

### Introduction

The ovary provides an ideal environment for egg survival due to its distinct structure that directly contributes to the growth and maintenance of the follicle. Because of its distinct structure, which directly aids in the follicle's growth and maintenance, the ovary is thought to provide an ideal milieu for the survival of the egg. Systemic metabolic diseases, chemotherapy, and ovarian pathologies including PCOS can all contribute to ovarian tissue malfunction. The purpose of this study is to compare ovarian decellularization with herbal and chemical detergents. Despite their effectiveness, these treatments are constrained by methodological issues such as the high implementation costs, the risk of cancer cell proliferation, the use of cytotoxic cryopreservation, and the destruction of oocytes during the freeze/thaw or cryopreservation procedures.

## Methods

One of the food sources in many countries is sheep meat. Therefore, after sacrificing these animals, their waste tissues can be used for research. The uterus of a female Sanjabi breed sheep was removed from the Kermanshah animal slaughterhouse, in Iran, after slaughtering these animals. The tissues were placed in phosphate-buffered saline (PBS) containing 2 % penicillinstreptomycin (P/S) and transferred to the laboratory, and then the ovaries were gently separated from the surface of the uterine tissue and made available for further processing. In this study, two chemical and herbal detergentswere used. A 1 % solution of sodium dodecyl sulfate (SDS) (chemical) was prepared with distilled water. The root of Acanthophyllum (Caryophyllaceae) ACP) (herbal), which grows in different regions of Iran [12], was used to prepare herbal detergent. The root of the plant was ground into powder and weighed. To prepare different percentages of herbal detergent according to the study, the solution (ACP powder in distilled

# **Innovation in ovary decellularization methods: Chemical and herbal detergents**

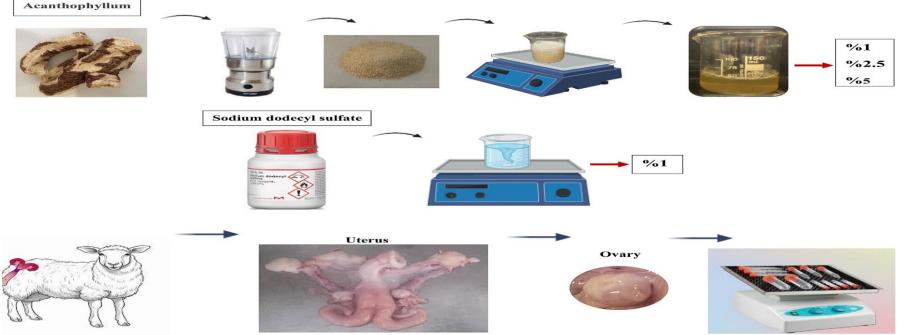
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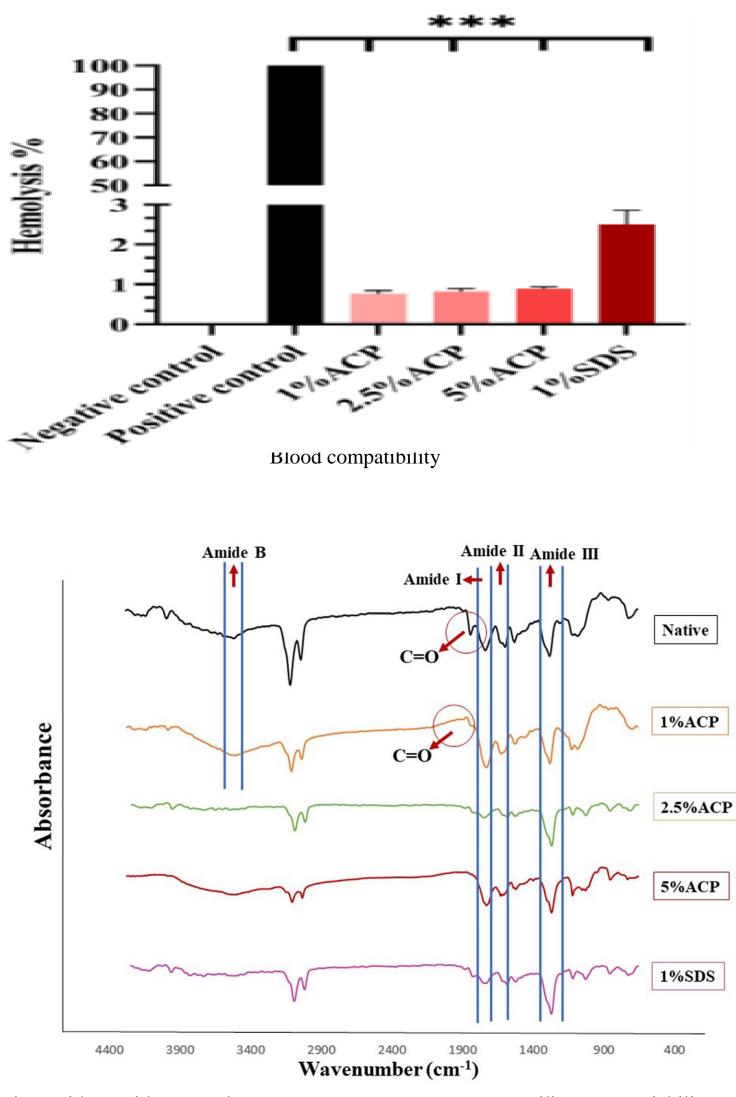
water) was placed on a stirrer for 24 h and then passed through a filter paper and kept in the refrigerator until use. DNA content, histological characteristics, attenuated total reflection Fourier transform infrared spectroscopy (ATR-FTIR), biocompatibility, antibacterial test, hemocompatibility, and scanning electron microscope (SEM) were investigated.

#### Results

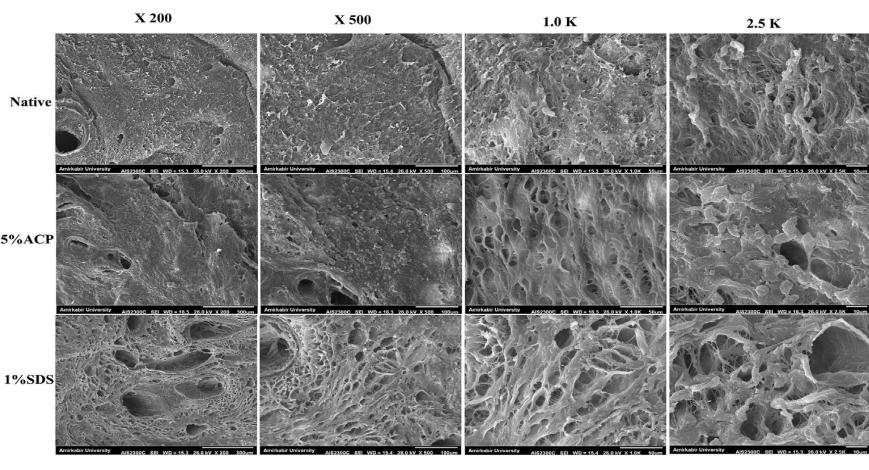
A significant difference in DNA concentration was reported between the native ovarian tissue group (907.33 ng/mg) compared to the decellularization groups including 1 % ACP (102.73 ng/mg), 2.5 % ACP (76.28 ng/mg), 5 % ACP (37.31 ng/mg) and SDS 1 % (37.05 ng/mg). The highest removal of DNA from the tissue was related to 5 % ACP and 1 % SDS groups. Disorganization of collagen fibers and tissue architecture was observed more in the SDS group than in the ACP group. No group reported cytotoxicity and the best blood compatibility in decellularization with herbal agents was reported. Protein bands are largely conserved in all methods. According to the histological results, among the decellularized tissues with herbal detergent, the 5 % ACP group was selected. Compared to the images of native ovarian tissue, decellularization with herbal detergent has been able to preserve the structure of thetissue to a large extent and the interconnection of fibers can be seen well. In the 1 % SDS method, fiber breakage is visible and tissue porosity has increased.



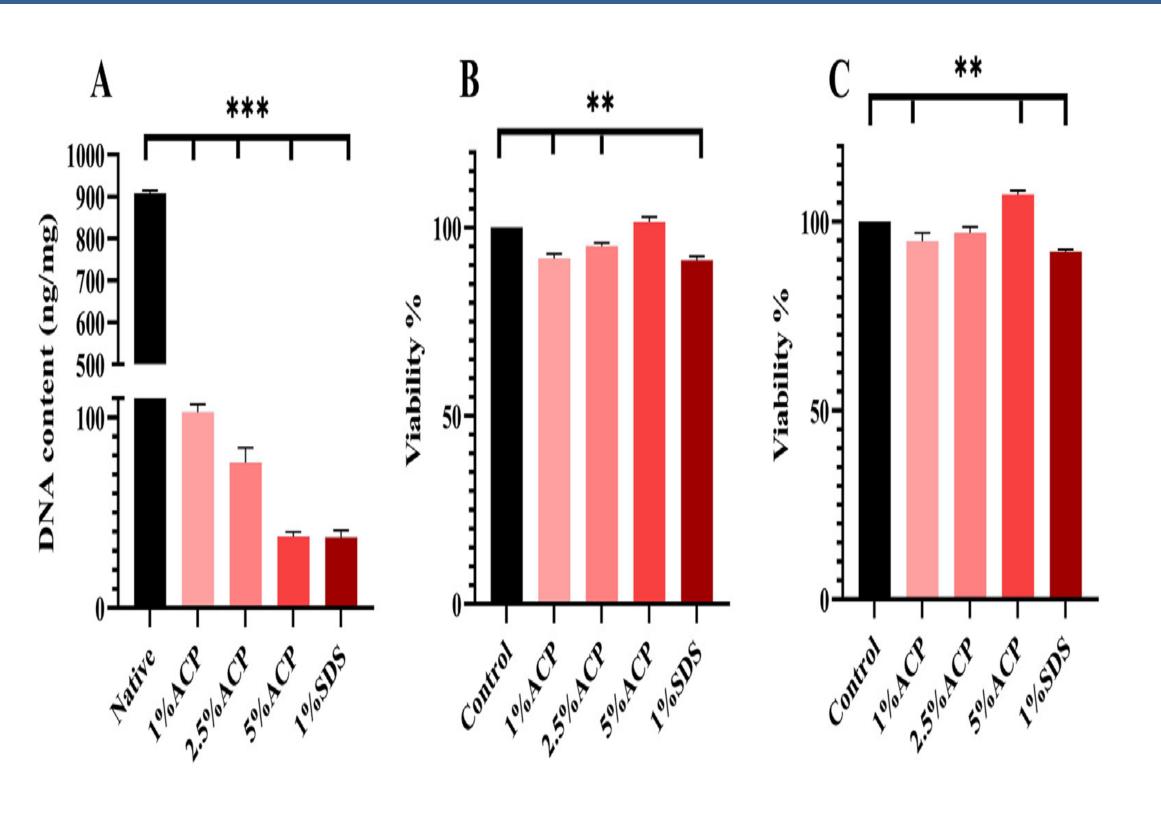
Preparation steps of detergents and decellularization



The effect of cadmium with or without carob extract on sperm count, sperm motility, sperm viability, sperm morphology



SEM images



The results of this investigation demonstrate how well 5 %Acanthophyllum (ACP) decellularizes ovarian tissue. The outcomes show that while 1 % SDS and 5 % ACP both efficiently eliminated cellular content, 5 % ACP was better at preserving the ovary's ultrastructure and collagen organization. ACP-treated scaffolds also showed improved blood compatibility, biocompatibility, and antibacterial qualities, which made them a viable substitute for chemical detergents like SDS. For ovarian investigations, ACP might be regarded as a feasible and potentially better herbal agent for decellularization in tissue engineering due to its capacity to decrease cytotoxicity and preserve protein integrity. By providing a safe and efficient process for ovarian scaffold creation, this work advances bioengineering techniques and opens new avenues for future research and possible clinical uses.

# Derived from the design no 4030399

#### Conclusions