

# Amnion and Amniotic Fluid Derived Orthobiologics

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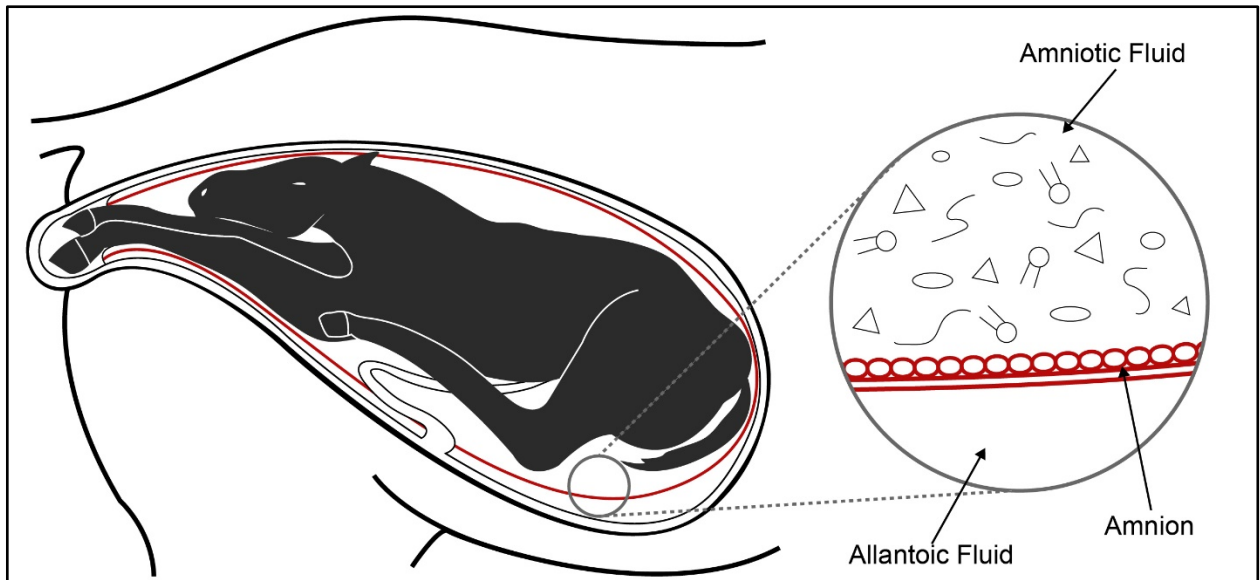
## KEY POINTS

- Amnion and amniotic fluid derived products have attracted attention as a source of multiple mediators applicable to regenerative medicine
- Amnion and amniotic fluid derived products have demonstrated the ability to reduce inflammation and enhance healing
- The biologically active factors include cytokines, growth factors, and tissue inhibitors of metalloproteinases
- Birth tissue-derived products may offer several advantages over other, comparably utilized, orthobiologics, including platelet-rich plasma

## Introduction

Amnion is a membrane, composed of fetal tissue, that closely covers an embryo when first formed. As gestation progresses, amniotic fluid is produced which causes the amnion to expand and become the amniotic sac that serves to provide a protective environment for the developing embryo or fetus.<sup>1</sup> The amniotic fluid is known to contain multiple cell types derived from the developing fetus, including broadly multipotent amniotic fluid stem cells.<sup>2,3</sup> In addition to the cells, the extracellular matrix of the amniotic membrane is inherently rich in growth factors, cytokines, and unique proteins like beta defensins and lactoferrin.<sup>4</sup>

The first report on the therapeutic use of amnion was published in 1910 when Davis reported on a series of cases using fetal membranes as skin grafts.<sup>5,6</sup> Amnion and amniotic fluid have attracted attention in recent years as the source of stem cells and mediators that may have widespread clinical application in the field of regenerative medicine. Several characteristics of birth tissue-derived products make them amenable to clinical applications: amniotic cells produce growth factors, cytokines, anti-bacterial peptides, anti-inflammatory agents, and the tissue exhibits low immunogenicity.<sup>7</sup> These characteristics have led to experimental and clinical investigations of birth tissue-derived products as therapy in many areas, including neurological<sup>8,9</sup>, orthopedic<sup>10,11</sup>, dermatological<sup>4,12</sup>, ophthalmological<sup>13</sup>, and dental disease.<sup>14</sup>



## The Role of Biologically Active Factors

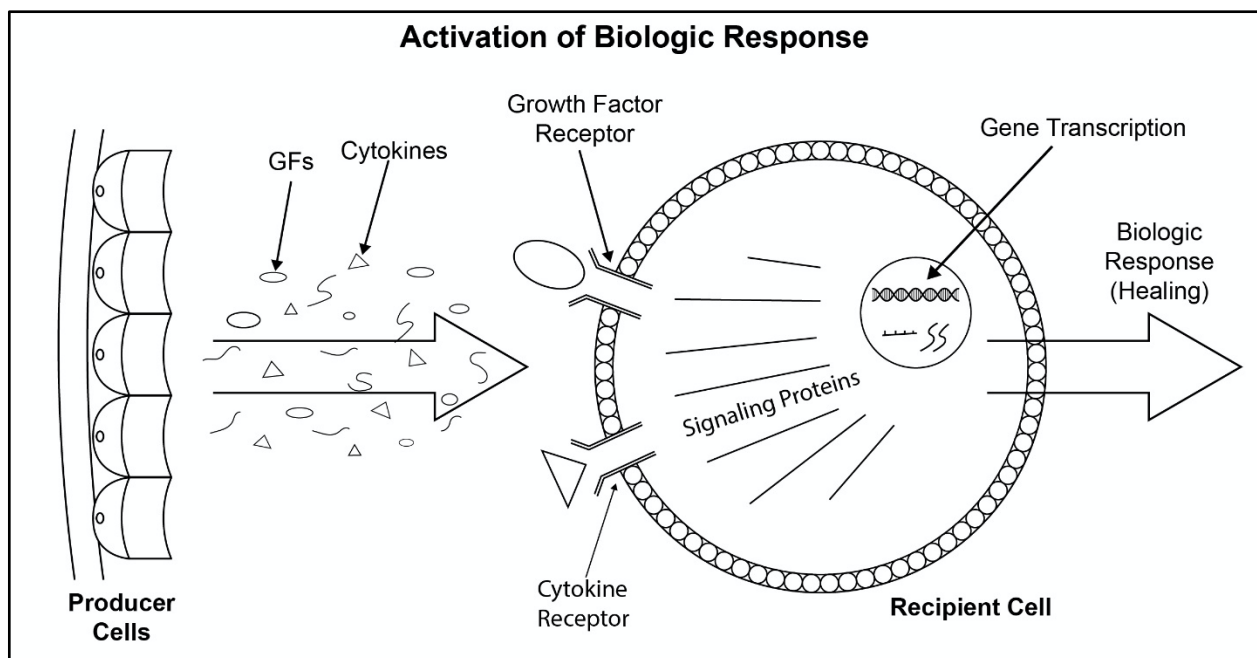
Amnion and amnion fluid-based products are derived by bioengineering the amniotic membrane and the amniotic fluid that surround the fetus during gestation, providing physical protection, antimicrobial protection, and the bioactive components in amniotic fluid may play an important role in fetal development and maturation.<sup>15</sup> These products are noted to have many beneficial effects when used as therapeutic agents. They have demonstrated the ability to reduce pain, scarring and inflammation, modulate angiogenesis, enhance tissue healing, possess antimicrobial properties, and the extracellular matrix can serve as a scaffold for cell proliferation and differentiation.<sup>7,12,16</sup>

The means by which birth tissue-derived products exert their effects are the growth factors, cytokines, and other mediators that are produced by the cells of the amniotic membrane and fluid. Quantifiable levels of more than 200 biologically active factors have been identified by enzyme-linked immunosorbent assay (ELISA) of one human preparation.<sup>10</sup> These factors can be divided into three major groups: cytokines, growth factors and tissue inhibitors of metalloproteinases (TIMP). Cytokines and growth factors are pleiotropic—they have many potential effects depending on the specific cells that they interact with and the physiological environment in which they exert their effects.

Many amnion and amniotic fluid derived products contain significant levels of cytokines including interleukin (IL)-1ra— an antagonist of the pro-inflammatory IL-1, IL-4 and IL-10— potent inhibitors of pathologic inflammation, and IL-6— a cytokine with both pro- and anti-inflammatory properties. While the anti-inflammatory mechanism of birth tissue-derived products is not completely understood, it is likely it involves the mediation of interleukins because of the key role interleukins play in regulating the inflammatory process.<sup>12</sup>

Amnion and amniotic fluid derived products contain a wide array of growth factors. Several of the growth factors identified in birth tissue-derived products are associated with the normal healing and reparation of damaged tissues, including epidermal growth factor (EGF), basic fibroblast growth factor (bFGF), transforming growth factors alpha and beta (TGF $\alpha$ , $\beta$ ), nerve growth factor (NGF), platelet-derived growth factor  $\beta\beta$  (PDGF- $\beta\beta$ ), and vascular endothelial growth factor (VEGF).<sup>12,17,18</sup> It is beyond the scope of this paper to provide in depth detail on the characteristics of each growth factor present in amnion and amniotic fluid derived products. However, the nature of cell culture and engineering of the medium allows for a degree of selectivity for specific growth factors included in an individual birth tissue-derived product. Birth tissue-derived products can be formulated for specific indications by manipulating the relative concentrations of growth factors present.

The third group of biologically active factors in perinatal based products are the tissue inhibitors of metalloproteinases (TIMPs). Matrix metalloproteinases (MMPs) play a key role in the normal physiology of connective tissues, but their unregulated activity has been implicated in numerous disease processes. MMPs have been demonstrated to inhibit healing and degrade growth factors.<sup>19</sup> TIMPs directly inhibit MMPs and are considered to be the key inhibitors of MMPs in tissues.<sup>20</sup> Quantifiable levels of TIMP-1, -2, and -4 are found in amnion and amniotic fluid derived products, at or above physiologic levels, and should facilitate healing.<sup>12,19</sup>



### Amnion and amniotic fluid derived Products as Orthobiologics

Amnion and amniotic fluid derived products have been employed as orthobiologics\* in recent years because of the ability to provide a source of biologically active factors (summarized above)

functioning to enhance and expedite healing of both soft tissue and articular injuries.<sup>21</sup> Along with platelet-rich plasma and stem cell-based products, in vitro studies and in vivo, clinical reports of positive outcomes using a variety of orthobiologics are widespread in both the human and veterinary literature. In recent years, the prevailing rationale behind the effects of orthobiologics has become based on the principle of growth factor-mediated therapeutic action rather than the previous understanding that cell migration, attachment, differentiation, and proliferation were the primary mechanisms of action.<sup>17</sup>

Amnion and amniotic fluid derived orthobiologics are frequently used in clinical cases in a similar fashion as platelet-rich plasma products (PRP), creating a natural opportunity for comparison. Birth tissue-derived orthobiologics may offer several advantages to PRP therapy. As opposed to birth tissue-derived products “off the shelf” availability, PRP requires drawing and processing of the patient’s blood. This introduces variability in the final product due to differing preparation methods and variable concentrations of platelets and growth factors. Significant differences in components have been observed with different separation methods and may influence clinical results on treated tissue.<sup>22</sup> Patient factors including age, sex, breed of horse, and concomitant use of nonsteroidal anti-inflammatory drugs (NSAIDs) have all been demonstrated to influence platelet numbers and growth factor concentrations in PRP.<sup>10,17,22,23</sup> Even intra-individual results of PRP preparations have demonstrated wide variations in platelet and cell numbers as well as levels of growth factors regardless of separation method.<sup>22</sup> In comparison to PRP, in which all active biologic factors are released into the joint or tendon simultaneously upon platelet activation, depending on the formulation, birth tissue-derived products may contain active factors that elute from the tissue more slowly over a period of days to weeks.<sup>10</sup> The presence of active factors over a prolonged period may enhance the in vivo, clinical response to these orthobiologics.

## Summary

Amnion and amniotic fluid derived products rely on the wide array of cytokines, growth factors, and TIMPs produced by amniotic cells and acting in concert to provide a range of beneficial clinical effects as orthobiologics. Technological advances in the means of recovering, processing, and culturing amnion and amniotic fluid derived cells and formulating a product containing factors biologically important for promoting healing of orthopedic tissues allows for creation of orthobiologics with known, consistent, and targeted factor profiles.

\* From “ortho” referring to bones, joints, muscles, and tendons and “biologics” in this context meaning naturally derived. Orthobiologics are substances found naturally in the body, that, when used in higher concentrations may help speed the healing process.<sup>21</sup>

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