# Amnion-Derived Fluid and Amniotic Fluid

[1]Bigbey, M., [1]Lee, E., [1]Diller, R. B., [1,2]Kellar, R. S.

[1]Axolotl Biologix, Phoenix, AZ
[2]Northern Arizona University, Flagstaff, AZ

**Introduction:**

Amnion-derived fluid and amniotic fluid are regularly mistaken as synonymous. Although amniotic fluid may consist of amnion byproducts, amnion-derived fluid *does not* consist of amniotic fluid. Amnion-derived fluid is manufactured using a concentrated source of mesenchymal and epithelial stem cells derived from the amnion, the inner lining of the amniotic sac. The subject of this article compares the differences between amnion-derived fluid and amniotic fluid; including their origins, properties, and isolation methods.

**Origin & Structure:**

The amniotic membrane is a part of the placenta that protects the fetus during pregnancy by nourishing it with supplements (Yu, 2015). Figure 1 illustrates the amniotic sac, which consists of the outer layer chorion and the inner layer amnion. The fluid within the amniotic membrane is termed the *amniotic fluid* which is comprised of multiple cell types, including cell lineages from all three embryonic germ layers (Yu, 2015). Moreover, amniotic fluid has an assortment of water, proteins, lipids, fetal, urine, and an electrolyte composition (Yu, 2015).


**Figure 1:** Amniotic Sac (Pregmed Editorial Team, 2015)

In contrast to amniotic fluid, amnion-derived fluid is produced from the culturing of amniotic mesenchymal stem cells and amniotic epithelial cells that are derivatives from the amnion of the amniotic membrane.

Figure 2 is a representation of the layers of the amnion. The first layer of amnion, an epithelial monolayer, makes up an innermost layer closest to the developing fetus. The epithelial layer is where amniotic epithelial cells originate. In addition, the epithelial layer actively secretes and transports nutrients to provide the developing fetus with protection (Gupta et al, 2015). The fourth layer of the amnion is the fibroblast layer, the source of mesenchymal stem cells (Gupta et al, 2015).

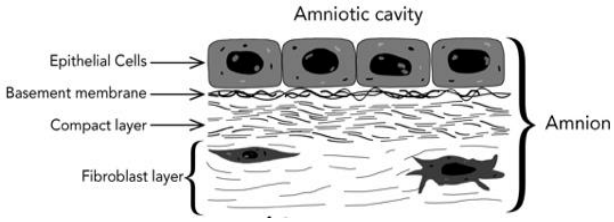


**Figure 2:** Diagram of layers of the amnion.

**Comparison of Amnion-derived Fluid and Amniotic Fluid:**

To create amnion-derived fluid first, the amnion is mechanically peeled away from the chorion since they are partially fused together to form the amniotic membrane. Mesenchymal and epithelial stem cells are then released from the amniotic membrane using enzymes. Following enzymatic release of the of the cells, they are centrifuged, then isolated to be cultured in an expansion media. Figure 3 illustrates a basic diagram of the cell isolation process that ultimately depicts the culturing of amniotic mesenchymal and epithelial cells.

The resulting expansion media becomes conditioned by the cells growing and releasing a wide variety of desirable growth factors into it, which is then harvested as amnion-derived fluid. This media contains, among

other factors, vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF), epidermal growth factor (EGF), and transforming growth factor beta (TGF-β) (Koizumi et al, 2000). VEGF is associated with angiogenesis and also has anti-apoptotic properties; bFGF and EGF are mitogens that stimulate growth of several different cell types, and TGF-β has immunoregulatory properties that suppresses the inflammatory response (Madrigal et al, 2014).

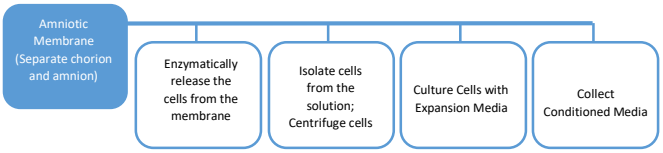


**Figure 3**: Isolation and culture of amnion-derived stem cells with subsequent culturing and conditioned media (amnion-derived fluid) collection.

These and other factors contained in the conditioned media are responsible for the properties of the media itself. The mechanism of repair is currently believed to be a combination of many things, which include inflammatory suppression, angiogenesis, and endogenous cellular proliferation, which can be explained by the factors contained in amnion-derived fluid (Hodgkinson, 2016).

In comparison, when amniotic fluid is harvested, there are several possible methods to create a final product. Some companies simply filter the fluid and use it as a treatment, as seen in Figure 4. Other companies isolate desirable factors and create a powder that can be rehydrated to create a similar growth factor cocktail.

Still others isolate the amniotic fluid cells and culture them to create a conditioned media similar to amnion-derived fluid. A very similar milieu of factors are released by amniotic fluid cells when grown in culture, including EGF, FGF, TGF, and VEGF. This grants the amniotic fluid and its derivatives similar properties to amnion-derived fluid (Rennie et al, 2012).


**Figure 4:** Amniotic fluid *(freely floating cells in the amniotic fluid in utero; amniotic-derived stem cells)*

## Conclusion:

The amniotic membrane is a rich source of mesenchymal stromal cells and amniotic epithelial cells. The cells found in amniotic fluid are a heterogeneous group of cells from the amniotic sac as well as the developing infant.

There are many similarities that exist between products harvested or made from the amniotic environment. Products resulting from the amniotic environment are rich in a variety of growth factors, cytokines, and soluble factors that are desirable for numerous regenerative medicine applications.

Both of these products are regulated under 21 CFR 1271 and section 361 of the Public Health Services Act and as such are minimally manipulated and indicated for homologous use.

## References

Gupta, A., Kedige, S. D., & Jain, K. (2015). Amnion and Chorion Membranes: Potential Stem Cell Reservoir with Wide Applications in Periodontics.

Yu, L. (2015). Human Amniotic Fluid-Derived and Amniotic Membrane-Derived Stem Cells (Z. R.C., Ed.). *Stem Cells: Basics and Clinical Translation*,*1*, 29-66.

Koizumi, N., Inatomi, T., Sotozono, C., Fullwood, N. J., Quantock, A. J., & Kinoshita, S. (2000). Growth factor mRNA and protein in preserved human amniotic membrane. *Current eye research*, *20*(3), 173-177.

Marvin, K. W., Keelan, J. A., Eykholt, R. L., Sato, T. A., & Mitchell, M. D. (2002). Expression of angiogenic and neurotrophic factors in the human amnion and choriodecidua. *American journal of obstetrics and gynecology*, *187*(3), 728-734.

Madrigal, M., Rao, K. S., & Riordan, N. H. (2014). A review of therapeutic effects of mesenchymal stem cell secretions and induction of secretory modification by different culture methods. *Journal of translational medicine*, *12*(1), 260.

Hodgkinson, C. P., Bareja, A., Gomez, J. A., & Dzau, V. J. (2016). Emerging concepts in paracrine mechanisms in regenerative cardiovascular medicine and biology. *Circulation research*, *118*(1), 95-107.

Rennie, K., Gruslin, A., Hengstschläger, M., Pei, D., Cai, J., Nikaido, T., & Bani-Yaghoub, M. (2012). Applications of amniotic membrane and fluid in stem cell biology and regenerative medicine. *Stem cells international*, *2012*.

Hoehn, H., & Salk, D. (1982). Morphological and Biochemical Heterogeneity of Amniotic Fluid Cells in Culture. *Methods in cell biology*, *26*, 11-34.