

Amniotic fluid processed with NovaSterilis' supercritical carbon dioxide (scCO₂) process retains its protein content after sterilization.

scCO₂ PROCESSING OF AMNIOTIC FLUID

Medical device research and development teams have explored the use of scCO₂ for the sterilization of allograft amnion tissue as an extension of the tissue banks growing use of scCO₂ for sterilization of allograft soft tissue and bone. Recent publications have focused on demonstrating an effective sterilization process for amnion tissue while preserving cytokines which are critical components of the tissue.^{1,2} O'Connell et al. demonstrated better retention of numerous cytokines in amniotic tissue sterilized with scCO₂ compared to those irradiated with gamma or electron beams. scCO₂ sterilization of amniotic fluid has been identified as an additional area of interest. NovaSterilis, in collaboration with iFyber, initiated a study to determine the effects of the scCO₂ process on the preservation of native cytokines.

While amniotic fluid is naturally sterile, collection of the material may introduce contamination. Additionally, amniotic fluid may contain viruses from the mother. Prior to use in medical treatments and research, amniotic fluid needs to be sterilized. Effective sterilization of amniotic fluid, like amniotic tissue, needs to be accompanied with conservation of proteins innate to its function.

scCO₂ PROCESSING AND EVALUATION OF AMNIOTIC FLUID

- **Sample Processing:** Amniotic fluid samples, in vented conical tubes (Figure 1), were subjected to NovaSterilis' scCO₂-based NovaClean™ process, which has been shown to be effective in the sterilization of liquid samples.³ Sterility assurance level (SAL) 10⁻⁶ can be achieved in liquid samples with fractional amounts of NovaKill™ additive. Amniotic fluid samples were processed with and without NovaKill additive.
- **Protein Content Assessment:** Amniotic fluid samples, processed with and without NovaKill as well as an unprocessed sample, were assessed with a Human XL Cytokine Array (R&D Systems, ARY022B). Figure 2 shows the results of the assessment.

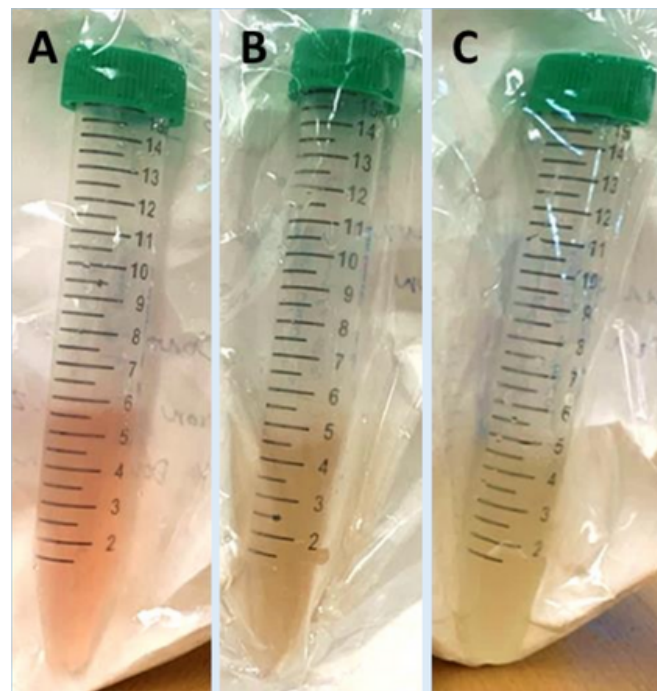


Figure 1. Appearance of amniotic fluid samples after scCO₂ processing. Processing amniotic fluid in vented conical tubes resulted in a color change from the pink unprocessed sample, A, to an orange color when processed with only scCO₂, B. When processed with scCO₂ and NovaKill additive, the amniotic fluid sample turned pale yellow, C.

Conclusions: The results were largely consistent among the amniotic fluid samples, showing conservation of the majority of the 53 proteins tested in the array. This study indicates that the protein content of amniotic fluid processed with scCO₂, with and without NovaKill additive, is not affected.

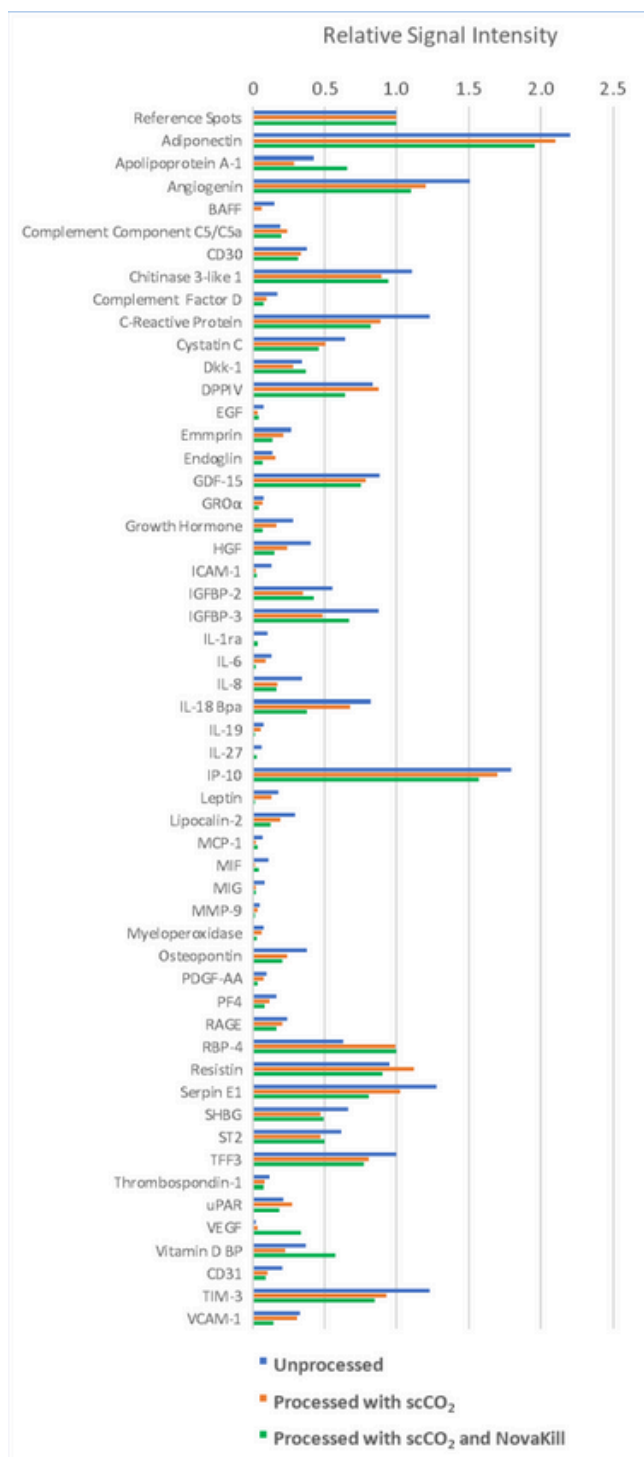


Figure 2. Cytokine array results. The protein content of amniotic fluid processed with scCO₂, with and without NovaKill additive, with a cytokine array. The majority of the 53 tested proteins were retained after processing with conditions know to sterilize.

References:

- ¹ Wehmeyer J, Natesan S, Christy RJ. 2015. Development of a sterile amniotic membrane tissue graft using supercritical carbon dioxide. *Tissue Eng Part C Methods*. 7:649-659.
- ² O'Connell J, Pentakaota J, Villeareal D, et al. 2025. Development of a sterilization process for amniotic membrane allograft tissue using supercritical carbon dioxide and NovaKill. *Cell Tissue Bank*. 26:4.
- ³ Sterilization of temperature-sensitive biomaterials, hydrated or submerged. *NovaSterilis Med01-04*.