Royal Commission on
New Reproductive Technologies

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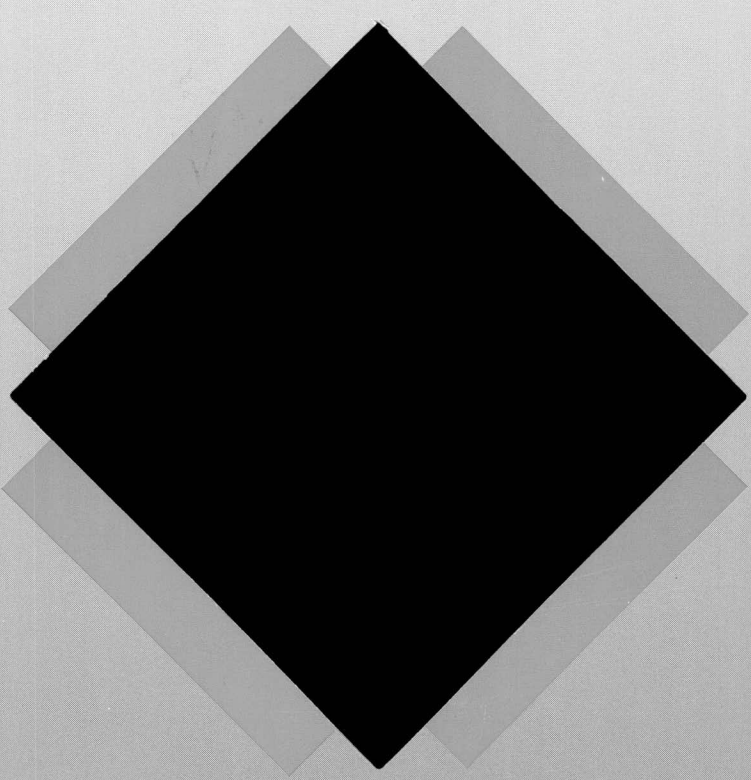

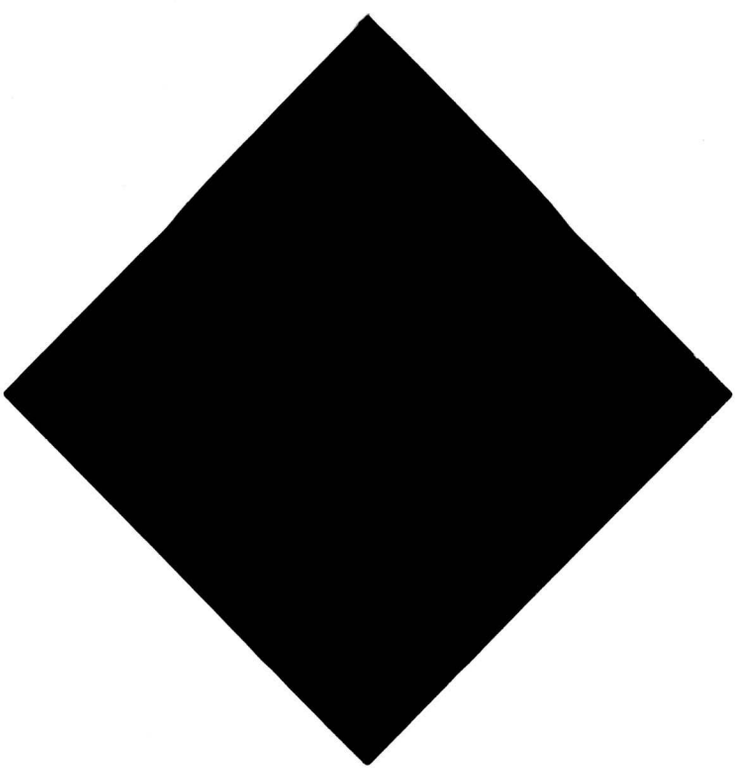

THE USE OF HUMAN EMBRYOS AND FETAL TISSUES:

A RESEARCH ARCHITECTURE

by

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The Royal Commission on New Reproductive Technologies was established in October 1989 to examine current and potential medical and scientific developments related to new reproductive technologies. In particular, the Commission has been asked to consider their social, ethical, health, research, legal, and economic implications for women, children, and society as a whole. The Commission is to report to the federal government by October 1992.

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Preface from the Chairperson

The federal government established the Royal Commission on New Reproductive Technologies in October 1989 and gave it a wide-ranging mandate. The mandate directs it to examine the issues surrounding a range of new reproductive technologies, considering in particular their social, ethical, health, research, legal, and economic implications.

The challenge facing the Royal Commission is to help Canadians understand and deal with the implications of new and powerful technologies related to human reproduction. Canadians have many questions about how the technologies are already being used in Canada, and why, and about what their role in society should be.

In many of the areas covered by the Commission's mandate, reliable data are simply not available on which to base recommendations as to what policies and safeguards should be applied. For this reason, the Commission set in motion a multi-disciplinary program of Research and Evaluation to provide rigorous, credible, and timely data about and critical analysis of the issues surrounding new reproductive technologies.

The Royal Commission is committed to an open and transparent research process with high standards and a protocol which includes peer review. Specialists in academic disciplines ranging across law, history, ethics, medicine, sociology, and philosophy are examining the implications of the technologies through a variety of methods. The Commission is in contact with various communities across the country to solicit advice and to commission research projects. Guidelines have been developed to help ensure the quality, integrity, and usefulness of all research studies. Research projects are subjected to rigorous internal and external review processes, first at the design stage and later at the report stage. Peer review for content and for methodology is a key feature of the process. In addition, researchers using human subjects are required to comply with appropriate ethical review standards.

Many academics, researchers, and groups who have participated in the Commission's work have requested access to the data and information generated by the Commission to help them consider their positions and make their recommendations to the Commission.

In response to these requests, the Commission sought and obtained permission to publish some of the research papers in advance of its Final Report. Reports such as this one will be released over the duration of its mandate to assist those working in the field of reproductive health and new reproductive technologies and to help inform the public.

Executive Summary

This paper reviews the special biological properties of the human embryo and fetus, their sources for research, and current medical and basic scientific research applications. Clinical and scientific applications are considered according to their relevance to a clinical model: thus, clinical research, pre-clinical research, and basic research represent the primary, secondary, and tertiary levels of this research taxonomy. Clearly, there are other models for organizing information — for example, in relation to disease type. The research architecture used in this paper is chosen to underline clinical and social relevance for public policy analysis. Limitations in the use of embryos and fetal tissues have been highlighted.

Certain unique biological properties, especially of fetal tissues, have fostered intense interest at both the clinical and the basic research level. The growing list of current and potential experimental applications for human embryos and fetal tissues suggests the possibility not only of supply and demand conflicts, but also of a range of social, legal, and ethical issues to be addressed.

Embryo research may raise questions of who is being treated — the couple who suffers infertility or carries genes for serious hereditary diseases, or the embryo itself. The distinction between being a patient and being an experimental subject may also be unclear. Further, competition in the use of scarce embryos for clinical and basic research may arise. Issues of informed consent for couples pursuing pregnancies that incorporate clinical embryo research are critical, and the role of such couples in providing proxy consent on behalf of their potential offspring for embryonic experimentation may pose many new and difficult ethical issues.

Current fetal tissue research indicates a growing list of possible therapeutic applications, including Parkinson's and Alzheimer's diseases, diabetes, and AIDS. Fetal tissues may become increasingly scarce as the scope of application widens and as earlier abortion techniques are developed. Currently, there is no agreement about whether the use of electively aborted fetal tissues can be separated from the act of abortion, morally or procedurally. This represents an area for further investigation and discussion.

Finally, an important limitation to this paper is that it reviews only those uses of embryos and fetal tissue reported in the academic and medical literature. Questions of whether and how embryonic and fetal tissues are used in the cosmetic, pharmaceutical, or other industries have not been examined. Social, ethical, legal, and regulatory issues have not been fully addressed. These are important domains of original research and discourse if effective and relevant public policies are to be developed.

Introduction

As areas of academic inquiry, the use of human embryos for research purposes (ER) and the use of human fetal tissue for therapeutic research (FTT) share several features. Both are interdisciplinary subjects, requiring a consideration of philosophical premises, medical and technical parameters, legal context, and ethical concerns. This relatedness is most significant at the normative or philosophical level of inquiry; debate concerning when human life begins, as well as the rights of the embryo and fetus, are central to the philosophical context.

The specifics of examining the medical and scientific uses of embryos and fetuses lead to quite separate lines of investigation, however. This paper briefly examines embryo research and fetal tissue research in terms of

I. the distinctive biological properties of embryonic and fetal material;
II. sources of these materials;
III. medical and technical applications — in both clinical and basic research terms; and
IV. alternatives to these materials in clinical and basic research modalities.

This paper embodies only a brief review of a rich and extensive literature in these areas. Finally, it provides a schematic research architecture intended to highlight the principal lines of scientific and clinical inquiry, as well as key sub-issues to be addressed.

It is useful to clarify some definitions of embryo, fetus, and fetal tissue at the outset. The zygote, cleavage, and blastocyst stages are best regarded as "pre-embryonic," and the term "embryo" technically applies to the structure that is present from the second through the eighth week after fertilization. Currently, most "embryo" research in fact involves the pre-embryo: the properties of totipotentiate development and pre-implantation status are most relevant to technologies of assisted reproduction, genetic manipulation, development of cell lines, and "twinning" procedures. This paper details the distinctive features of the pre-embryo and illustrates relevance to the various scientific and clinical procedures that are performed.

It is equally important to note that this paper deals with the use of fetal tissues for research, rather than with fetal research. Fetal tissue research involves the investigation of fetal tissue properties and possible applications in therapy, transplantation, or industry. Clearly such research is not intended to benefit the fetus from which the tissues are derived. By contrast, fetal research is directed at understanding the growth and development of the fetus in both healthy and pathological conditions; this research has resulted in fetal therapies, such as intra-uterine fetal surgery and the management of fetal cardiac arrhythmias *in utero*.

Biological Characteristics of the Human Embryo

Certain unique properties of the embryo are of great value in basic and applied research. These characteristics change with the gestational age of the embryo, and it is useful to outline these changes and their significance.

Conception and Zygote Development

The processes that lead from the uniting of human germ cells — ovum and sperm — to the implanted, or true, embryo are continuous and complex. The process can be divided into stages: the new generation begins with the zygote, or newly united egg and sperm. Entry of a single sperm cell into the ready oocyte is of itself made possible only by a series of biochemical and physiological alterations in both germ cells: the ovum continues to mature after rupture from the ovarian follicle and is optimally receptive to fertilization (*in vivo*) some 6 to 12 hours after ovulation, or after artificial retrieval (for fertilization *in vitro*). Similarly, the sperm cell must undergo the changes involved in capacitation (molecular changes undergone by sperm cells after ejaculation that permit the sperm cell to respond to substances accompanying the ovum) and acrosomal reaction (fusion of the acrosomal sites on the sperm cell to allow the formation of "portals" and hence the biochemical release necessary for passage through into the ovum).

The response of the egg to penetration is activation, signifying the initiation of embryonic development and incorporating both functional and structural changes in the newly fertilized egg. These include the induction of blockade to polyspermy (permeability loss in the zona pellucida to prevent entry of more than one sperm cell), opening and evacuation of cortical granules, emission of the second polar body, and formation of the two pronuclei, each with its discrete package of genetic information contributed by the parent cells.[1] These subtle biochemical and structural processes are, as yet, only dimly understood, and this "cross-over" time (between dealing with separate germ cells and the newly fertilized ovum) is an area of great research interest, particularly in terms of understanding fertilization failure (both male and female factors) and errors of fertilization (such as polyspermy).

The Genome

The completion of the union of sperm and egg (zygote formation) and the development of a new nucleus fusing the genetic contribution of the parent germ cells constitute the new hereditary generation, with its new genome. The zygote is remarkable in its theoretical potential to give rise to a distinct and unique member of the human community. This potential is both theoretical and statistical, because only about one in three zygotes created *in vivo* will accomplish this, and the potential is conditional on successful uterine implantation.[2]

The information contained in this new genetic entity is replicated in each somatic cell in the developing embryo and in each cell of the human being that may result. The identical replication of the genome in each cell is essential to the study of genetic markers, whether at the stage of

pre-implantation embryo, pre-natal diagnosis (chorionic villus sampling and amniocentesis), or after birth.

Cleavage and the Blastomere

The advent of the new genome is followed by the process of cleavage. During this time the initial single cell and its nucleus divide successively: one cell becomes two, then four, then eight, and so on. Each of these successive equal divisions occurs with little or no intervening growth in the overall mass of the developing entity. Thus each successive product cell (blastomere) becomes increasingly smaller as the size of the total aggregate remains nearly constant throughout the early stages of division.[3]

Totipotentiality

For a given individual, nearly all somatic cells contain the identical genome, or hereditary information, but the cells of the early pre-embryo are equipped with a unique property — totipotentiality. Stated simply, each cell in the early stages of division has the theoretical potential to develop into a full adult. This property has implications for twinning and fusion and for the biopsy of the early embryo for diagnosis. This property endures through the 8-cell stage, when all the blastomeres are equally potentiate for further development. By the 32-cell stage this totipotentiate quality is lost, and as cell numbers increase, differentiation into particular cell types occurs.

Twinning and Fusion

Animal experiments using mouse embryos up to the 8-cell stage have demonstrated that each blastomere has the potential to develop into a complete adult, if separated from the other cells. This phenomenon may occur naturally, as in the case of human identical (monozygotic) twins. Thus the very early human pre-embryo has the potential to become none, or one, or more than one distinct human being.[4] Similarly, experiments in the mouse have demonstrated that if two 8-cell embryonic aggregates of different parentage are fused, then a single adult may result. Genetic contributions from four parents can be recognized in the resultant individual in fusion experiments.[5] Thus at the 8-cell stage, the developmental singleness of one individual person has not yet been established. It may be inaccurate to speak of the early embryo, which is neither singular nor plural.

Implantation and Differentiation

Within two divisions of the 8-cell stage, at 32 cells, totipotentiality is lost. The cells have become more adherent and are densely packed. With increasing cell numbers, multi-cellular forms can be identified (differentiation) — certain cells forming an outer layer surrounding a less differentiated inner cell mass. The outer cells are developing into the trophoblast or feeding layer. This material will become extra-embryonic and is engaged in completing the placental interactive layer with the maternal uterus. The inner cell mass may continue to develop into the

true embryo. The whole of this continually developing mass is termed the blastocyst; the cavity is termed the blastocoele.[6] *In vivo*, it is at about this stage that the entity completes its travel, entering the uterus where implantation may occur.

Growth Factors

The very early embryo is not inert with respect to its environment, either *in vivo* or *in vitro*. Studies reveal that the pre-embryo produces and releases growth factors and other chemical messengers from at least the 4-cell stage. Identified substances include beta-human chorionic gonadotropin (beta-hcg — the diagnostic pregnancy hormone) and embryo-derived platelet activating factor. The role of various factors is poorly understood; however, certain released factors appear to be necessary for successful implantation in the uterine wall.[7,8] Identifying and understanding the functioning of growth and implantation factors derived from the early embryo remains an important area of infertility and implantation research.

Biological Characteristics of the Fetus and Fetal Tissues

This section examines the scientific basis for the desirability of fetal tissue for research and therapeutic purposes, especially the use of fetal tissues for transplantation. The relevant properties of fetal tissue are described in detail.

Differentiation/Dedifferentiation

Fetal tissue cells exhibit a remarkable capacity for growth and differentiation. This is particularly true of early fetal cells as rudimentary organ functions are developing and the capacity diminishes with increasing gestational age of the fetus.[9] This is in distinct contrast to adult human tissues, which exhibit very little capacity for redifferentiation. This property of cells derived from fetal tissues enhances their potential for functional differentiated growth *in vivo* and *in vitro*.

Culture in vitro

The great potential for growth of certain fetal cell types *in vitro* is directly related to the relatively undifferentiated status of those cells. Again, this is in contrast to cells derived from solid adult organs; cells derived from some of these organs may be completely resistant to culture *in vitro* — brain and cardiac cells are examples — while others, such as liver cells, will undergo some replication under appropriate culture conditions. That fetal cells can be cultured suggests research directions in the development and maintenance of specific fetal cell lines in culture, with cryopreservation of these cells as a potential source for continued research and use for therapeutic purposes.[10]

Potential for Growth and Restoration of Function in the Host

Animal models have been used extensively to study the growth and functional capacity of transplanted fetal cells of various types.[11,12] The results of these studies indicate that there is demonstrable and clinically significant growth and functional recovery by transplanted fetal cells in disease models for Parkinson's disease and Type I (juvenile, or insulin-dependent) diabetes. Such studies provide the rationale for clinical experimental trials using fetal tissue transplantation in human patients when conventional therapies fail.

Resistance to Oxygen Deprivation

The sensitivity of post-natal solid organs to oxygen deprivation is an important limiting factor in transplantation; organ harvest and transport must be carefully timed if the grafting procedure is to have any chance of success. Primitive fetal cells exist under uterine conditions with substantially limited vascularization and oxygenation, and consequently have increased resistance to lack of oxygen at harvest and transplantation.[13] Some investigators report that human fetal neural tissue is functional after several hours in saline solution at room temperature. This property renders fetal tissues a particularly attractive source of transplant material.

Ease in Transplantation

Compared with the technical demands of adult solid organ transplantation, the handling of fetal cells for transplantation is remarkably easy. Solid organs demand an intricate surgical approach, particularly in their dissection from vasculature, when harvested for transplantation. Fetal cells can be grossly dissected by cell type, then mechanically manipulated into suspension. This allows transplantation by virtual "injection" into the target host organ and requires no efforts to connect the cells to an existing blood supply.[14]

Immunogenicity

Perhaps the most important obstacle to successful clinical outcome in all transplant procedures is the rejection of foreign transplant material by the recipient. The host immune system recognizes the transplanted material as genetically distinct — as it does with infectious bacteria or viruses — and initiates a destructive response. Transplant outcomes have been dramatically improved by the use of more advanced tissue typing and immuno-suppressive drugs such as cyclosporin. Immuno-suppression is not always successful, however, and serious side-effects are not uncommon; tissue and organ rejection remains a serious clinical challenge. Fetal tissues, by contrast, are characteristically immunologically immature and may provoke little or no immune response in the host.[15] This feature of fetal tissue is key to its desirability as a transplant material.

Supply

The scarcity of human organs and tissues for transplantation and treatment represents a grave challenge to health care professionals and patients awaiting transplant. First-trimester abortion is a common procedure in Canada; currently, the uterine contents are disposed. First-trimester elective abortion represents a potentially vast pool of tissues that might be harvested for therapeutic purposes.[16]

Sources of the Human Embryo

In Vitro Creation of Embryos — Assisted Reproductive Technologies

Human pre-embryos for research are generally created *in vitro* in clinical reproductive biology units (both free-standing clinics and hospital- and university-based infertility treatment centres). In these contexts, embryos for research purposes arise in two ways. First, embryos may be designated as "surplus" where ova and sperm have been united in laboratory conditions in the hope of creating embryos suitable for transfer to the uterus of an infertile woman. Ovarian hyperstimulation by drugs such as Clomid, Pergonal, Profasi, and Metrodin may result in the creation of too many ova for safe transfer (an upper limit of four embryos is considered reasonable to ensure maternal and fetal safety).[17] Depending on the clinical program, surplus embryos may then be designated for cryopreservation (for implantation in a future cycle), donation to another patient, research, or disposal.

A further source of human pre-embryos for research results from the rejection of these embryos for embryo transfer, cryopreservation, or donation because of an obvious defect. Such defects include polyspermy (as demonstrated by the microscopic appearance of more than two pronuclei within 24 hours after insemination) and damage (rupture) of the zona pellucida with cytosolic leakage.[18] Such defects contra-indicate embryo transfer and are a potential source of embryos for use in research. Investigation of these pre-embryos may help elucidate the mechanisms of fertilization errors and other disruptions contributing to infertility.

Second, embryos can be created for the specific purpose of research, where donors consent to their gametes/embryos being used in this way. This situation is less common, owing to the relative scarcity of gametes for persons undergoing infertility treatment. However, certain patients who do not wish cryopreservation of spare embryos, or where cryopreservation or donation is not available, may consent to the use of their excess embryos for research purposes.

Uterine Flushing and Embryo Harvest

Human pre-embryos can be non-surgically "flushed" from the uterus of a newly pregnant woman, less than 14 days after fertilization.[19] These techniques were first developed for the recovery of early embryos in agricultural breeding stock, for subsequent transfer to less valuable stock for gestation. Only a limited number of uterine flushings have been performed in humans.

The application of this technique theoretically includes very early embryonic diagnosis of genetic defects in high-risk couples, for example, carriers of the cystic fibrosis gene. It should be noted, however, that guidelines developed by the Society of Obstetricians and Gynaecologists of Canada recommend against the use of uterine flushing for retrieval of ova and embryos for the purpose of donation, because of the risks to a woman undergoing this procedure.[20]

Selection for Cryopreservation

Excess embryos created *in vitro* by assisted reproductive technologies may be selected for cryopreservation, based on gross morphological characteristics including cell number, equal blastomere size, and lack of fragmentation. A confirmation of normal fertilization by visualization of two pronuclei on the first day after insemination is necessary to exclude polyploidy. Embryos failing to meet these criteria are rejected for freezing and are a potential source of research embryos.[21]

Sources of Human Fetal Tissue

Therapeutic abortion, spontaneous abortion (miscarriage), and ectopic pregnancy are all potential sources for fetal tissue. Whatever the source, to be suitable for transplantation and most basic research studies, fetal tissue must be viable, free from major genetic defects, and uncontaminated by infectious agents — bacterial, viral, or fungal. These requisite features must be considered when evaluating the potential sources.

Therapeutic Abortion

The usual source of human fetal tissue for research and therapeutic application is the elective first-trimester abortion. In the literature, details are scant with respect to the administrative aspects of collecting this material; many hospital consent forms for the procedure contain a global clause, in rather general terms, permitting the use and/or disposal of the abortus material. It is known that up to 1.6 million abortions are performed annually in the United States and up to 85 000 in Canada: about 90% of these take place within the first trimester.[22] Vacuum aspiration is considered the method of choice for first-trimester abortion at this time. During the second trimester, dilatation and evacuation is usually employed; this method results in whole fetus recovery, while the vacuum aspiration approach causes significant fragmentation of the contents of pregnancy. Very late abortions can be performed by replacing the amniotic fluid with concentrated saline and stimulating uterine contractions with pitocin or prostaglandins.[23]

Spontaneous Abortion

Another potential source of fetal material for research is spontaneous abortions (miscarriage). Difficulties in procuring this material include loss of control over timing of harvest, fetal death and tissue necrosis, and concern about the genetic normalcy of spontaneous abortions. Additionally, infections such as syphilis, rubella, and mycoplasma may cause spontaneous

abortion.[24,25] For these reasons, fetal tissue from spontaneous abortion is not used for research or clinical applications.

Ectopic Pregnancy

Fetal tissue derived from ectopic pregnancies has been suggested as a practical and ethical source of this material. Between 40% and 64% of ectopic pregnancies abort spontaneously in the first trimester; this abortus material is rarely recognizable or viable in culture.[26] The balance of ectopic pregnancies become clinically apparent before the ninth week of gestation, and intervention is necessary to save the mother from this life-threatening condition. This intervention may involve surgical removal of the gestation; more recently, however, non-surgical local injection of methotrexate (lethal to the fetus) has been used, so as to preserve the Fallopian tube for future reconstruction.[27] This injection procedure renders the fetal tissue unsuitable for research.

Medical and Technical Considerations and Indications: The Embryo

This section constitutes the major portion of this paper. Differences between the issues pertinent to the use of human embryos for research purposes and the use of human fetal tissue for therapeutic research are more salient when clinical and research applications are considered. Embryo research, in general, is directed at medical and scientific inquiry relevant to human reproduction, so that, at least potentially, embryo research may serve to benefit the larger population of human embryos, if not the specific embryo as research subject. By contrast, fetal tissue for therapeutic research is generally, but not exclusively, directed toward therapy for adults.

Primary Applications — Assisted Reproductive Technologies

The development of new assisted reproductive technologies (ARTs) has relied extensively on human pre-embryo research. While animal models were used for many years to investigate the mechanisms of mammalian fertilization *in vitro*, the advent of *in vitro* fertilization-embryo transfer as a clinical treatment for infertility has fostered a peculiar environment where infertility research and treatment take place simultaneously. Examples of this include the application of modified techniques such as GIFT (gamete intrafallopian transfer), ZIFT (zygote intrafallopian transfer), and PROST (pronuclear oocyte salpingo transfer).[28,29]

Quality Control

Quality control is an essential feature of infertility laboratories processing human gametes and pre-embryos. Rigorous laboratory methods demand daily monitoring of *in vitro* culture conditions, including temperature, ph (relative acid/base index of the culture environment), humidity, sterility, and quality of culture media. Where more than one incubator is available, comparing parameters from one machine to the other provides an internal quality control. (It is noteworthy that availability of more than one incubating chamber is an important precaution in

human embryo culture, so that an immediate back-up is available in the event of a system failure.)[30] In this sense, all human gametes and pre-embryos in a given clinical culture setting exist as internal controls for one another. In a given laboratory, fertilization failure with one set of gametes may be more confidently ascribed to the inherent properties of those gametes when germ cells from other patients fertilize and develop successfully. When all patients' gametes fail to fertilize or undergo cleavage, this points more strongly, though not exclusively, to failure of the culture conditions.

Culture Media

Many programs employ simultaneous assay of animal culture as a measure of quality control, particularly in the preparation of culture media, with mouse embryo the most common model. Subtle but critical problems with media may be detected this way: in one example, quality control tests demonstrated that a commercially available culture medium allowed normal development of two-cell mouse embryos, while one-cell mouse embryos (pronuclear oocytes) failed to undergo cleavage.[31] Such results point to the highly sensitive requirements for successful culture of mammalian embryos and the need for continuing research into improved and reliable culture techniques.

Cryopreservation

The cryopreservation of human embryos represents an additional area of ongoing primary research in infertility treatment. The creation of supernumerary embryos in a given *in vitro* fertilization treatment cycle results from current ovarian stimulation approaches. Morbidity and mortality associated with multiple pregnancy have led to policies recommending the transfer of only three embryos on a given cycle, with a maximum of four under exceptional conditions. Successful freezing, thawing, and transfer of human embryos may potentially serve to improve assisted reproductive technology results for a given ovarian stimulation treatment.

Some findings suggest that pregnancy results are improved when embryos are transferred at the appropriate time in a "natural" or non-exogenous ovarian stimulation cycle. Thus the patient may benefit from increased chances of pregnancy with fewer exposures to exogenous ovarian stimulation and invasive oocyte retrieval, fewer associated medical risks, and lower financial cost.[32,33] The results of embryo cryopreservation programs around the world vary, however. Australian results indicate a significant improvement in continuing pregnancies when frozen and thawed embryos were transferred during the natural cycle when compared with embryo replacement during a stimulation cycle.[34] A recent report from the Chaim Sheba Medical Centre IVF Program in Israel found that pregnancy potential is diminished using frozen embryos.[35] Research activities in this area include the comparison of different cryoprotectants (DMSO and 1,2-propanediol) and staged temperature-lowering protocols with rapid and ultra-rapid freezing techniques.[36] Again, investigations into improved methods of embryo freezing in various treatment programs can be seen as a simultaneous embryo research and infertility treatment endeavour.

Male Factor Infertility

Finally, novel clinical approaches aimed at improving fertilization and pregnancy rates where poor sperm quality is a problem illustrate another area of simultaneous treatment and research. "Male factors" as the sole cause of infertility account for at least 25% of infertility experienced by couples and are implicated in at least 40% of infertile couples as a co-factor in infertility. *In vitro* fertilization has been used for a number of years to treat male factor infertility, as fertilization with certain male factors is improved *in vitro*, especially where poor sperm motility is present. Clinical research in this area centres on the development of techniques to facilitate fertilization. One approach has been the development of differential chemical gradients to select the healthiest sperm for insemination *in vitro*, while other methods employ disruption (by drilling or fracture) of the ovum zona pellucida to ease entry of sperm. Micro-injection of a single sperm cell into the cytoplasm of the ovum has been attempted.[37,38] It is noteworthy that the subtle effects of these manipulations on the health of the potential child and adult cannot yet be known, although some apparently successful pregnancies and deliveries have resulted following the use of each of these methods.

Secondary Research — Embryo Growth

There is great interest in elucidating the subtle mechanisms of embryo growth, differentiation, implantation, and immunology for the purpose of elaborating existing theoretical knowledge and discovering improved approaches to infertility and healthy reproduction.

Embryo-released Factors

Very little is known about the factors released by the early embryo and the nature of their function in development and interaction with the maternal environment (*in utero*). Human pre-embryos manufacture and release both beta-hcg and platelet activating factor in culture, although the reasons are still not understood. It is known that the embryo binds glycoproteins from its environment as it travels along the fallopian tube; again, the role of these phenomena are matters of speculation. Insulin appears to have a growth factor effect on pre-embryos in certain culture conditions. The *in vivo* effects of circulating drugs from exogenous ovarian stimulation on the newly developing pre-embryo are not known.[39]

Identification and functional analysis of pre-embryonic growth factors and chemical messengers may provide clues to understanding "unexplained" infertility and may help to define subtle parameters necessary for normal growth and interaction with both culture and uterine environments. Preliminary research has begun to reveal the metabolic behaviour of human pre-embryos through the development of assays to show what type and how much nutrient the embryo takes up from culture media.[40]

The mechanisms of differentiation from totipotent pre-embryonic cells (fewer than 32 cells) into placental interactive tissue and into undifferentiated true embryonic cell mass remain largely elusive, as do the triggers for the differentiation of the inner cell mass into specialized tissues and

then organs during gestation. Elucidating these mechanisms is of great interest to basic biologists and those scientists involved in normal and abnormal human reproduction. Currently, only certain gross morphological characteristics of the pre-embryo have been correlated with observed healthy development of a human life.[41]

Implantation

A final area of secondary human pre-embryo research centres on the mechanisms and necessary conditions for successful implantation of the embryo in the uterine wall. These investigations may prove instrumental in understanding failed implantations and the relatively high early embryonic waste seen in humans. Further, understanding implantation may help explain ectopic pregnancies where no obvious tubal or structural defect can be demonstrated.[42] Ectopic pregnancy remains an important clinical problem in gynaecology, in terms of both acute patient management and the future fertility of a woman who experiences an ectopic pregnancy.

Tertiary Research — Embryo Biology

One of the most dramatic advances in the area of basic embryo biology has been the very recent development of techniques for pre-implantation diagnosis of genetic composition and disease in the human pre-embryo.

Pre-implantation genetic diagnosis

Pre-implantation genetic diagnosis is an experimental diagnostic technique designed for application to embryos that may be replaced in the uterus. Very early embryos contain cells of equal potential development, and it is possible to remove at least one cell (blastomere) from a 4-cell or 8-cell pre-embryo, without compromising the normal development of a pregnancy, if the "biopsied" embryo is transferred to a receptive uterus.

The method requires removal of the zona pellucida, washing the embryo in a calcium-free medium to loosen cellular adherence, then gently lifting away one cell. In a mouse model for this research, biochemical assay was performed to determine the presence of the gene-product enzyme HPRT. Results of the assay were available in 24 hours, and the biopsied embryo was transferred successfully.

HPRT deficiency is a sex-linked genetic defect in humans causing a severe neurological disorder (Lesch Nyhan disease) in affected males.[43] It is also possible to freeze the biopsied embryo until such time as laboratory tests of the collected cell are complete, should test results take more than 24-48 hours. The embryo can then be thawed and transferred. Thus, application of this procedure may well necessitate the incorporation of embryo cryopreservation techniques. The method could be applied to embryos created *in vitro* or harvested by uterine lavage for the purpose of genetic diagnosis.[44]

Genetic Diagnosis

The development of pre-implantation diagnosis for genetic defects will undoubtedly accelerate the debate on social and ethical issues relevant to genetic testing. Some may feel that pre-implantation testing and selection of only healthy pre-embryos for uterine transfer would be less traumatic — physically, psychologically, and socially — than current methods of pre-natal diagnosis and subsequent abortion of defective fetuses.[45]

In 1989, for example, the gene for cystic fibrosis was identified.[46] Cystic fibrosis is the most common genetic disease affecting caucasians; the potential for pre-implantation diagnosis may become a very attractive option for couples where both are carriers but wish to have children. The same procedure may be possible in the future for the many inherited diseases for which the gene has yet to be identified. At this time, pre-implantation diagnosis requires biopsy and embryo freezing, because the biopsied test cell must be cultured to generate sufficient tissue to identify the appropriate deoxyribonucleic acid (DNA).[47] Application of these techniques is likely to be severely limited, however, since they require couples to undergo assisted reproduction.

Sex Selection

Pre-implantation diagnosis has been used in a limited number of cases to ascertain the sex of the pre-embryo, so as to identify embryos at risk for sex-linked diseases such as muscular dystrophy and haemophilia. A number of sex-linked genetic diseases have been identified; females are generally carriers for these diseases, and affected offspring are generally male.[48] Identified male embryos from couples at risk would be not be implanted; only female embryos would be replaced in the uterus. It is clear, however, that this technique could also be used to select for sex alone in healthy embryos, where there is a strong preference for a child of a specific sex.

Gene Therapy

The ultimate application of pre-implantation diagnosis for genetic disease would entail the correction of the defect. Gene therapies unheard of only a few years ago have been developed for specific diseases and are at the stage of clinical trial in a number of models.[49,50] Pre-implantation genetic correction has not been attempted, however, and remains a remote possibility at this time. There is little motivation to develop these techniques, because it is possible simply to implant embryos found free of disease, rather than risk transfer of an embryo that has been genetically manipulated. One possible advantage of attempting gene insertion and therapy with the pre-implantation embryo is that there are far fewer cells to be manipulated, each of which has great potential for differentiation and proliferation. However, given the choice of implanting only normal embryos, this is a research avenue unlikely to be pursued.[51]

Medical and Technical Applications: Fetal Tissue

The use of human fetal tissues for research, industry, and therapy is not new; however, there has been rapid growth in the potential uses in recent times. Primary applications are those in established use and include vaccine development, viral research, and clinical application. Secondary uses of fetal tissue are clinical treatments where a considerable volume of animal research has led to proposals for or initiation of preliminary clinical trials. Examples are the use of fetal tissues for transplantation in Type I diabetes and Parkinson's disease. Tertiary research includes those possible clinical uses of fetal tissue that are in the earliest stages of investigation.

Primary Applications

Industry

Human fetal tissue in culture has been used for many decades by pharmaceutical and biotechnology companies in the development of vaccines and to test the efficacy and teratogenicity of new pharmaceutical products.[52] The human polio vaccine was developed in the 1950s using fetal tissue cells in culture. The ability of fetal cells to divide rapidly and proliferate in culture, their decreased immunogenicity, and their capacity to grow if transplanted into a host are important properties in the selection of fetal tissue for industrial applications. It is noteworthy that the literature in this area is very scant: a search of the International Pharmaceuticals Research Data Base for the past 10 years revealed abstracts related to the effects of drugs on the fetus, but none on the use of fetal tissues in research. It is tempting to speculate that industrial research using fetal tissues is rarely published, possibly owing to the controversial nature of using these tissues (especially if the source is aborted fetuses). By contrast, the academic literature (Medline) on the use of fetal tissues is substantial, perhaps as a result of the onus placed on academic researchers to publish the results of their investigations.

Viral Research

Fetal tissues are used extensively in the investigation of viruses. The rapid proliferation of fetal cells in culture permits rapid replication of a virus for detection, genetic assay, genetic manipulation, and tests of viral facilitators and inhibitors. Such research is performed on human influenza viruses, hepatitis B virus, the measles virus, and human immunodeficiency virus (HIV).[53,54,55] Fetal cells used include those derived from lungs, hepatocytes (early liver cells), and thymus. Fetal tissues are a critical tool in viral research into these clinically important infections.

Treatment of DiGeorge's Syndrome

This syndrome is characterized by a congenital absence of the thymus. The use of human fetal thymus is the only "routine" application of fetal tissue for transplantation; all other transplant procedures using human fetal tissue remain highly experimental. For more than 20 years human fetal thymus transplant has been recognized as the treatment of choice for DiGeorge's syndrome.[56,57]

DiGeorge's syndrome is a rare congenital abnormality, resulting when the third and fourth branchial pouches fail to develop normally. A complex of anomalies results, including facial deformities, cardiac malformations, kidney disease, hypoparathyroidism, and an inadequately developed thymus.[58,59] The severity of the syndrome varies markedly; the mildest forms require only corrective cardiac surgery, with Vitamin D and calcium supplements to counteract hypoparathyroidism.

Severe forms of the syndrome are characterized by profound immunodeficiency because of the lack of thymic function and resultant lack of T-cell function. It is this aspect of the disease that is amenable to correction by the transplantation of fetal thymus. The results of fetal thymic transplantation indicate that 8 of 26 patients treated by transplant enjoyed extended survival.[60] Rapid restoration of function has been observed in some patients (within days), while several months are required in other patients. Some success has also been achieved with the use of cultured fetal thymic cells. Transplantation of tissue rather than extract appears to be required for optimal results in the more severe forms of the disease. DiGeorge's syndrome remains a rare paediatric disease, but it is important as a model of therapy where the transplantation of human fetal cells is the treatment of choice. This success story has generated hope for the effective application of fetal tissue transplantation for a variety of other disorders.

Secondary Applications

Transplantation in Parkinson's Disease

Parkinson's disease is a commonly recognized condition with characteristic signs: tremors, muscular rigidity, and slowness of body movement (bradykinesia). The underlying dysfunction reflects a deterioration of the brain's dopamine-producing substantia nigra cells, cells critical to processes involved in the initiation and control of movement. There is no known pharmacological treatment to halt or reverse the neuronal degeneration. Varying degrees of symptomatic control can be achieved with combinations of drugs, including anticholinergics, Propranolol, Amantadine and, most important, L-Dopa — a precursor to dopamine. Typically, symptomatic control with various drug protocols fails through the progress of the disease, and the side-effects of drug therapy may be formidable.[61,62] More than 70 000 persons in Canada suffer from Parkinsonism, and about 5 500 new cases are diagnosed each year. At any point in time it is estimated that nearly 7 000 cases are refractory to conventional drug therapies; not all of these are suitable candidates for fetal tissue transplantation.[63]

Experimental Parkinsonism has been induced in a number of animal models; although the etiology of Parkinson's in humans is not clearly understood, it is possible to induce the degeneration of substantia nigra cells. This has been accomplished in rats using 6-hydroxydopamine (6-OHDA), a chemical analogue of dopamine known to cause selective deterioration of the substantia nigra pathways.[64] Similarly, a recent experimental model for Parkinson's was developed, quite by accident, in non-human primates. A profound Parkinson syndrome was clinically observed in a number of individuals who had ingested N-methyl 4-phenyl 1,2,3,6-tetrahydropyridine (MPTP), a homemade semi-synthetic narcotic. The brain

damage suffered by these persons appeared identical to that observed in idiopathic (without a known cause) Parkinson's disease. Further investigations demonstrated that MPTP causes selective degeneration of substantia nigra, and elicits motor symptoms very similar to those seen in Parkinson's patients, when administered to non-human primates.[65]

Both rodent and non-human primate models for Parkinson's are extremely valuable in the development of clinical management approaches to the disease, although these chemical-lesion models are far more limited in revealing the etiology of Parkinson's. The non-human primate model is particularly important in the development of neurosurgical approaches, including the transplantation of fetal neural tissue. Fetal mid-brain material from both animal and human sources has been demonstrated to survive and function when transplanted into the rodent model for Parkinson's disease.[66,67] There are limits to the extent to which results from animal models can be generalized to fetal neural transplantation in humans; however, the results of such studies form the basis for considerating clinical trials using fetal tissue transplantation in human subjects.

The transplantation of human fetal mid-brain material has been reported in a small number of patients from Britain, Mexico, Sweden, and the United States, almost exclusively in Parkinson's patients. The first published report came from Mexico, where two Parkinsonian patients were transplanted with fetal tissue derived from a spontaneously aborted fetus of 13 weeks gestation.[68] The report indicated that both patients experienced substantial symptomatic improvement, yet these cases have been strongly criticized on the grounds that fetal neural tissue of 13 weeks gestation is too mature to survive the transplant procedure and undergo proliferation and functional development in the host.[69]

In Britain, the successful transplantation of fetal substantia nigra in two Parkinson's patients has been reported, and at least 12 patients are claimed to have received a transplant, although details are not available.[70,71] In the United States, one case report has come from the University of Colorado, and a randomized controlled clinical trial involving 20 Parkinson's patients is under way at Yale University; both studies are supported by private funds, owing to the National Institutes of Health ban on government funding of transplantation research using electively aborted fetal material.[72]

The most detailed reports of transplantation of fetal substantia nigra material in two patients with Parkinson's disease comes from Sweden. Both patients received immuno-suppressive drug therapy; six months after the transplant, only minimal improvement in the patients' subjective experience of the disease was reported. No reduction in conventional medication requirements was seen, although neurophysiological examination indicated small, significant improvements on the side of the body opposite the side of the brain graft. The investigators concluded that the therapy as performed did not effect a significant clinical improvement, but that neurophysiological findings provide a strong rationale to pursue the approach, both in animal studies and in clinical trials.[73]

In Canada, researchers at Dalhousie University and the Victoria General Hospital in Halifax have developed a stringent patient selection protocol for a preliminary clinical fetal tissue transplantation trial that was scheduled to begin in 1991. The following criteria for patient selection have been set: confirmed diagnosis of idiopathic Parkinson's disease by clinical findings and abnormality on positron-emission tomography (PET scan), progressive disease to point of dependence, good initial response to drug therapy with maximum dosage no longer providing relief or where side-effects are intolerable, and availability and commitment to regular detailed assessment prior to surgery and for follow-up throughout the post-operative period. Patients are to be selected from the hospital's Movement Disorder Clinic, and technical assistance in fetal tissue handling will be provided by basic researchers at Dalhousie University.

This clinical trial represents a pilot project involving fewer than 10 patients. The president of the hospital emphasizes that ethical and scientific consultations have been conducted across the country, and that the trial involves patients for whom conventional therapy can now offer only a marginal existence.[74] The results of this carefully planned and public investigation may soon provide much needed direction concerning the future of fetal neural transplantation as a legitimate therapy for certain end-stage Parkinson's patients.

Transplantation in Type I Diabetes

Diabetes mellitus is a serious and common disease with two major forms — insulin-dependent (juvenile - Type I) and non-insulin dependent (Type II). Both disease states are characterized by persistent elevation of blood sugar; the term diabetes mellitus means "sweet urine."

Type I diabetes accounts for about 10% of all diabetes cases. A decrease or complete loss of pancreatic insulin results from the selective destruction of the pancreatic islet cells responsible for the production of insulin. The average age of onset is 12 years, and symptoms include weight loss, increased urination and thirst, and severe fatigue. Acutely, the disease can be fatal if blood ketone levels increase as a result of failed carbohydrate metabolism. Some 15 years after the onset of the disease, many patients acquire secondary complications, including retinopathy (loss of vision), neuropathy (destruction of peripheral nerves), renal disease, cardiovascular disease, severe peripheral vascular disease, and susceptibility to infections. The reason for the loss of pancreatic cells is not known, although an immunological reaction is suspected and a genetic component may be present. The disease has high rates of morbidity and mortality in those afflicted. There is no cure, and patients are treated with daily injections of exogenous insulin.

By contrast, non-insulin dependent (Type II) diabetes has an average age of onset of 40 years and is strongly associated with obesity, with clear heritable tendencies. Retinopathy, renal disease, and cardiovascular disease are long-term complications. Weight control and oral medication can control the disease in most patients; only a few require insulin to obtain normal blood sugar levels.[75]

Pancreatic transplantation from cadaveric and living related donors has enjoyed some success in the treatment of Type I diabetes: worldwide, 1 549 pancreas transplants were performed in 1 440 patients between December 1966 and June 1988. A one-year graft function was observed in 49% of patients, with a survival rate of 85%. Unfortunately, these results decline rapidly following the first year after the transplant. Significant immuno-suppressive regimens are often required, and the long-term use of these drugs contributes significantly to major organ failure.[76,77] More recently, investigators have considered the use of pancreatic tissue from aborted fetuses as a more attractive source of transplant material. The growth potential of the tissue, its possible reduced immunogenicity, and the consistently available supply suggest important therapeutic potential.

The development and functioning of the human pancreas *in utero* takes place between the eighth and twentieth weeks of gestation. Islet cells can be observed in the eighth week, alpha (glucagon-producing) cells at nine weeks, delta cells (somatostatin) in the tenth week, and beta (insulin-producing) cells at 11 weeks, with further growth and differentiation of minor cell types after 11 weeks. Great growth potential is exhibited by fetal islet cells: insulin content increases from 2 units/gram at 11 to 13 weeks gestation to 6 units/gram at 23 to 24 weeks.[78,79] The ability of developing fetal pancreatic islet tissue to respond physiologically to glucose by secreting insulin does not occur until late gestation, maturing over a period of months. Frozen fetal pancreatic tissue can be stored successfully using DMSO (70% survival) and retains its endocrine function and histological characteristics on thawing.[80]

Animal studies have been used to investigate the effectiveness of fetal pancreatic transplantation in rodent models of diabetes. The transplant of one fetal rat pancreas may be sufficient to normalize blood sugar, ameliorate frequent thirst and urination, and assist weight gain in the diabetic rat. Several fetal pancreas transplants are required to achieve overall normalization of carbohydrate metabolism, with reversal of secondary complications possible.[81] The limitations of cadaveric and living related donors transplantation and the results of animal studies form the basis for clinical experimentation in fetal pancreatic transplant for human diabetic patients.

The first report of human fetal pancreatic transplantation appeared in 1938; no demonstrable effect was seen in two patients.[82] Possibly 600 Type I diabetes patients have received fetal pancreatic transplant worldwide; results reported in the International Registry have been disappointing. Most recipients received pancreas from fetuses at 16 to 20 weeks gestation.[83] One study followed five patients for one year; insulin-producing cells were recovered from the patients at 9 to 14 months after transplant, although histological evidence of rejection was noted.[84]

Clinical transplantation research in this area was premised on the notion that fetal pancreatic tissue would prove less immunogenic than that from adult sources. Evidence is mounting that fetal pancreatic tissue may be at least as immunogenic as adult tissue.[85] The clinical failure of fetal pancreatic transplants when compared with animal studies is not understood: immunogenecity may be responsible, or technical considerations in the handling and processing

of the fetal pancreatic cells may be involved. Further basic research is needed to assess whether fetal pancreatic tissue transplant holds any real promise in the treatment of juvenile diabetes, particularly when compared with promising alternative research in the treatment of diabetes.

Tertiary (Basic) Applications

Human fetal tissues have been used or considered for use in a variety of clinical conditions. This part of the paper outlines these uses and reports the results of fetal therapy where known. It is important to note that these applications are generally primitive in terms of our experience and knowledge of their future potential.

Severe Combined Immune Deficiency (SCID)

SCID encompasses a number of rare congenital disorders where gene defects result in the failure of one or both lymphocyte lines (T or B) to function. Children born without lymphocyte function usually die as a result of infection before their first birthday. SCID became more widely known as a result of the press coverage of the "boy in the bubble," who lived in complete sterile isolation for most of his life. These diseases may show increased frequency in identifiable ethnic groups.[86]

Complete cure for this condition can be effected by HLA (tissue-typing) identical bone marrow transplant, and this is the treatment of choice. The result of the transplant is the appearance of a T-cell population carrying the donor's genetic markers. This therapy is limited by the fact that related HLA-matched donors cannot be found for more than 60% of patients.[87] In such cases the transplant of allogeneic human fetal liver, with or without fetal thymus, may be attempted; one report claimed survival of six out of eleven patients transplanted with liver and thymus tissues from the same fetus. No tissue matching was attempted in this study. A summary of 64 SCID patients who received fetal liver transplantation at various centres indicates that 22 patients had durable engraftments.[88] Thus fetal liver transplantation with or without fetal thymus may prove an effective treatment alternative in SCID where HLA-matched bone marrow is not available for transplant.

Leukemia

The term leukemia refers to a number of malignancies of the various types of white blood cells. Four major classes are identified: ALL (acute lymphocytic leukemia), AML (acute myelogenous), CLL (chronic lymphocytic), and CML (chronic myelogenous). The incidence of these forms varies with age, with ALL most common in children and young adults. AML is the leading cause of cancer deaths in children.[89]

Transplantation of bone marrow after destruction of the patient's own bone marrow by radiation and chemotherapy has greatly improved cure rates. Where HLA-matched or partly matched donors have been unavailable, fetal liver has been transplanted in a small number of leukemia patients. The results are difficult to interpret, because the numbers are small, patients

varied widely in severity of illness, and the criteria used to establish engraftment vary from centre to centre.[90,91] It is believed that recovery of the patient's own bone marrow occurs earlier after chemotherapy and fetal liver transplantation than with chemotherapy alone. Further basic investigation is warranted to elucidate the mechanisms of this recovery and to assess the future of fetal liver transplantation for certain leukemic patients.

Aplastic Anemia

This term refers to a variety of disease states characterized by the failure of the bone marrow to produce normal amounts of formed blood elements — red cells, white cells, and platelets. Approximately 50% of cases are idiopathic, with the balance attributed to agents such as viruses, chemical exposure, drug side-effects, and radiation accidents. Untreated, 50% of patients die within one year of diagnosis. Immuno-suppressive therapy with steroids, immune globulin, and HLA identical bone marrow transplant are therapies.

Human fetal liver was transplanted into 122 patients with aplastic anemia between 1960 and 1986, and 66 of these patients experienced partial or complete recovery. The mechanism of the recovery is not understood, as tissue engraftment was poor (much poorer than that seen when fetal liver is transplanted in leukemia patients).[92] Controlled randomized clinical trials would yield much needed data about the efficacy of fetal liver transplantation in treating aplastic anemia, and basic laboratory research is needed to reveal the mechanism of its success.

Inherited Metabolic Storage Disorders

Such disorders are one type of a broad classification of diseases labelled inherited metabolic diseases, where the principal defect is an absent or abnormal gene failing to produce a required enzyme. The absence of the enzyme results in disruption of function at both cellular and organ levels. Such storage disorders include Tay-Sachs disease and a rapidly growing list of disorders newly identified by molecular probes. The defect in metabolism prevents normal breakdown and elimination of biochemical substances, and the accumulation of materials at the cellular level may result in skeletal deformation, physical and intellectual retardation, and neurological abnormalities leading to severe dysfunction and death.[93]

A range of therapies for the management of metabolic disorders exists, but they are generally very limited in effectiveness. A report of 21 patients transplanted with human fetal liver cells indicates partial and transient benefits, with stabilization of tissue deposits, although few data are available.[94] The use of transplanted fetal material remains an area of basic research into the treatment of these rare diseases.

Radiation Poisoning

Radiation poisoning from thermonuclear bombs, nuclear power plant accidents, and medical diagnostic and treatment accidents produces acute and long-term health effects. Bone marrow in particular is exquisitely sensitive to damage by radiation; bone marrow suppression is one of the earliest acute effects of exposure to radiation, even at relatively low doses. Leukemias are a common long-term complication of radiation exposure.

Both bone marrow transplantation and fetal liver transplantation were used to treat victims of the Chernobyl accident. The effectiveness of the treatment is difficult to evaluate at this time, owing to limited numbers and follow-up time and wide discrepancies in patient age, health status, and severity of illness. The use of fetal cells may yet prove important in the treatment of this peculiarly modern menace.[95]

Feto-fetal Therapy

Two animal models for the *in utero* transplantation of fetal hematopoietic cells have been developed. Diseases that may be amenable to treatment by this method include beta-thalassemia, sickle cell anemia, Wiskott-Aldrich syndrome, chronic granulomatous disease, Kostman's syndrome, infantile malignant osteopetrosis, Chédiak-Higashi syndrome, Maroteaux-Lamy syndrome and SCID.[96] The method involves the transplantation of fetal hematopoietic stem cells to fetal rhesus monkeys and fetal sheep *in utero*. The preliminary results of these studies indicate successful tissue engraftment and freedom from graft versus host disease for up to two years.[97] Such investigations point to yet another application of fetal liver cells in transplantation therapy for disease in humans.

Alzheimer's Disease

Alzheimer's disease is an incurable progressive condition resulting in increasing loss of higher cognitive functions. The cause of Alzheimer's is not known; however, clinical findings are well defined, and a complement of neurological pathologies are consistently noted at post-mortem examination of the brain tissue of Alzheimer's patients. These neuropathologies include neurofibrillary tangles and plaque formations in cholinergic neurons, increased cellular aluminium content associated with these formations, and diffuse neuronal degeneration.

While the cause of Alzheimer's is not known, certain pathological features of the disease have led some researchers to suggest a common etiology with Parkinson's disease.[98] In recent investigations using a marmoset monkey model, memory loss for learned tasks was induced by surgical lesion of cholinergic neurons. The animals were then treated by surgical grafting of embryonic cholinergic tissue to the forebrain. The ability to perform the previously learned tasks was restored. The authors are concerned about generalizing the findings to Alzheimer's patients, however, since the brain degeneration associated with this disease is quite diffuse.[99,100] This is in contrast to the relatively discrete lesion seen in Parkinson's disease.

The application of fetal cholinergic transplantation in humans with Alzheimer's remains some years away; the societal importance of this disease and the present lack of effective treatments suggests that fetal tissue transplantation will be given serious consideration as a therapeutic possibility.

Acquired Immune Deficiency Syndrome (AIDS)

Acquired Immune Deficiency Syndrome (AIDS) reached pandemic proportions in the 1980s. The human immunodeficiency virus (HIV) was demonstrated to be the etiologic agent in 1984, yet there is neither cure nor vaccine. Certain drugs may prolong immune function in some patients, but AIDS is a uniformly fatal disease. Fetal cells have been used in the culture and manipulation of the HIV in culture, and there is some evidence that fetal hepatic cells may suppress the virus. Such research remains at the most basic level of investigation; yet it is certain that fetal cells will continue to be used for study of the HIV, and the use of fetal cells for AIDS therapy is a possibility.[101]

Plastic Surgery

Quite recently, fetal connective tissue and cartilage have been studied in a pig model of mini-autograft dermal injections. The tissue was compared with the conventional materials collagen and silicon. The results indicate that the fetal tissue should not be recommended for soft tissue filling in the face, because of localized inflammatory reaction. The report did, however, recommend research into the processing of collagen from fetal connective tissue for further investigation in this area.[102]

It is likely that other potential applications of fetal tissues in the treatment of disease and in industry will arise, as understanding of the remarkable properties of fetal tissues increases.

Alternatives and Limitations: Embryo Research

This segment of the paper briefly highlights major limitations and possible alternatives to the use of human embryos for research.

Perhaps the single most important factor limiting the use of human embryos in research, particularly basic research, is the relative scarcity of access to early pre-embryos. Human embryos created *in vitro* are created almost exclusively for the purpose of transfer to the uterus, in the hope of attaining a successful pregnancy. This is also true of the few instances where uterine lavage can be used for pre-implantation genetic diagnosis. Primary embryo research (the clinical trial) has been noted as a broad category of human pre-embryo research; this is the research that takes place in infertility treatment programs offering services such as IVF, GIFT, ZIFT, PROST, and embryo cryopreservation. Secondary embryo research comprises those clinical experiments where no precedent human observations or only minimal clinical data form the basis of the intervention, yet a clear possibility of important clinical benefit is discernible.[103]

Clinical research in both the primary and the secondary category circumscribes a peculiar area of biomedical endeavour that overlaps both treatment and investigation. It is likely that both categories will continue to be active areas of human pre-embryo research, given the demand for improved clinical results with the new assisted reproductive technologies. Some have argued that when the embryo is regarded as the sole research subject, the woman undergoing treatment for infertility may be lost sight of. Further arguments have been advanced that research that is intended to be therapeutic for the embryo may be non-therapeutic for the woman. Critics of this type of research argue that regulation of embryo research is necessary and that it must take account of the relationship between a woman's treatment, the dependence of embryos on women for gestation, and "the empirical uncertain nature of biomedical knowledge."[104] Regulation of pre-embryo research is being implemented in a growing number of jurisdictions. It may prove an important limiting factor in future embryo research.

The third category of human pre-embryo research is pre-clinical basic research that cannot be pursued adequately by the use of animal models; pre-implantation genetic diagnosis is one example. It is distinguished from the first two categories in that there is a declared intent from the outset that the experimental subjects will not be transferred into a receptive uterus.

Animal models provide the starting point for such investigations and should be exploited aggressively. However, there is ample evidence of limitations of generalizing animal model results to the human embryo. For example, there has been great interest in developing techniques for the cryopreservation of human oocytes as an alternative to embryo freezing, in part to circumvent ethical, social, and legal concerns about embryo freezing. It is now possible to freeze, thaw, fertilize, and transfer mouse ova, with normal resultant offspring. Investigations into the freezing of human ova have been far less successful, owing to subtle but critical structural differences between ova from the two species. Therefore, freezing of embryos or zygotes is the preferred procedure at this time.[105]

Thus, while animal models are essential for preliminary basic embryo research, there are strong procedural reasons for tertiary human embryo research in order to safeguard offspring and parents. Again, the major limiting factor to such research continues to be the scarcity of human embryos for any purpose other than attempting pregnancy.

Alternatives and Limitations: Fetal Tissue Research

Currently, there appears to be more than sufficient fetal tissue available for research and therapy from elective abortions alone. In the United States 1.6 million legal elective abortions are performed annually, and approximately 85 000 in Canada. More than 90% of these pregnancies are terminated in the first trimester.[106] Most research applications require fetal tissues between 8 and 14 weeks gestation, the exception being fetal pancreatic transplantation for juvenile diabetes.

Limitations to this apparently adequate supply are real, however. There is no accepted consent and screening process in place for obtaining aborted fetal material from women undergoing abortion. Concerns have been voiced about how consent could be obtained in a non-coercive fashion and about whether the woman who undergoes abortion has a moral right to donate the fetus. The current method of choice for first-trimester abortion is vacuum aspiration; this method severely macerates the collected conceptus material, so that distinct cell types are recognizable in only about 10% of cases. Alternative methods of abortion (D&C) yield improved fetal specimen quality but pose an increased morbidity risk for the woman.

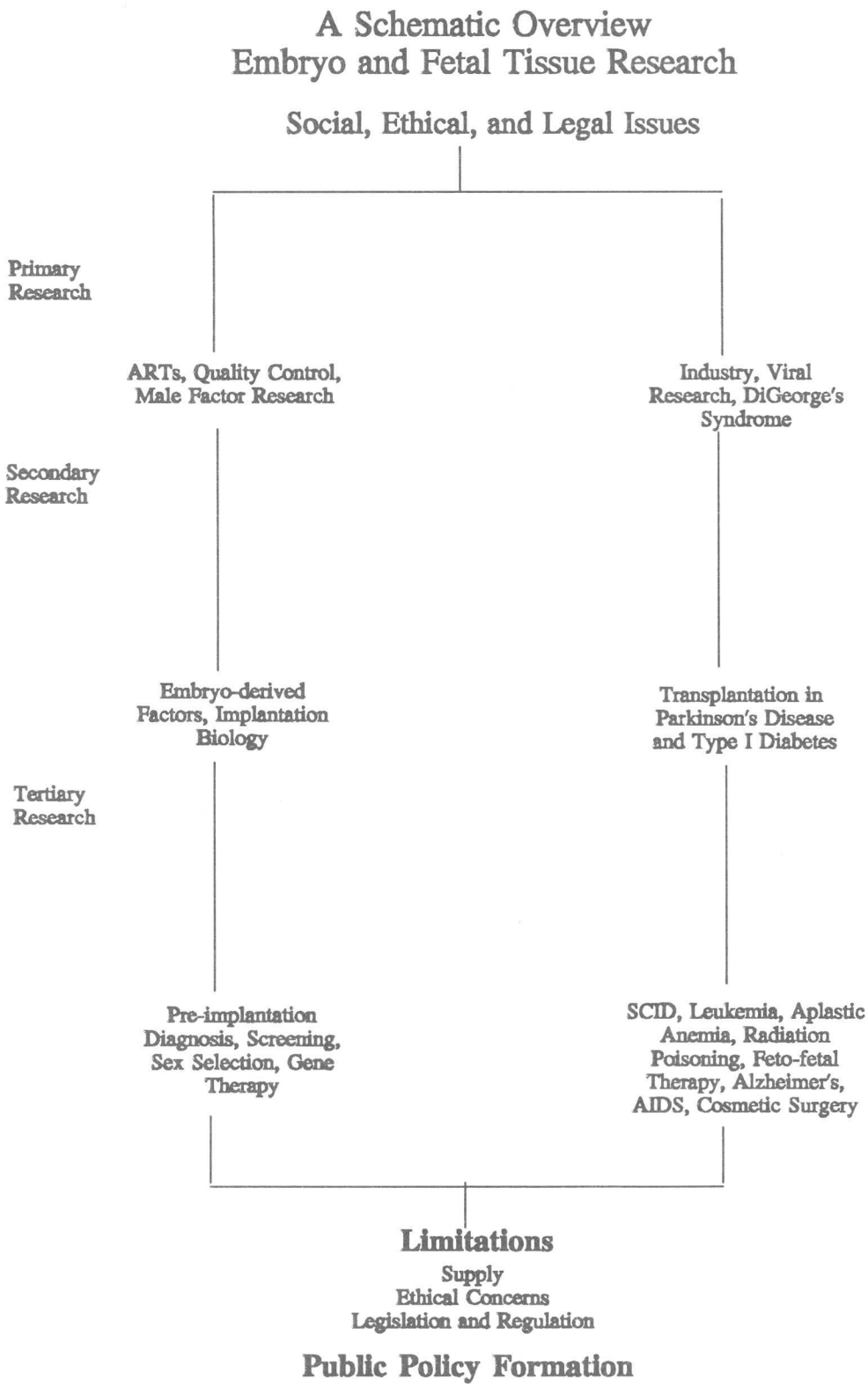
Perhaps in competition with a perceived need for fetal tissue for research and treatment is current research directed at new, earlier, and safer methods of first-trimester abortion — menstrual extraction and the abortifacient pill. Refinement and implementation of these or other early abortion methods would severely compromise the amount of fetal tissue available for research. In addition, there is debate about whether the use of aborted fetal tissue for research is ethically permissible, and this may sully the acceptability of using fetal tissues from elective abortions.[107]

These concerns have rekindled interest in the use of fetal material from spontaneous abortions. The potential problems of timing, tissue necrosis, genetic defect, and infection have been outlined; nonetheless, investigation continues into the potential exploitation of this source.[108] Non-surgical treatment of ectopic pregnancy is likely to eliminate this as a potential source of fetal tissues.[109]

A number of investigations are currently under way to examine the use of animal fetal tissue for transplantation and therapy in humans (xenografts). In the early 1960s, grafting of baboon and chimpanzee kidneys was attempted, as were liver grafts from chimpanzees. Four attempts have been made to graft animal hearts to humans. The results of these clinical experiments have been extremely poor and largely abandoned, owing to the severe graft rejection that results. The relatively non-immunogenic fetal tissue of non-human primates may prove more successful in transplantation experimentation.[110] The use of non-human primate fetal tissues for therapy will undoubtedly raise a host of ethical and social controversies in its own right.

Some research has shown promise that fetal cells may be successfully cultured and developed as continuing cell lines, thus ensuring a supply of tissues for both basic cell research and transplant. Cryopreservation of fetal tissue is successful for some cell types.[111] The refinement of these methodologies may serve in the future to address many of the practical, ethical, and social issues surrounding the use of fetal tissues.

In policy terms, the use of fetal tissues for treatment ideally does not detract from the basic research tasks of determining the underlying pathology responsible for diseases such as Parkinson's, diabetes, Alzheimer's and the inherited disorders, and discovering cures for these diseases. Today, Canada has no public policy governing the use of fetal tissues. In view of the rapid proliferation of applications for fetal tissues in industry and biomedicine, the need for public policy development is pressing.[112]


A Schematic Overview
Embryo and Fetal Tissue Research
Social, Ethical, and Legal Issues
Primary Research
ARTs, Quality Control, Male Factor Research
Industry, Viral Research, DiGeorge's Syndrome
Secondary Research
Embryo-derived Factors, Implantation Biology
Transplantation in Parkinson's Disease and Type I Diabetes
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Pre-implantation Diagnosis, Screening, Sex Selection, Gene Therapy
SCID, Leukemia, Aplastic Anemia, Radiation Poisoning, Feto-fetal Therapy, Alzheimer's, AIDS, Cosmetic Surgery
Limitations
Supply
Ethical Concerns
Legislation and Regulation
Public Policy Formation

Glossary of Terms

Allogeneic: cells and tissues from the same species.

Blastomere: the distinct, individual cells of the early embryo.

Chorionic villus: the projections of the outermost coating or layer encasing the developing embryo or fetus.

Cleavage: the division of a given cell, giving rise to two distinct cells.

Cryopreservation: the long-term preservation of organisms by specialized freezing and thawing techniques.

Cytosolic leakage: leakage or spill of cellular contents resulting from disruption of the cell membrane.

Cytogenetics: the study of genetics at the cellular level.

Dopamine: a biochemical messenger released by substantia nigra cells; important for normal initiation and control of movement.

Fetal cardiac arrhythmias *in utero*: abnormal fetal heart rates, detected before the fetus is born.

Hematopoietic cells: those cells involved in production of blood cells and blood components.

Human chorionic gonadotropin: also hcg, a hormone of early pregnancy; testing for hcg in maternal blood and urine is a routine diagnostic test for pregnancy.

Human germ cells: the human reproductive cells, namely sperm and egg cells, and the progenitor cells giving rise to them.

Hypoparathyroidism: congenital or acquired, failure or absence of the parathyroid glands; linked to failed development of the thymus in DiGeorge's syndrome.

Immunogenic: provoking an immune response.

Immunogenicity: the tendency or degree to which any material (including biological materials) will provoke an immune response in a recipient of that material.

Intra-uterine fetal surgery: surgical procedures performed on a fetus while it remains in the maternal environment.

Non-exogenous ovarian stimulation cycle: ovarian cycle where the natural follicular development is neither induced nor enhanced by treatment with hormones or drugs (normal cycle).

Polar body: a cellular extrusion resulting from the last meiotic (final maturation) division of the oocyte.

Polyploidy: appearance of more than two pronuclear bodies in a newly fertilized ovum; see polyspermy.

Polyspermy: impregnation of an ovum by more than one sperm cell. When this occurs, more than two pronuclei can be seen by microscopic visualization in the early hours following fertilization.

Pronuclei: the separate nuclei of the sperm and ovum, before these unite to form the single definitive nucleus of the fertilized ovum.

Somatic cells: the cells of the human body, excluding the germ cells (ova and spermatozoa).

Substantia nigra cells: literally, cells of the "black substance"; these brain cells produce dopamine and are implicated in Parkinson's disease. The name reflects their appearance.

Teratogenicity: that quality of any agent — chemical, biological, or physical — that contributes to or causes malformation of a developing organism.

Totipotentiality: the capacity of a single cell to develop into or to generate a complete organism.

Twinning procedures: technical processes used to divide a mammalian embryo in its early stages (usually 4 or 8 cells), so that two identical embryos result; if these are successfully transferred to a receptive uterus, identical offspring may result. This effect also occurs naturally, if rarely, in humans.

Zona pellucida: the transparent membrane forming the cell wall in the mammalian ovum.

Zygote: the organism resulting from the union of the human germ cells — ovum and the sperm — with its new and distinct genetic constitution.

Notes

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Royal Commission on
New Reproductive Technologies

CANADA

Commission royale sur les
nouvelles techniques de reproduction

COMMUNIQUE

FOR IMMEDIATE RELEASE
13 February 1992

ROYAL COMMISSION ON NEW REPRODUCTIVE TECHNOLOGIES RELEASES PAPER ON THE USE OF HUMAN EMBRYOS AND FETAL TISSUES

OTTAWA — There is a pressing need for Canada to develop public policy to address the rapid proliferation of applications for human embryos and fetal tissues in both industry and biomedicine, according to a paper released today by the Royal Commission on New Reproductive Technologies.

The paper, entitled **The Use of Human Embryos and Fetal Tissues: A Research Architecture**, was prepared for the Commission by Michelle Mullen, BSc, MHP, of the University of Toronto Centre for Bioethics. The study reviews the special biological properties of the human embryo and fetus which have fostered intense interest at both the clinical and basic research level. It identifies the possible applications in therapy, transplantation, and industry of human embryos and fetal tissue.

In the paper, Mullen says embryo research is a key factor in the development of new technologies for assisted reproduction, such as cryopreservation (embryo freezing). She points to dramatic advances in the areas of genetic diagnosis and gene therapy, and outlines possible research directions to study embryo growth, differentiation, implantation, and immunology.

As well, she examines the use of human fetal tissue which has been used for decades by pharmaceutical and biotechnology companies in the development of vaccines and to test the efficacy of new pharmaceutical products. Mullen notes that it has more recently been a critical tool in viral research on infections such as human influenza, hepatitis B, measles, and human immunodeficiency virus (HIV).

The author concludes that, while the scope of application for human embryo and fetal tissue research is increasing, there is the lack of public policy to address the social, ethical, legal, and regulatory issues their use raises.

The Royal Commission on New Reproductive Technologies was established by the federal government in October 1989. Its mandate directs it to examine medical and scientific developments around new reproductive technologies, in particular their social, ethical, health, research, legal, and economic implications, and their impact on women, children, and society as a whole.

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The Commission has established a multi-faceted Consultations program to enable it to hear the views and opinions of people from all sectors of Canadian society. It has also set in motion a comprehensive and multi-disciplinary program of Research and Evaluation to provide rigorous, credible, and timely information about, and critical analysis of the issues surrounding new reproductive technologies.

Many academics, researchers, and groups who have participated in the Commission's work have requested access to the data and information generated by the Commission on these subjects to help them consider their positions. In response to these requests, the Commission has obtained permission to publish some research papers in advance of its Final Report. Reports such as this one will be released over the duration of its mandate to assist those working in the field of reproductive health and new reproductive technologies, and to inform the public.

Copies of this publication, as well as information about the Royal Commission on New Reproductive Technologies, are available by calling the Commission, toll-free, at 1-800-668-7060.

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(For further information, or for a copy of **The Use of Human Embryos and Fetal Issues: A Research Architecture**, please contact the Communications Division at (613)951-3706.)

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POUR DIFFUSION IMMÉDIATE
Le 13 février 1992

LA COMMISSION ROYALE SUR LES NOUVELLES TECHNIQUES DE REPRODUCTION PUBLIE UN DOCUMENT SUR L'UTILISATION D'EMBRYONS ET DE TISSUS FOETAUX HUMAINS

OTTAWA -- Selon un document de recherche publié aujourd'hui par la Commission royale sur les nouvelles techniques de reproduction, il est urgent que le Canada se dote d'une politique générale pour contrôler la rapide prolifération de la recherche sur l'embryon et le foetus humains, tant dans l'industrie qu'en biomédecine.

Intitulé **La recherche sur les embryons et les tissus foetaux humains : organisation de la recherche**, le document a été rédigé pour la Commission par M^me^ Michelle Mullen, B.Sc., MHP, du Centre de bioéthique de l'Université de Toronto. M^me^ Mullen y examine les propriétés biologiques propres à l'embryon et au foetus humains qui ont suscité un intérêt intense tant en recherche clinique qu'en recherche fondamentale. Elle aborde également les diverses applications auxquelles ces propriétés pourraient donner lieu en thérapie, pour la transplantation et dans l'industrie.

M^me^ Mullen affirme que ce type de recherche est essentiel à la mise au point de nouvelles techniques de reproduction assistées, comme la cryopréservation (congélation d'embryon). Elle souligne les progrès énormes réalisés dans les domaines du diagnostic génétique et de la thérapie génique, et indique quelques orientations possibles pour la recherche, par exemple sur la croissance, la différenciation et l'implantation de l'embryon, ainsi qu'en immunologie de l'embryon.

Elle examine également les diverses utilisations du tissu foetal, dont se servent depuis des dizaines d'années l'industrie pharmaceutique et l'industrie de la biotechnologie pour mettre au point des vaccins et pour vérifier l'efficacité des nouveaux médicaments. M^me^ Mullen précise que, ces dernières années, le tissu foetal a été un facteur-clé de progrès dans le domaine de la recherche sur les infections virales, dont la grippe humaine, l'hépatite B, la rougeole et le virus de l'immunodéficience humaine (VIH).

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M^me^ Mullen conclut que, même si le champ d'application de la recherche sur l'embryon humain et le tissu foetal s'élargit sans cesse, il n'existe aucune politique générale s'appliquant à ses incidences sociales, morales, juridiques et réglementaires.

Créée par le gouvernement fédéral en octobre 1989, la Commission royale sur les nouvelles techniques de reproduction a pour mandat d'examiner les progrès médicaux et scientifiques touchant les nouvelles techniques de reproduction, et en particulier leurs incidences sociales, morales, juridiques et économiques, leurs incidences en matière de santé et de recherche, de même que leurs répercussions sur les femmes, les enfants et la société dans son ensemble.

La Commission s'est dotée d'un programme diversifié de consultation afin de recueillir les points de vue et les opinions de Canadiens et Canadiennes de tous les milieux. Elle a également mis sur pied un vaste programme multidisciplinaire de recherche et d'évaluation pour recueillir des renseignements rigoureux, crédibles et pertinents sur toutes les questions qui entourent les nouvelles techniques de reproduction, et pour pouvoir en faire une analyse critique.

De nombreux universitaires, chercheurs et groupes qui ont participé aux travaux de la Commission ont demandé à avoir accès aux données et renseignements accumulés par la Commission pour les guider dans leur réflexion. En réponse à ces demandes, la Commission a obtenu l'autorisation de publier certains documents de recherche avant son rapport final. La Commission publiera donc, pendant la durée de son mandat, d'autres documents similaires, tant pour aider les professionnels et travailleurs des domaines de la santé génésique et des nouvelles techniques de reproduction que pour renseigner le public.

On peut se procurer des exemplaires de cette publication, et aussi se renseigner sur la Commission, en composant sans frais le numéro 1-800-668-9781.

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(Pour plus d'information ou pour obtenir un exemplaire de la publication **Recherche sur les embryons et les tissus foetaux humains : organisation de la recherche**, appelez la division "Communication" au numéro (613) 957-0655.)

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