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Stem Cell Therapies in Obstetrics and Gynaecology: The Female Urogenital Tract and the Fetus as Sources and Targets for Molecular and Regenerative Medicine

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This is the first edition of this paper.

1. Introduction

Reproductive tissues are now recognised as sources of stem/progenitor cells and as targets for regenerative medicine. This paper briefly reviews the progress and future challenges of applying regenerative medicine to the urogenital tract and the use of stem cells for the treatment of inherited genetic diseases, especially those with irreversible perinatal damage. Stem cells sourced from reproductive tissues have been used or investigated for their potential use in other areas such as haematological disease, traditionally treated with haematopoietic stem cells (HSC) from adult sources but for which toxic adjuvant treatments, or bone tissue engineering, are concurrently needed.¹ However, applications of such methods, together with the use of stem cells for gamete generation, are beyond the scope of this paper.

Briefly, stem cells have two properties. The first is the ability to self-renew or undertake numerous cell divisions, while maintaining an undifferentiated state. The second is that of multipotency; the capacity to differentiate into a mature cell type. Totipotent stem cells, from the morula, can differentiate into embryonic and extraembryonic cell types, and can produce a complete and viable organism. Pluripotent stem cells descend from totipotent cells and differentiate into tissues derived from any of the three germ layers, including fetal tissues (amniotic fluid cells, the amnion, umbilical cord and placenta). Embryonic stem cells are pluripotent, having been derived from the inner cell mass of a blastocyst. Multipotent stem cells differentiate into various tissues originating from a single germ layer, for example, mesenchymal or haemopoietic stem cells. Unipotent cells such as muscle satellite cells on the other hand, produce only their own cell type but show greater self-renewal than fully mature cells. Theoretically, the more primitive or “potent” a stem cell is, the more predisposed it is to uncontrolled cell division, and the greater its potential for oncogenesis. Although there is some concern regarding the oncogenic potential of pluripotent stem cells such as embryonic stem cells and induced pluripotent stem cells, nonpluripotent cell sources are not inherently oncogenic.

Embryonic stem (ES) cells offer the prospect of novel treatments in regenerative medicine although progress here has been impeded by controversies surrounding the source. However, multipotent cells are now being isolated from several fetal tissues, readily obtained as products of diagnostic tests, at disruption of pregnancy and at birth. In the field of gynaecology, regenerative medicine approaches to repair or replace damaged or diseased urogenital tract organs, such as the urinary sphincter, pelvic floor, uterus, ovaries and vagina, are currently in the preclinical and clinical phases of study. In obstetrics, the area of stem cell transplantation has been largely focused on fetal therapy.

2. Stem cells from reproductive tissues

Over the past decade, stem cells have been isolated from embryonic, fetal and extra-fetal tissues, as well as adult gonads. Extra-fetal tissues such as the amniotic membranes and placenta share a common origin; the inner cell mass of the blastocyst, which gives rise to the embryo, yolk sac, mesenchymal core of the chorionic villi, chorion and amnion. Most likely because of their shared origin, amniotic fluid and the placenta contain a heterogeneous population of progenitor cells. This includes mesenchymal, hematopoietic, trophoblastic and, perhaps, more primitive stem cells. Although the chemical and cellular composition of the amniotic fluid varies with gestational age and fetal health status, mesenchymal stem cells (MSC) can be consistently isolated at any gestation. Placental and amniotic fluid MSC have been

shown to differentiate into most cell types of mesodermal lineage, as well as into a few cells of ectodermal and endodermal lineages.² The full spectrum of differentiation remains to be defined in its entirety. Additional cell types have been isolated from amniotic fluid, including a population of CD-117 positive cells which express markers of pluripotentiality, demonstrate self-renewal through more than 50 population doublings while maintaining telomere length, are nontumorigenic and can differentiate into tissues of all three germ layers.³ Their ability to be used for autologous and allogeneic cell sources for regenerative medicine applications are currently being explored but will require a facility dedicated to their characterisation, immunogenicity properties and storage.

Fetal stem cells have been isolated from various parts of the fetus. Early gestation HSC from the bone marrow and liver are well characterised,⁴ with placental HSC being described more recently.⁵ Primitive human fetal MSC (hfMSC) have been isolated from virtually every part of the developing fetus,⁶ have higher proliferative capacity,⁷ express significant higher amounts of telomerase and have longer telomeres⁸ compared to their adult counterparts. Additionally, they differentiate efficiently into neuronal and muscle lineages and have shown more robust differentiation down the osteogenic lineage than the later perinatal and adult sources of MSC,⁹ suggesting their utility for postnatal applications for bone tissue engineering.¹ Primitive hfMSC are readily transduced by integrating vectors and do not express HLA-II or costimulatory molecules CD80 and CD86,¹⁰ indicating their utility for ex vivo gene therapy and allogeneic use.¹¹

3. Sample collection and stem cell banking

The use of fetal and perinatal stem cells in regenerative medicine should be regulated through appropriate institutional and regulatory boards. Protocols for optimal collection of such tissues should maximise the quantity and quality of stem cells derived prior to their banking within Good Manufacturing Practice (GMP) facilities. The banking of umbilical cord blood (UCB) is an established process in many centres worldwide, is a source of HSC¹² and MSC¹³ and has established utility for allogeneic postnatal treatment of haematological diseases such as leukaemia and bone marrow failure.¹⁴ Although there is a trend towards nondirected autologous banking of UCB in low risk families promulgated largely by private cord blood banks, this practice has not been supported by several academic institutions.^{15,16} However, there may be an advantage to private banking for directed use in a family with a sibling affected by a medical condition that can potentially benefit from UCB.¹⁷

Harvesting of stem cells from fetal tissue following medically indicated pregnancy termination should be guided by the specific cell targeted, if the harvested cells are intended for a particular application. hfMSC have been collected from the liver for intrauterine transplantation targeting osteogenesis imperfecta and for the treatment of haemoglobinopathies, both of which should be processed in GMP conditions.¹⁹ However, as a source for donor cells, the majority of the parts of a fetus can be harvested; from the central nervous system for neural stem cells, to the skin for epidermal progenitors.^{20,21}

4. Approaches for regeneration of the urogenital tract

In the past decade, several studies have focused on the use of regenerative medicine to correct insufficiencies in the urogenital tract.

4.1 Stem/progenitor cell treatment of stress urinary incontinence (SUI)

Biomaterials aim to resolve SUI by providing structural/mechanical bladder neck support. This is executed via the injection of autologous stem or urethral tract progenitor cells, which has been culture-expanded before retransplantation, into the urethral sphincter. This aims to restore and regenerate rhabdomyosphincter muscle content and function. Continuing clinical and animal studies suggest that injected autologous cells may integrate into the sphincteric complex and differentiate, leading to “sphincter regeneration”.²² However, it is unclear as to the precise fate of injected cells, such

as the extent to which injected cells integrate and adopt a functional myogenic phenotype, or perform a growth factor secreting feeder function to stimulate regeneration. Nevertheless, the concept of stem cell injection for sphincteric muscle regeneration is the subject of research in a number of centres and the development of improved forms of treatment for SUI might yet prove to be one of the major clinical benefits of regenerative medicine.

4.2 Bladder reconstruction

A tissue-engineered and urothelial-lined bladder provides a functional barrier against urine exposure and could help to overcome most of the serious complications associated with conventional entero-cystoplasty. The requirements of such “engineered” tissue are more complex than just structural support and need to fulfil the functions of the normal healthy bladder wall by combining compliance (normally conferred by the detrusor smooth muscle) with a urinary barrier (normally provided by the specialised urothelial lining).²³

Three fundamentally different strategies have been investigated to augment or reconstruct the urinary bladder:

- *Use of acellular natural or synthetic biomaterials*

With this approach an acellular biomaterial graft is used as a tissue implant which becomes incorporated through the ingrowth of cells from the surrounding native host bladder. As biomaterials can be produced, stored and used as “off-the-shelf” materials, this approach circumvents technically demanding and expensive cell-based and patient-specific procedures. The best studied of these biomaterials are small intestinal submucosa and bladder-derived acellular matrix. Success varies with these materials and seems to be dependent on the graft size and biomaterial processing.²⁴

- *Implantation of scaffolds pre-incubated with autologous cells in vitro*

Tissue is engineered by seeding cultured cells (usually autologous urothelial and smooth muscle cells) onto a biodegradable scaffold in vitro prior to implantation. In 2006, Atala et al.²⁵ reported the first clinical tissue-engineered bladder augmentation procedures in seven patients aged 4–19 years with neuropathic bladders in a follow up period ranging from 22–61 months. Autologous urothelial cells from bladder biopsies were cultured in vitro for 7–8 weeks prior to seeding on collagen or collagen coated polyglycolic acid (PGA) scaffolds. Biopsies taken from the engineered augments showed “adequate structural architecture and phenotype” without causing metabolic problems, urinary calculi or abnormal mucus production. Functional urodynamic data revealed best outcomes in patients receiving cell-seeded collagen coated PGA scaffolds wrapped in omentum as a vascular bed at the time of reconstruction. Although this is the first report indicating the feasibility of creating a full thickness bladder wall from in vitro propagated urothelial and smooth muscle cells in patients, important questions remain, particularly regarding the fate of the seeded cells in the regenerated organ.

- *Combining tissue-engineered urothelium with a host vascularised smooth muscle segment (“composite cystoplasty”)*

Composite cystoplasty describes an approach to combine autologous urothelial cell sheets grown and expanded in vitro with a de-epithelialised pedicled smooth muscle segment from the host.²⁶ There are advantages of this strategy over a completely tissue-engineered organ. The first is that the in vitro component of the procedure is confined to the propagation of urothelium and the second is that a single, highly regenerative cell type is combined with a preformed, vascularised smooth muscle tissue. The rationale stems from long term complications of conventional entero-cystoplasty which arise almost entirely from the unsuitable properties of the intestinal epithelium rather than the smooth muscle component of the bowel wall.

4.3 Biomaterials for pelvic floor prolapse (POP) and urinary incontinence (UI)

There are two distinct requirements of bioengineered materials in this area. The first requirement is to provide mechanical support to the pelvic organs. The second is to generate new muscle which can perform in an integrated manner with the existing organs, such as with new sphincters. The main disadvantages of synthetic meshes for POP and pelvic floor related UI are the complications of erosion and extrusion. Biomaterial developed to replace meshes would need to contain the strength of meshes and also be bioabsorbable. Hybrid biomaterials may be fabricated using a combination of synthetic and naturally derived polymers and can possess many desired characteristics of replacement tissues, including good biocompatibility and appropriate biomechanical and biochemical properties. Fibre diameter can be altered by changing the relative concentration of the two polymers, which in turn could be used in producing the most appropriate biomaterial for use.²⁷ In order to fulfil the aim of producing an optimised biomaterial for restoring pelvic floor function and resolving SUI in all patients, further *in vivo* testing is essential.

4.4 Uterine reconstruction.

Factors that affect the integrity of the uterus, such as congenital/acquired malformations or disease, often compromise a woman's reproductive potential. As referenced earlier the uterus is a source of progenitor cells that enhance the ability of self-repair.²⁸ Studies by Taylor's²⁹ group have demonstrated that bone marrow stem cells can engraft and produce endometrium from the beginning. This finding, and that of Tanaka et al.³⁰ regarding sustained engraftment of primate embryonic stem cells in the ovine uterus, suggest that cell therapy may be used to regenerate uterine tissues for women with uterine factor infertility.

4.5 Vaginal reconstruction

The first-line treatment for vaginal agenesis is vaginal dilation therapy. However, vaginal reconstruction is often performed as a treatment for vaginal agenesis using nonvaginal tissue substitutes, such as segments of large intestine or skin. These materials are not functionally or anatomically ideal. Using a rabbit model, De Filippo et al.³¹ reported the construction of a functional vagina using autologous cells expanded from a small vaginal biopsy. Six months after total vaginal replacement, radiographic analysis of rabbits implanted with the neo-vagina demonstrated wide, patent vaginal calibres without strictures. Histological analysis revealed well organised epithelial and muscle cell layers. Physiological studies showed normal range responses to electrical stimulation or to an adrenergic agonist.

5. Opportunities for intrauterine stem cell transplantation (IUSCT) for monogenic diseases

5.1 Rationale for IUSCT

The objective of IUSCT is to correct a genetic disorder early in the evolution of disease through the engraftment of normal functional stem cells. There are several aims of IUSCT; replacing the missing or aberrant protein before permanent organ damage occurs, rescuing an affected fetus from a perinatally-lethal condition or improving postnatal survival and preserving vital functions. The fetal milieu offers the best chance of cure for diseases that cause end-organ damage in utero, because of the opportunity to correct pathology at the early stages of cellular damage. Tolerance toward the transplanted cells may be induced in the pre-immune fetus before antigen-recognition develops at the end of the first trimester, in order to facilitate engraftment in an immature bone marrow compartment where there is little competition from host cells. Because of the physical limitation on the quantity of donor stem cells that can be harvested and transplanted, fetal size offers a distinct advantage over the several-fold larger neonate. This advantage is that it allows a greater concentration of stem cells to be achieved within the target organ compared to a larger postnatal recipient. Potential target organs in early development such as the central nervous system have a greater susceptibility to transduction by vectors or engraftment by stem cells. This may be a function of the temporal appearance of cell surface receptors and metabolic pathways. Restrictive barriers such as the blood-brain barrier are also more permissive in early development which may contribute to more efficient stem cell engraftment in the fetus.³²

5.2 Applications of IUSCT

The most cogent argument for IUSCT would be to treat diseases which can result in perinatal lethality, such as α -thalassaemia and those which cause irreversible end organ damage such as some mucopolysaccharidoses (MPS). However, the ontological advantages, especially immune naiveté, which allows for the use of normal allogeneic cells, will make this attractive to diseases of early postnatal onset, such as β -thalassaemia and sickle cell anaemia, muscular dystrophies and other lysosomal enzyme disorders (Appendix 1). Experimental data in small and large animals demonstrate varying degrees of engraftment following IUSCT.³³ Although, because genetic knockout large animal models are currently unavailable, efficacy of this treatment in arresting or reversing pathology remains to be seen. Human experience with in utero haematopoietic stem cell transplantation (IUHSC), performed for a spectrum of genetic diseases, informs us that successful treatment has only been achieved in a few fetuses with inherited immunodeficiencies.³⁴ The success is likely to be mainly due to the availability of a stem cell niche and the lack of an immune response to the donor cells. IUSCT has been performed unsuccessfully in fetuses with haemoglobinopathies or enzyme deficiency disorders at a time in gestation when immune-competency had been attained. More information is required concerning the factors governing the efficacy of IUSCT and obstacles to successful engraftment. Important barriers include a normal host haematopoietic microenvironment that competitively inhibits donor stem cell proliferation in bone marrow niches, explaining the success seen almost exclusively in immunodeficient syndromes.³⁵ In mouse models, maternal humoral or cell-mediated response to the transplanted cell or to the transgenic protein can interfere with cell engraftment and transgene expression in transplanted fetuses.^{36,37} While this has not been validated in large animal models, it is likely that the use of paternal HSC and the avoidance of breastfeeding may be required to negate these immune barriers. Successful IUSCT will potentially negate the need to find a haplo-identical allogeneic donor and avoid the myeloablative side effects associated with postnatal bone marrow transplantation.

The use of hfMSC has been explored for diseases involving a mesenchymal origin, where they undergo site-specific differentiation and contribute to the repair of the tissues after IUSCT with hfMSC in both muscular dystrophy and osteogenesis imperfecta (OI) mouse models.^{38,39} While there was no cure in the case of muscular dystrophy, hfMSC-IUSCT in a murine model of OI led to normalisation of bone indices, and a two thirds reduction in fracture frequencies.⁴⁰ Clinical transplantation of hfMSC in a case of Type III OI resulted in a better than expected clinical outcome.⁴¹ A fetus with prenatally diagnosed OI has been transplanted in utero in Singapore with preliminary results suggesting an augmentation of the growth rates (Chan, personal communication). It is likely that this approach will be adopted as an open-label Phase I trial.

5.3 Allogeneic versus autologous approaches

The fetus can be a recipient for autologous or allogeneic transplantation of HSC to treat monogenic disorders which have been shown to benefit from bone marrow transplantation in the postnatal patient, such as β -thalassaemia major, severe combined immunodeficiency (SCID) or selected lysosomal storage disease (LSD).⁴²⁻⁴⁴ Allogeneic donor stem cells that are transplanted before the onset of fetal immune maturity can achieve central tolerance and avoid rejection. An ex vivo gene transfer approach via the harvest of autologous stem cells through fetal blood sampling or fetal liver biopsy in early gestation is another approach. Here, defective autologous stem cells are corrected through gene transfer technologies before reintroduction into the fetus, reducing the risk of immune rejection.⁴⁵ Whilst this approach may appear attractive, it may also present significant technical obstacles for clinical translation. This is because it would require confirmation of pregnancy, molecular diagnosis, fetal stem cell harvesting, gene correction and reintroduction into the fetus towards the end of the first/early second trimester window. Experience with large animal models in this field has been limited to the fetal sheep model, where both allogeneic and autologous first and second trimester fetal liver mononuclear cell transplantation resulted in similar rates of engraftment. However, the harvest of these fetal liver cells through a 20G needle resulted in a loss of 7 out of 15 (47%) fetuses. A published clinical series showed that ultrasound or

fetoscopy guided human fetal liver biopsies result in a far lower procedure-related pregnancy loss rate.⁴⁶ Highly proliferative first trimester fetal HSC circulate in significant numbers, have favourable engraftment kinetics, and are thus a possible source of autologous HSC. Although, only limited safety data has accrued to date with 25–33% incidence of bradycardia and a loss rate between 5–8% at 12 weeks.⁴⁵ Amniotic fluid may be a feasible and safer alternative source if significant HSC numbers can be derived.⁴⁷

5.4 Tissue engineering using fetal stem cells for specific intrauterine applications

Fetal stem cells, as a source of material for tissue bioengineering, have the potential for far-reaching application. These applications include the utility of fetal mesenchymal stem cells for bone therapies,¹ and the potential application of amniotic membrane and fluid-derived stem cells for diseases of the skin, liver and heart.⁴⁸ Although a detailed discussion is not within the scope of this paper, stem cells derived from fetal sources may have specific intrauterine application for sealing amniotic membranes following preterm rupture or amniocentesis. They may also be a valuable source for developing autologous implants to be used in reconstructive surgery for congenital heart disease, craniofacial and neural tube defects.^{50–52}

6. Opinion

Stem cells from reproductive tissues have now been isolated and well characterised, with significant advances made in directing their differentiation, genetic manipulation and integration into scaffolds and bioreactors.

We are beginning to understand the potential role of regenerative medicine in the treatment of urogenital diseases. The opportunity to widen such applications within reproductive medicine is becoming apparent. Progenitor cells are likely to play an important role in normal uterine and ovarian physiology and are probably involved in the response of these tissues to injury and disease. The potential to exploit these processes for the fabrication of tissue or organ implants is promising, such as the utilisation of urogenital tract cells in the construction of the urinary bladder. These cells may also be used as a platform for in place organ regeneration. In addition, stem cells are likely to play a role in reproductive tract pathology in some cancers, endometriosis and other diseases. Greater understanding of stem cell biology and the processes that result in uncontrolled reproductive cell proliferation may prove helpful in the treatment of these conditions and could yield novel alternatives to standard treatments for urinary incontinence, infertility and structural repair.

In the field of obstetrics, particularly fetal medicine, the twin advances made in prenatal molecular diagnosis and the advent of stem cell transplantation and ex vivo gene transfer may impact greatly in the treatment of a wide range of inherited genetic disorders. Some clinical success has already been reported for immunodeficiency disorders and skeletal dysplasia, while emerging data from preclinical models in mice and in particular nonhuman primates will inform future clinical translation of this technology for other genetic diseases. The first human experiments will commence where there is a strong clinical indication for intervention, when the potential benefits outweigh putative risks and an understanding that there may still be issues that cannot be addressed with animal models is in place. Given the burden of β -globinopathies and the progress made in the preclinical field, it is likely that the haemoglobinopathies will be one of the first diseases to reach broad clinical translation.

Women with a prenatal diagnosis of severe or lethal genetic disease are currently faced with the choice of having an affected baby with only palliative postnatal therapy available or pregnancy termination and possibly future pre-implantation genetic diagnosis and embryo selection. The heretofore unrealised potential of IUSCT may be the only therapeutic option. On the other hand, there is limited evidence that a complete cure can be achieved, and therapeutic benefit may only be seen after repeated pre and postnatal dosing. Thus, partial IUSCT may alter the outcome from perinatal demise to survival of a severely disabled child who would still require postnatal therapy. Bystander maternal effects will also be an important consideration which may mitigate the desire to treat the fetus. Potential adverse effects may be related to the procedure by which the material is injected, including transplacental cell trafficking.

The authors recommend that clinicians are aware of the various ethical issues at play when IUSCT is contemplated. It is also recommended that a multidisciplinary approach is adopted with a transparent discussion about the known limitations, putative benefits and unknown or unquantifiable risks. We recommend that each clinical case be considered on its individual merits until there is a greater body of evidence on the efficacy of fetal therapy from which to draft guidelines. Centres of excellence in fetal medicine and therapeutics research should take the lead in developing the scientific expertise in this field and the clinical guidelines for future trials, in discussion with the regulatory and ethical authorities.

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Appendix 1. Summary of potential candidate diseases for IUSCT

Disease type	Disease	Aberrant gene/protein	Postnatal treatment with stem cell transplant recommended?	IUSCT attempted in human fetuses?
Haemoglobinopathies	α -thalassaemia	α -globin	Yes	Yes
	β -thalassaemia	β -globin	Yes	Yes
	Sickle cell disease	HbS	Yes	Yes
Lysosomal storage disease	MPS I (Hurler syndrome)	α -L-iduronidase	Yes	Yes
	MPS VI	Arylsulfatase B	Yes, for severe disease	No
	MPS VII	β -glucuronidase	Yes	No
	X-linked Adrenoleukodystrophy	ABCD1 gene	Yes, for cerebral X-ALD	No
	Globoid-cell leukodystrophy	GALC gene	Yes	No
	Gaucher disease	β -glucosidase	Type 1 only	No
	Niemann Pick disease	SMPD1 gene	Type B only	Yes
Immunodeficiency syndromes	X-SCID	IL-2R γ (common gamma chain)	Yes	Yes
	ADA-SCID	adenosine deaminase	Yes	No
	Chronic granulomatous disease (CGD)	p91-PHOX	Yes	Yes
Genetic bone disorder	Osteogenesis imperfecta	COL1A1	No	Yes (hfMSC)

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