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# The Placental story: assessing the clinical promise and estimating a loss

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## Abstract

The Placenta, an interface organ between the mother and the foetus, that acts as an in-utero life support system for the foetus, for years in different tradition had its role as an elixir having the ability to repair, restore and regenerate dead and damaged cells. But over the years the placenta has lost its relevance post-expulsion and been reduced to mere biological waste .With increasing evidence of placental tissue showing regenerative potential, the present study assesses the various properties and assimilates the evidences of effective clinical applications and provides an annual estimation of the amount of precious biological resource that is lost in a tertiary care centre in eastern India.

**Keywords:** Placenta, Biological Waste, Cord Blood, Stem Cells, Amniotic Membranes.

## Introduction

Motherhood , one of the most endearing emotion ,sees a fertilised ovum transform into a new life that goes through an enigmatic journey engulfed in a support system that nurtures it in- utero. This nutri-dense, hormone rich, interface organ between the mother and the foetus that subserves multi-pronged action is the Placenta.It is treated reverentially in different traditional post- birth rituals. The Placenta, claimed as finest biological sieve, has been found to be a potentially rich resource of biological tissue that has the ability to change the course of dying, degenerating and dead tissues and organs and lead to repair, restoration and regeneration of the same.

## Morphology

The Placental resources comprise of the placenta, amniotic membrane, amniotic fluid, umbilical cord, umbilical vessels, and the umbilical cord blood that remain largely unutilised. The term placenta consists of a foetal part called Chorion Frondosum and a maternal part called the Decidua basalis[1].The umbilical cord emerges from the mid region usually. A 2008 study found the mean surface area of the human placenta to be within 286cm$^2$ with an average volume of 600cc and weight of 500g [2].The amniotic membrane has a surface area of approximate1300-1500cm$^2$.The amnio-chorionic membrane forms the outer limits of the sac that encloses the foetus. The amniotic membrane consists of an epithelial layer, basement membrane and an avascular stroma .It remains loosely attached to the chorion and hence may be separated [3].On the epithelial side it is bathed by the amniotic fluid. The volume of amniotic fluid is at its maximum around 34 weeks of

gestation at 800 ml approximately, which reduces to 600ml at term [4].The umbilical cord connecting the foetus to the placenta, contains two arteries and one vein, surrounded by a hyaluronan enriched Wharton Jelly. Though variable, on average the cord length comes to 61cm approximately [5]. The Wharton's jelly of the umbilical cord is rich in hyaluronan (HA) in a concentration of 4mg/ml [6].The other important resource is the Umbilical cord blood. The mean volume of cord blood collected varies with the mode of delivery, with 104ml(approx.) for caesarean delivery and 85ml(approx.) for vaginal deliveries [7].

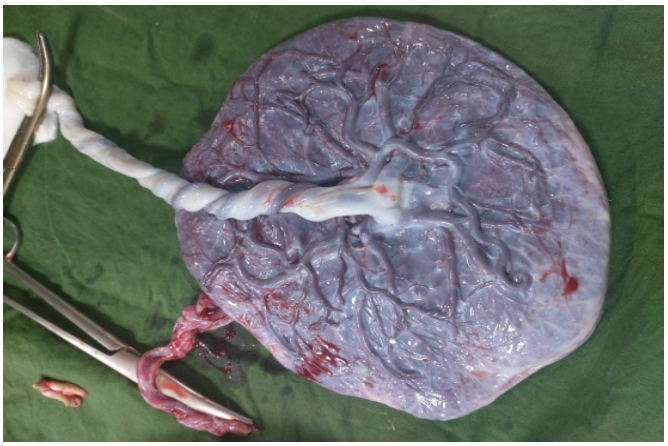

Fig. 1a: Foetal side of Placenta with umbilical cord

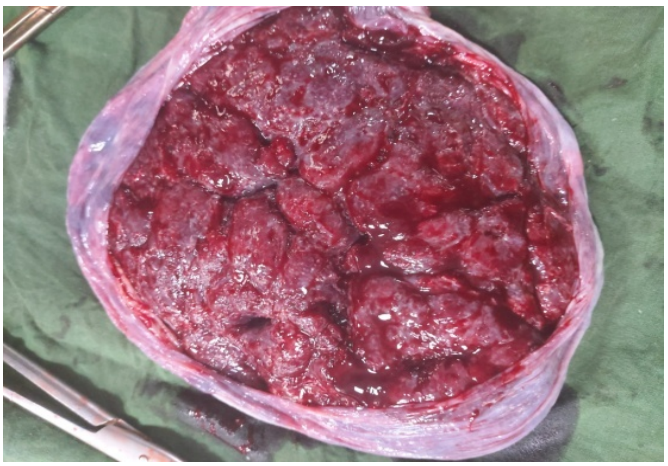

Fig 1b: Maternal side of placenta

**Immuno-modulatory properties**

Placental structures, are a natural model of allogeneic engraftment that are capable of persisting in a genetically foreign organism [8]. This favours the idea that cells from placenta could possess intrinsic immunological characteristics [9].The trophoblast has almost no expression and very low amounts of classical MHC (HLA-A, HLA-B, and HLA-C) are expressed by the other cells of placenta. Hence the immune system finds it difficult to recognize these cells [9,10]. Besides, trophoblast expresses HLA-E, HLA-F, and HLA-G ,the nonclassical MHCs [11].HLAs that are expressed in the foetal membranes are tolerogenic rather than immunogenic. Majorly it has been found that Mesenchymal stem cells(MSCs) obtained from all the placental sources express CD105, CD90, CD73, CD29, CD13, CD10, and do not express CD14, CD34, CD45, and HLA-DR 12. The cells retain the ability to synthesize chorionic gonadotropin and express cytokeratin-7 and CD200 [13].

**Stemness**

The placental cells combine these tolerogenic properties along with the ability to differentiate. The two professed pillars of cell transplantation are stem cell differentiation potential and lack of rejection. The early embryological origin of placenta is in favour of the hypothesis of stem-cell potential. In the First International Workshop on Placenta-Derived Stem Cells held in Brescia, Italy in 2007 ,the foetal placental regions identified as potential stem/progenitor cells , were the amniotic epithelial, amniotic mesenchymal, chorionic mesenchymal, and chorionic trophoblastic tissues. Besides, components of the umbilical cord too were mentioned [14,15]. Different studies found that placental MSCs possess the capability of differentiation not only into three classical mesodermal lineages (adipogenic, osteogenic, and chondrogenic) but were found to differentiate in myogenic, angiogenic, pancreatic, cardiogenic, and neurogenic cell types 15,16. .The human placental MSCs represent stem cell

types which have properties of pluripotent embryonic stem cells alongside properties of multipotent mesenchymal stem cells. Owing to the close ontogenic relationship to embryonic stem cells, placental MSCs not only have immunoprivileged characteristics, but possess a greater plasticity, and faster proliferation than adult MSCs [17,18].

**Secretome**

The possibility of identifying the bioactive factors responsible for the therapeutic effect rendered by these MSCs sourced from placenta and thus translating them into medicine is making the rounds too. The secretome is the set of factors secreted by a cell into the extracellular space, that include free nucleic acids, soluble proteins, lipids, and extracellular vesicles [19] . The secretome of placental MSCs is specific and it is subject to changes in the physiological or pathological conditions and stimuli [20]. The use of cell free,"conditioned secretome" is of promise and being studied [21]. Hence the Mesenchymal cells are increasingly looked upon more as "Medicinal signalling cells" [22].

**Storage**

Cord blood serum, placental extracts, cell suspensions, chorionic and amniotic membranes, and placental tissue are all suitable for cryopreservation procedures making them easy to store [23,24]. The present day UCB banks bases their concept on the findings of the Broxmeyer *study* that found, controlled UCB cryopreservation for over 20 years had no significant effects on the cell viability or the function [25] .

**History of Clinical applications**

The use of human placenta as medicinal material dates back to early 400 BC, during the period of Hipocrates and as healing agent in 200BC in China [26]. In *1593* ,Li Shi-Zhen, a great Chinese Biologist in his treatise on substances with medical properties called "The *Compendium of Materia Medica" mentions* the medical uses of human placenta "zi he chi" [27]. Few centuries down the line in 1910, Davis J [28] showed that the use of amniotic membrane (AM) in skin grafting was able to achieve better results than xenograft or cadaveric coverings. Shortly afterwards in *1913*, Stern [29]and Sabella [30] also used amniotic membrane (AM) for treating skin wounds as biological dressing having differentiation potential. The placental tissue has been used as a biosorbent for the treatment of varied chronic inflammatory diseases like suppurative cholangitis, mastitis, pancreatic necrosis,etc. It resulted in improved general condition, reduced bacteremia, improved blood flow, and lesser trauma to cells in comparison to other conventional sorbents [31].

The amniotic membrane find use in ophthalmology too, for the closure of corneal pathology defects,due to its transparency and the mobilisation of endogenous stem cells from the limbus area, along with its ability to suppress vascularization [32,33] . The amniotic and chorionic membranes has also found application in the management of nonhealing trophic ulcers, vaginal reconstruction surgery, enterocutaneous fistula, prevention of adhesions, orthopedic pathology, etc [34,35] .The cells from the amniotic and chorionic membranes, and umbilical cord have been successfully isolated, phenotyped, and applied after ex-vivo expansion [36,37] . In 1982 Hitherto neglected cord blood stem cells found a focus as source of stem cells after the first successful transplantation of cord blood cells in Fanconi anemia by Eliane Gluckman [38]. Umbilical cord blood was put forward as an easy to access alternative source of hematopoietic stem cells compared to the bone marrow [39]. Allogenic transplantation of umbilical cord blood cells found successful application in various

hematologic and metabolic pathologies[40]. The use of freshly collected cord blood in the management of anaemia in cases with malaria was also documented [41]. The use of fresh autologous UCB cells for use in infants with hypoxic-ischemic encephalopathy were also reported [42]. Wharton jelly was also found to be a cocktail of growth factors, extracellular matrixes and exosomes, which improves the proliferative capacity, motility, mitochondrial degeneration, endoplasmic reticular functions etc in both type 1 and type 2 diabetes-derived BM-MSC (DM-MSC) [43].

**2. Methodology**

The present study takes an overview of empirically accumulated data regarding the anatomy, functional properties, documented scopes of clinical applications of placental components. The selected studies were compared and summarised on the basis of evidence based theories and models and interventions. The study focused on contrasting and combining results from these different studies in an endeavour to trace the story of the journey of the placenta over the years.

Furthermore, a cross-sectional observational study was undertaken in a tertiary care centre in eastern India over a study period of one year, to find the present mode of disposal of placenta and assess the loss of prospective biological resource having the potential for application in the rapidly progressing field of regenerative medicine and make a difference in the resource poor health care setting .A pre-tested schedule was developed to gather the relevant information. The study population covered the antenatal mothers who had their routine ante-natal check-ups and was found HIV ,Malaria, syphillis and hepatitis B negative ,going into spontaneous vaginal deliveries and caesarean sections and excluding the cases of IUFD, still birth and neonatal deaths.The data gathered was analysed and interpreted taking the accepted values regarding the varied components of the placenta as mentioned earlier.The mothers were interviewed regarding their consent regarding the placenta and the cord blood extracted thereof for preservation and clinical application for managent of ailments.

**Result**

The study covered the deliveries taking place in a calendar year in a Tertiary care centre .Month wise data was compiled for the number of deliveries conducted there, type of delivery, number of live births, number of still birth, number of neonatal deaths and intra-uterine foetal deaths(IUFD).

Table 1:The annual distribution of deliveries taking place in a tertiary care centre

| Month | Total deliveries | C/S | Live birth | Still birth | Total neonatal deaths | IUFD |
|---|---|---|---|---|---|---|
| Jan | 730 | 348 | 703 | 04 | 03 | 20 |
| Feb | 754 | 299 | 719 | 02 | 03 | 30 |
| March | 665 | 282 | 649 | 01 | 01 | 14 |
| April | 602 | 265 | 578 | 03 | 01 | 20 |
| May | 653 | 295 | 629 | 03 | 0 | 21 |
| June | 703 | 325 | 672 | 05 | 09 | 17 |
| July | 764 | 317 | 744 | 01 | 0 | 19 |
| August | 845 | 332 | 817 | 04 | 0 | 24 |
| Sept | 785 | 320 | 760 | 04 | 0 | 21 |
| Oct | 810 | 310 | 780 | 02 | 0 | 28 |
| Nov | 793 | 324 | 753 | 04 | 0 | 36 |
| Dec | 866 | 360 | 842 | 02 | 02 | 22 |
| Total | 8970 | 3777 | 8646 | 35 | 19 | 272 |

The total number of deliveries taking place in a year was 8970 of which caesarean section was performed in 3777 cases with 8646 of them being live biths. There were 35 still birth, 19 neonatal deaths and 272 cases of Intra-uterine foetal deaths. While estimating the amount of annual loss biological resource lost in terms of placenta and its varied components these deaths were excluded.

The Table 2 reflects the probable amount of Umbilical cord blood lost in both caesarean cases and spontaneous vaginal deliveries, cord length and area of amniotic membrane lost because of placental disposal as biological waste.The average volume of umbilical cord blood lost during caesarean section was taken as 104 ml ,while during spontaneous vaginal delivery it is 85ml on average [7].The cord length was taken as 61cm on average [5] ,while amniotic membrane area was taken as 1300cm$^2$ ,the lower value of the range provided by a previous study [2].

Table 2: Annual estimation of placental resource lost .

| Year / months | Cord Blood Volume(ml) | | | Cord length (61cm) | AM Area (1300cm2) |
|---|---|---|---|---|---|
| | No. Of C/SX(104ml) | No. Of SVDX (85ml) | Total | | |
| Jan | 36192 | 30175 | 66877 | 43249 | 913900 |
| Feb | 31096 | 35700 | 66796 | 43859 | 934700 |
| March | 29328 | 31195 | 60523 | 39589 | 843700 |
| April | 27560 | 26605 | 54165 | 35258 | 751400 |
| May | 30680 | 28390 | 59070 | 38369 | 817700 |
| June | 33800 | 29495 | 63295 | 40992 | 873600 |
| July | 32968 | 36295 | 69263 | 45384 | 967200 |
| August | 34528 | 41225 | 75753 | 49837 | 1062100 |
| Sept | 33280 | 37400 | 70680 | 46360 | 988000 |
| Oct | 32240 | 39950 | 72190 | 47580 | 1014000 |
| Nov | 33696 | 36465 | 70161 | 45933 | 978900 |
| December | 37440 | 40970 | 78410 | 51362 | 1094600 |
| Total | 359,528 | 413865 | 773393 | 527772 | 11239800 |

It was estimated that 359.5 litres of umbilical cord blood in cases that underwent caesarean sections and 413.8 litres in the placentas of cases of spontaneous vaginal deliveries were lost annually,with 773.3 litres of umbilical cord blood lost annually in a tertiary care centre alone.The cord length lost was 5.2 kms ,and 1123.9 sq metres of amniotic membrane area were lost.The cord blood loss in the 326 cases of still birth, neonatal death and and intra-uterine foetal death ,of them 142 being caesarean cases (14768 ml), while 184 being vaginal deliveries(15640ml),were 30.4 litres.The total umbilical cord blood loss assessed was 702.9 litres.

In the interview conducted among 10% mothers among the study population, chosen by simple random sampling, all the mothers agreed to the use of their expelled placenta for clinical applications in the management of various disease profile.82% were unaware of the fate of their placenta.Only 1.3% mothers expressed their desire for burying the placenta at their home courtyards and plant trees on top in memory of the parted placenta.

**Discussion**

The present study found with the placenta being largely held as biological waste and incineration and pit burial being fated in most ,the clinical promise that is documented till date , could be pursued with much less ethical dilemma which shrouds the other sources of progenitor cells.It holds true for most countries and women in general in the current study showed no objection to the clinical use of placent or its components , otherwise considering the material as a “waste.” Donating the placenta does not affect the donor (mother) in any aspect , be it physical or physiological.At the same time, it provides a large amount of material suitable for direct application and for long-term storage in initial state or after processing, as well as for the preparation of extracts and isolation of cells or individual components [44]. Andrew Burd and Lin Huang [1] has taken an estimate of 100,000,000 healthy live births annually with a 15% rate of caesarean section deliveries, and on the basis of that calculated a loss of umbilical cord blood in to be 1560,000 litres caesarean sections,7225,000 litres in vaginal deliveries and 8785,000 litres in total, globally. The present study based on actual deliveries and type of

deliveries conducted in these cases found an estimated loss of 359.5 litres of umbilical cord blood in cases that underwent caesarean sections and 413.8 litres in the placentas of cases of spontaneous vaginal deliveries , 773.3 litres of umbilical cord blood lost annually in the concerned tertiary care centre alone.

Burd and Huang [1], estimates an annual global loss of 61000kms of cord length and amniotic membrane area of 15 million msq.Vis-a- vis the current estimation approximated a loss of 5.2 kms of cord length and 1123.9 sq metres of amniotic membrane area which could have contributed to the clinical application of varied settings.

The late 1990s saw the inception of unrelated UCB banks that accepted donations from term deliveries and stored Umbilical Cord Blood units for allogenic transplantations and accessible to public use. It is claimed that, estimated 730 000 UCB units have been donated and stored to date and 35 000 UCB transplants have been performed worldwide [45] . The average yield of obtained hAECs (human amniotic epithelial cells) having stem cell potential, from a placenta, ranged from 190×106 cells with a typical range of 90–280 million cells, the average obtained viability was 87% 46. Whereas Mesenchymal stem cells (MSCs) represented 15.67% (±0.29%) and 2.14% (±0.65%) of cells isolated from the membrane and placenta, respectively 47. The minimum intended dose of stem cells given to the patient is quoted as having a range of 2.5-5.0 x10 6 of cells 48. Given the calculations one can estimate the staggering loss of potential biological resources that is simply being labelled as ‘waste’ waiting for disposal.

**Conclusion**

In face of the debate regarding the ethical justification of using human primordial stem cells for tissue transplantation, cell replacement, and gene therapy, the afterbirth “placenta”, emerges as intriguing alternatives to using embryonic stem cells.This claim rests on the ability of placenta and its various components and the cells and bio-active factors to differentiate ,ease of storage and evidence based effectiveness clinical applications. The lack of ethical dilemma owes to the current scenario, where it is largely treated as a biological waste with incineration or burial being the usual fate. The amount of placental tissue yield that is possible from a single tertiary care centre in a year itself is indicative of the immense untapped biological resource that may be explored further regarding clinical applications. It can prove to be of great benefit in the health care scenario ridden with cases of hematopoietic, non-hematopoietic or metabolic origin .A placental bank like the umbilical cord blood banks could be visualised as an upcoming alternative that may be accessible to all irrespective of their socio-economic conditions.

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