online journal at the time of the claim (soon shown to be fraudulent) by the South Korean group to have developed patient-specific cell lines by SCNT. What this paper shows is that they were able to conduct nuclear transfer of a human ES cell nucleus to an enucleated ovum, and develop a blastocyst, but take it no further. They had one success in 36 attempts, and concluded that they need ova within one hour of collection. None of this has been reported in any adequately peer-reviewed journal, and apparently it remains the case that they have not developed human cell lines by SCNT (therapeutic cloning). They have used a lot of human ova in their attempts though, and their experience seems to suggest that attempts at SCNT with human material will require working with very fresh eggs – obtained for work within an hour of sample collection from women.

3. Klimanskaya, I et al, Human embryonic stem cell lines derived from single blastomeres. Nature online August 23, 2006

This paper has no relevance to the need or otherwise for therapeutic cloning. It describes the establishment of ES cell cultures from single cells removed at the 8-cell embryo stage, such as can be obtained with the procedure of “Preimplantation Genetic Diagnosis” (PGD), where a single cell can be taken at the 8-cell stage to make a genetic diagnosis in high risk cases. If this became much more efficient than shown in this paper, it could provide for establishment of ES cell lines derived from IVF embryos that are not chosen for implantation on the basis of e.g. genetic disorder. It would thereby have the potential therefore of application to the study of a select number of single gene diseases. Although PGD is high risk, it is undertaken for specific reasons. The legislative requirement for this would be to allow work on embryos rejected at PGD as unsuitable for implantation.

4. Takahashi, K and Yamanaka, S, Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. Cell, Vol 126, 1-14, Aug 25 2006

If ever a new approach were to abolish the need for therapeutic cloning, this is it. I have referred to it in context in my Submission – page 4, section 2 (v). The paper reports that it is possible to "reprogram" an adult cell by providing it with a set of specific genes - 4 in number - and finish with cells that can behave virtually as ES cells in the tests that were applied. This is work carried out in mouse cells, it obviously needs to be confirmed, and there will be much more to be done to refine the method and establish whether the reprogramming is complete, and fully reproduces the ES cell. What can be said though, is that it is an exciting "proof of concept" that a pluripotent cell could be generated from an adult cell without cloning. It remains to be translated to human cells, and the approaches used for the mouse work will be invaluable in informing that work. If it is successful , it would be likely to be particularly relevant to the development of “disease specific’ clonal cells.

5. Barberi, T et al, Neural subtype specification of fertilisation and nuclear transfer embryonic stem cells and application in parkinsonian mice. Nature Biotechnology, vol 21(10) October 2003

In this paper either standard mouse ES cells or cloned ES cells were used to treat chemically induced Parkinson’s disease in mice. There was no advantage gained with the cloned cells, although there were only 6 mice per group, possibly because the brain is a relatively “immune privileged” site. This experiment was only of 8 weeks duration , thus insufficient to provide for development of tumours , which have occurred so commonly in recipient mice in other, similar, published experiments. Any proof of therapeutic concept such as this must be prolonged sufficiently to allow a conclusion about safety. especially excluding the possibility of tumour development and of chromosomal changes.

6. Blelloch, R et al, Nuclear cloning of embryonal carcinoma cells. PNAS sept 28 2004, vol 101(39) pp13985-13990

This very interesting work transfers the nucleus of a primitive mouse cancer to an enucleated ovum, and both therapeutic and reproductive cloning were carried out. The tumour cells retained their malignancy, and embryos died or were abnormal. Previously these same scientists carried out similar experiments, using nuclear transfer from mouse melanoma tumours to create embryos. The melanoma malignancy first appeared to regress, but then all embryos and mice developed tumours. These approaches could be very informative about the genetic changes in cancer, but they have no relevance to the need for therapeutic cloning in Australia. It would be absolutely essential that all such work be confined to mouse models for the foreseeable, and probably very long-distant future. A major reason is that meaningful research along these lines can only be carried out if the generated embryos are allowed to develop much further, including in vivo after implantation into the uterus. All of that work will be necessary in animals to assess the contribution made to such cancer biology of the inherent genetic instability of ES cells derived by SCNT.

7. Strelchenko, N et al, Reprogramming of human somatic cells by embryonic stem cell cytoplast. Reproductive Biomedicine Online, 2006 Jan; 12(1), 107-11

This online paper describes an attempt to bypass the need for therapeutic cloning. It is a preliminary technical report, in which the authors are seeking to find factors in ES cells that can be

used to “reprogram” adult cells to behave like ES cells. They fused ES cells with adult cells and found some evidence that they could transfer some “stem “ behaviour to the adult cells, but they finished with a mixture of cells , fused and non-fused, that were clearly difficult to work with. The reprogramming work by Takahashi and Yamanaka (item 4 above) is at a more advanced stage of achievement, although still in mouse cells.

8. Cooper, D, The Lockhart Review: Where now for Australia? Journal of Law & Medicine14 : 27, 2006.

The PhD student author in this superficial analysis embraces warmly the full recommendations of the Lockhart Committee Report, quoting selectively from it, including no comment on its many shortcomings, and concluding that “the potential benefit to countless Australians of stem cell therapies should be accorded more weight than the objections of some sections of the Australian