Effect of the Cutting Method and Transport Temperature on the Quality of Ovarian Tissue

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Abstract

This study aimed to assess the impact of different tissue sectioning techniques and transport temperatures on the structural integrity of ovarian tissue. Two primary variables were evaluated: the sectioning method (manual, non-rough surface tool, rough surface tool, and vibratome) and the transport temperature (5°C, 20°C, and 37°C).

Introduction

Ovarian tissue preservation is a key tool in reproductive biology and fertility preservation strategies, particularly for women and girls undergoing gonadotoxic treatments. Cryopreservation followed by ovarian tissue transplantation enables the restoration of both hormonal and reproductive functions and represents the only viable option for prepubertal patients or those requiring urgent treatment [1–3].

Factors such as the sectioning technique and transport temperature can critically influence tissue viability. Optimizing these parameters is essential to improve both clinical and experimental outcomes, and to advance toward standardized and efficient fertility preservation protocols [1,4].

Objectives

- To evaluate the effect of different tissue sectioning methods (manual, non-rough surface tool, rough surface tool and vibratome) on the morphological and cellular integrity of porcine ovarian tissue.
- To analyze the influence of various transport temperatures (5 °C, 20 °C, and 37 °C) on ovarian tissue viability.
- To determine optimal handling combinations that best preserve tissue structure and minimize apoptosis, aiming to standardize ovarian tissue processing protocols.

Methodology

Ovarian cortex samples from prepubertal sheep were sectioned using four distinct methods: manual cutting, outdated device, updated device, and vibratome. Each sample was then transported at one of three controlled temperatures: 5 °C, 20 °C, or 37 °C. Four replicates were performed for each method-temperature combination, totaling 48 samples.

Following transport, the samples were fixed in 4% paraformaldehyde, embedded in paraffin, and processed for histological and immunohistochemical analysis. Hematoxylin-eosin (H&E) staining was used to assess general tissue morphology, while immunohistochemistry was employed to detect caspase expression as a marker of apoptosis.

Samples were coded according to the applied treatment, enabling a systematic comparison of the effects of sectioning methods and transport conditions on ovarian tissue quality.

Results

The results revealed significant differences in ovarian tissue quality depending on the treatment applied. Certain combinations of sectioning method and transport temperature favored improved morphological preservation and reduced apoptotic signaling in primordial follicles, suggesting that these factors directly influence tissue viability.

Regarding apoptotic labeling, a clear effect of the sectioning method was observed. The use of the vibratome resulted in a substantial increase in cellular damage, with 62% of primordial follicles showing apoptotic markers, whereas the other evaluated methods preserved 100% of follicles without apoptotic signal. This highlights the detrimental impact that an inappropriate sectioning technique can have on tissue viability (Table 1).

Transport temperature also played a key role in tissue preservation. Conditions at 37 °C were associated with the highest levels of tissue damage compared to lower temperatures(Table 1). These findings underscore the importance of careful protocol selection in both sectioning and transport to optimize ovarian tissue quality for future applications.

Conclusions

-Sectioning method and transport temperature critically affect ovarian tissue viability, with vibratome use and transport at 37 °C being particularly detrimental, significantly increasing cellular damage and apoptotic signaling.

-Proper selection of tissue handling conditions—including less invasive cutting techniques and lower transport temperatures—is essential to preserve the morphological and functional integrity of ovarian tissue in both clinical and experimental contexts.

Future Steps

Future work will focus on determine if transport temperature and sectioning method quality of the tissue has a positive effect on tissue quality after thawing. Additionally, evaluating the functionality of preserved tissue through in vitro culture will be essential to confirm follicular viability and activation after manipulation.

References

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Table 1. *Results of the % of marked primordial follicles*. Values are presented as: X (n/N), where:X is the percentage of marked follicles, n/N indicates the number of marked follicles over total counted. Green "L" indicates that the error is within ±1.5, suggesting the value is not statistically significant. Red "H" indicates that the value is higher than expected and statistically significant. For both temperature and type of section, the p-value is under 0,001.

	RESULTS					
	% of Marked Primordial Follicles					
	Manual	Non-Rough Surface Tool	Rough Surface Tool	Vibratome	Total	
5	0 L (0/658)	0 L (0/320)	0 L (0/372)	4,2 H (10/229)	0,6(10/1589)	p=<0,001
20	0 L (0/434)	12,5 H (3/24)	0 L (0/320)	2,4 H (10/412)	1,1(13/1190)	
37	6 L (11/182)	0 L (0/434)	0 L (0/371)	97,4 H (1054/1082)	51,5(1065/2069)	
Total	0,9 (11/1274)	0,4(3/778)	0(0/1063)	62(1074/1733)		
	p=<0,001					