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# Fetal Chimerism and Fetal Thymic Transplantation

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**Abstract**

Fetal tissue transplantation is an attractive field of modern medicine that can have immense application in treating several refractile conditions. Fetal tissue transplantation is an allogeneic transplantation procedure and like any allogeneic transplantation they contribute towards the formation of a chimera at the cellular and tissue level grossly and can be classified as a macro chimerism. The fetus during the time of pregnancy also takes part in micro-chimerism through fetomaternal cell trafficking where there is an exchange of the maternal and the fetal cells through the blood-placental barrier. Apart from the creation of a stable chimerism, fetal tissues also play a role in healing. Fetal thymic transplantation is one of the most exciting applications of regenerative medicine which has also been discussed briefly with some case studies.

Keywords:

Fetal tissue; Blood-placental barrier; Di George's syndrome

## Introduction

On November 10, 1988, Dr. Curt Feed and his colleagues did a fetal brain tissue transplantation in a 52-year-old patient suffering from Parkinson's disease. Since then fetal tissue transplantation has come a long way [1]. According to the Guttmacher institution between 2010-2014, approximately 56 million abortions occur globally [2]. Most of these fetuses are thrown away in the hospital pits and incinerators as a biological trash material without realizing its true potentialities. Since 1999, Bhattacharya et al. has been practicing the application of fetal tissue transplantation freshly collected and fully screened from consenting mothers in a variety of refractory illnesses including fetal thymus [3]. Fetal thymic transplantation deserves special mention as it has been successfully used to rectify patients suffering from Di George's Syndrome, Hodgkin's and Non-Hodgkin's Lymphoma. These applications of allogeneic fetal thymus transplantation led to the formation of a stable chimeric model without its rejection.

## Chimerism and Fetal Tissue

The fetal chimerism can be of two types the micro and macro chimera. During pregnancy, fetomaternal chimerism is a well-documented phenomenon where there is believed to be some exchange of cells between the mother and the fetus through the blood-placental barrier. Herzenberg et al. showed the presence of fetal genetic material in the peripheral circulation of the mother [4]. These fetal cells in the maternal system can reside for a long time and its earliest detection can be reported at 4-5 weeks of postpartum with a further decline in the maternal blood post 6 months [5,6]. Pertaining to the previous point, some believe, there can be a 100% clearance of chimerism at the post-partum stage [7]. Others believe that this chimerism can persist in the maternal system for long even after post-partum [8]. Other examples of fetal micro-chimerism have shown to exist in the maternal bone marrow system also [9,10].

Macro-chimerism is a phenomenon that is expressed or observed at the cell tissue level. Fetal tissue transplantation below 15 weeks or so in the pre-HLA development phase has shown to survive without any graft versus host rejection for longer periods [11,12]. Tolerance and accommodation can be probably two important biological phenomena contributing towards the successful survivability of the macro-chimerism. Further, it was observed that the fetal tissue can develop its own microenvironment by downgrading the molecular antennas responsible for recognition and rejection by the host's T-cells [13].

Accommodation normally up-regulates the complement system thereby developing a resistance to cytotoxic activities of the host's immune system [14,15]. PI3K and heme oxygenase pathways are responsible for the process of accommodation and successful fetal survival during the time of pregnancy which can be correlated with the survival of the fetus without rejection post-transplantation in the patient's body. The concept of whole embryo transplantation is also very interesting as the trophoblastic layer of the embryo can act as a barrier against the T-cell activities.

## Advantages of Fetal Tissue Transplantation

The fetus including the fetal tissues is rich in stem cells, progenitor or fetal cells. They express low mRNA transcripts of HLA-G and the mesenchymal stem cell content of the fetus can have an anti-inflammatory effect as observed under *in vitro* conditions [16,17]. Fetal tissues can even heal injuries efficiently and rapidly without the formation of scar tissues or fibrosis. These cells or tissues, when transplanted in vivo, has shown to develop their own microenvironment without getting rejected. This is due to the fact that most of the fetal tissues are collected before 15 weeks of gestation and

as they remain in a pre-immune phase or a pre-HLA stage it remains untargeted by the host's immune system [18].

Application of fetal tissue in medicine and surgery can be also an alternative to organ transplantation. Currently, organ transplantation can be allogeneic or xenogeneic transplantation. Currently, the waiting period and the cost of treatment for receiving an organ transplant globally is a major limiting factor often leading to high mortality rates. Another major problem in organ transplantation is graft versus host rejection. Organ transplantation requires HLA matching and more the match, more is the less use of immunosuppressant and anti-inflammatory drugs and vice versa. Long-term use of such drugs can often lead to side effects like host immunosuppression [3]. Also, the correct age match of the organ donor and recipient is an important parameter while performing organ transplantation. Zoogenesis or transmission of animal infection to humans can be a cause of concern in xeno-transplantation.

Keeping in mind the above limitations of organ transplantation the clinical applications of fetal tissues and whole fetal organs can be a very effective therapeutic approach due to three main reasons 1. Pre-HLA nature of the fetus 2. A potent hub for potent progenitor and stem cells 3. Collection is easy if proper ethics is maintained.

## Case Studies of Fetal Thymus Transplantation

In 1974, a 12-week fetal thymus tissue was collected and transplanted in small sections at the abdominal muscles of an infant patient suffering from Di George's Syndrome, follow up studies after 2 weeks showed remission of the respiratory infection with no evidence of Graft versus Host Diseases (GvHD). Within a month's time, the lymphocytes reached a normal level in the blood circulation [19-22].

Similar studies were conducted in additional patients where 5 years follow up studies of the patients reported normal T-cell count except in few cases where the T-cell count decreased post-transplantation. Long-term follow-up studies further revealed that 5 of the subjects had a long-lasting beneficial effect with the restoration of normalcy even after 20 years. One of the patients however expired because of cardiomyopathy [23-26].

Syngeneic fetal thymic cells have been successfully transplanted in case of a child suffering from severe combined immunodeficiency (SCID) due to the unavailability of an HLA matched identical donor for bone marrow transplantation. The patient is now leading a healthy and normal life without the need of any further treatment [27-29].

Between 1999 and 2006, Bhattacharya et al., transplanted fetal thymus (age between 12 to 16 weeks) in the axilla of 7 patients (age between 13 and 64 years, 2 females and 5 males) who were suffering from both Hodgkin's and Non-Hodgkin's Lymphoma after patient informed consent and ethical clearance was provided. These fetal thymuses were all freshly collected and screened for HIV-I, II, Hepatitis, Malaria, CMV, Syphilis in 7 patients. Paracetamol was given to all the patients post-transplantation. One month follow up studies revealed the WBC count to be between 24,000 to 42,000/$mm^3$ without any prevalence or observation of graft versus host disease. Histology studies further revealed the development, growth, and differentiation of the fetal thymus in vivo. In one of the Non-Hodgkin's lymphoma case, the 16-week old fetal tissue was removed due to a massive rise in the WBC and was thought to be of oncogenic in nature. Post removal there was a decrease in the abnormal WBC level and restoration of a normal count [30].

## Fetal Thymus Transplantation and Chimerism

As already mentioned before, like other fetal tissues, none of the fetal thymic transplants exhibited any host versus graft disease along with mono-nuclear invasion or endarteritis. This led to the successful creation of a thymic fetal and host macro-chimerism. Alleviation of symptoms in almost all the cases was observed probably due to the homing of the fetal thymic progenitor cells or its cytokine-based support system. The fetal thymic tissue like other fetal tissues is also resistant to stress and hypoxic conditions [31]. The less vascularity of the fetal tissue can also avoid the T-cell surveillance and other immune complexes. The extracellular matrix (ECM) plays a major part in healing and likewise, the fetal tissue ECM also provides support for cytokines and a unique matrix consisting of integrin and non-integrin receptors for cell adhesion, mobilization, and migration [31,32]. These are expected to be some of the major factors that may play in the creation of a successful fetal chimeric model with immense potential in clinical medicine although it is still in its infancy stage.

Applications of embryonic stem cells and induced pluripotent stem cells to rectify thymic disorders

Human embryonic and induced pluripotent stem cells are another attractive field of stem cell therapy to rectify many disorders of the immune system including the thymus. Both being pluripotent in nature have the ability to differentiate into the entire three germ layers the endoderm, ectoderm and the mesoderm and can therefore be used to differentiate into thymic cells for treating thymic disorders.

In one such study by Inami et al. in 2011, mouse induced pluripotent stem cells (miPSC) were able to create thymic epithelial progenitor cells (TEPCs) and were further differentiated into medullary TECs (mTECs) in the presence of fibroblast growth factors, bone morphogenetic protein, receptor activated nuclear factor B ligand and LiCl. Gene expression studies further analyzed the presence of Hoxa3, Pax1, Pax 9 along with cell surface markers like EpCAM, MTS24 on the 14th day of the iPSC differentiation [33].

Normally the thymus is the main organ for developing the immune tolerance by eliminating the auto reactive T cells. The thymic epithelial cells or TECs are the major cells of the thymic microenvironment responsible for the functional development of the T cells [34]. Thymic epithelial cells (TECs) obtained from iPSCs could also be used to develop tolerance against iPSC derived graft through the process of co-transplantation. This process can help in the production of T cells that are resistant to stem cell auto-antigens and neo-antigens thereby potentially preventing the transplantation rejection. Further, it can have a positive impact on cell-based regenerative medicine therapies [35].

Like iPSCs, human Embryonic Stem Cells (hESCs) are attractive sources for the production of thymus epithelium *in vitro*. Xiaoning et al. reported the production of thymic epithelium progenitor-like cells (TEPLCs) in the presence of Activin, Retinoic Acid, and BMP. Gene expression studies revealed the presence of thymic specific genes like FOXN1 and even functional thymic markers such as MHC II, AIRE post-transplantation. The group also noted that these TEPLCs derived thymic epithelium could further aide mouse thymopoiesis in T-cell deficient mice and also generate human T cell in NOD/SCID mice when transplanted with human hematopoietic stem cells [36]. Similar works pertaining to generation of thymic epithelial progenitors (TEPCs) are also reported by Parent et al., The generated TEPCs further went on to form matured and functional thymus epithelial cells (TECs) which supported the T cell development in thymus deficient

mice. These T cells were functional *in vivo* and showed the ability to proliferate under *in vitro* conditions also [37]. According to Min Su et al., the efficiency of differentiating human embryonic stem cells (hESCs) into functional thymic epithelial progenitor's cells (TEPCs) and functional thymic epithelial cells (TECs) is low. Also, *in vivo* co transplantation studies with human hematopoietic precursors have reported very low human T cell generation [34]. They further developed a process where TEPCs from hESCs can be differentiated into matured TECs and form the thymic architecture including long-term development of functional mouse T cells or high level of human T cells after co-transplantation with human hematopoietic precursors [34].

Thymus transplantation still remains the best method to treat thymic defect. However, acute shortage of thymus transplant donors including the need for HLA, ABO matching is a major hurdle in thymus transplantation [38,39]. Newer treatment concepts like regenerative medicine have ushered a new era in clinical science. In cell therapy, human embryonic stem cells and induced pluripotent stem cells have shown potentialities to produce thymic epithelial progenitor cells TEPCs and functional thymic epithelial cells TECs. However, most of them are limited to *in vitro* and *in vivo* animal studies as the scope of translating these studies into clinical trials is yet not feasible. It still remains unclear to what extent these hESCs, iPSCs can differentiate into functional TECs from TEPs and support thymopoiesis *in vivo* [40,41]. Considering the limitations of iPSC, hESC, fetal thymus transplantation can be an extremely attractive option. The process of fetal thymus transplantation and its collection is simple and can be easily done by a trained physician in a surgical OT with minimal infrastructure.

## Discussion

### Ethics of fetal tissue, hESC and iPSC application

Induced pluripotent stem cell, human embryonic stem cell research, and fetal tissue transplantation are currently under ethical scrutiny by different religious, political and scientific groups. Fetal thymus in all the above case studies was collected from volunteers or mothers who gave their consent to donate their aborted fetuses for the betterment and advancement of medical science and research. Just like mothers have the right to decide to terminate or continue with their pregnancy similarly medical researchers and clinicians should also have the liberty to decide whether to utilize a discarded healthy fetus for a better clinical outcome in many end stage diseases when all current standard mode of treatment has been exhausted and failed [3]. Said that there are many instances where moral ethical values are not always practiced especially in developing countries where human exploitation is recorded the highest. In poor and developing nations, many pregnant mothers can be forced to undergo abortion in exchange for monetary gains resulting into massive unethical malpractices and exploitations. Such challenges can be countered only through strict vigilance and formulating stringent guidelines and laws condemning such malpractices and exploitations through creations of severe punishment if found guilty.

The second moral and ethical question that arises in fetal tissue transplantation is when should be a fetus considered to be an individual with life and whether it is justified to destroy a life for medical gains? Normally most of the fetal tissues are collected from the first trimester upto the early second trimester. Ethical dilemmas and issues come into play when mid second trimester fetuses are collected as it is believed that during this period a fetus starts to attain personhood and become live. However, fetal tissues collected from early second trimester abortions should be immediately used for fetal tissue transplantations after the woman has given her consent [42]. Nonetheless, the above topic is still highly debatable and requires a thorough validation before coming to a particular view.

Embryonic stem cell research from the beginning itself has been associated with ethical issues pertaining to the destruction of an embryo. Formation of teratomas as reported in some cases has further increased the doubt about the potentialities of hESCs as a source for clinical stem cell therapy [43,44]. Similar, ethical dilemmas are also associated with induced pluripotent stem cells and its clinical applications like the use of proto-oncogene transcription factors klf-4, c-myc, use of viral vectors. One study stated that human iPSCs develop teratomas more efficiently and faster than hESCs irrespective of their site of injection [45-48]. Compared to this, fetal tissue, which is also a source of pluripotent, multipotent stem and progenitor cells can be a better alternative to regenerative medicine.

## Conclusion

Successful fetal tissue transplantation be it thymic or any other tissue is a unique example of a stable chimera model where two different genetic constitutes stay together *in vivo* without the incidence of rejection. However, there are still many unanswered questions. The mechanism by which a stable chimerism is maintained by the fetus and the fetal tissue *vivo* is still under intense speculation and further molecular investigations are required. To what degree or extend the rejection does not occur is also another interesting area of molecular immunology. The fate of the fetus post-transplantation *in vivo* is another important phenomenon. Whether the fetus gets dissolved or remains intact or grows is an important scientific quest. In the above study the fetal thymus tissue was shown to develop over time and in another study by the same group, Bhattacharya et al., has shown that a fetal tissue removed after 12 years since its transplantation in a patient cured from the disease has shown to be intact in nature without any signs of rejection [13].

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