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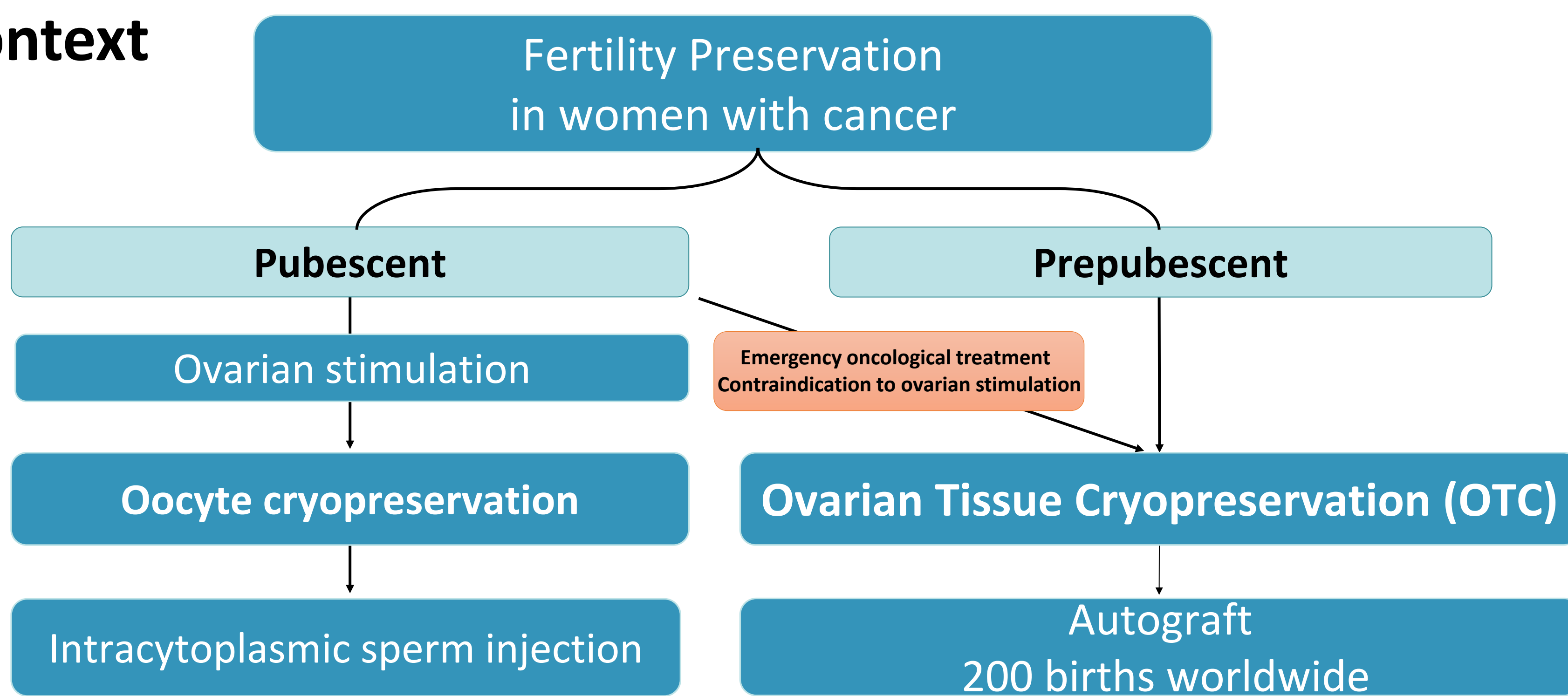
HAL Authorization

Optimization of freezing and thawing protocols for human ovarian tissue cryopreservation through thermophysical characterisation of freezing medium by Differential Scanning Calorimetry

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1. Context



OTC: significant improvements are required since freezing and thawing protocols vary according to laboratories without any clear justification of selection criteria

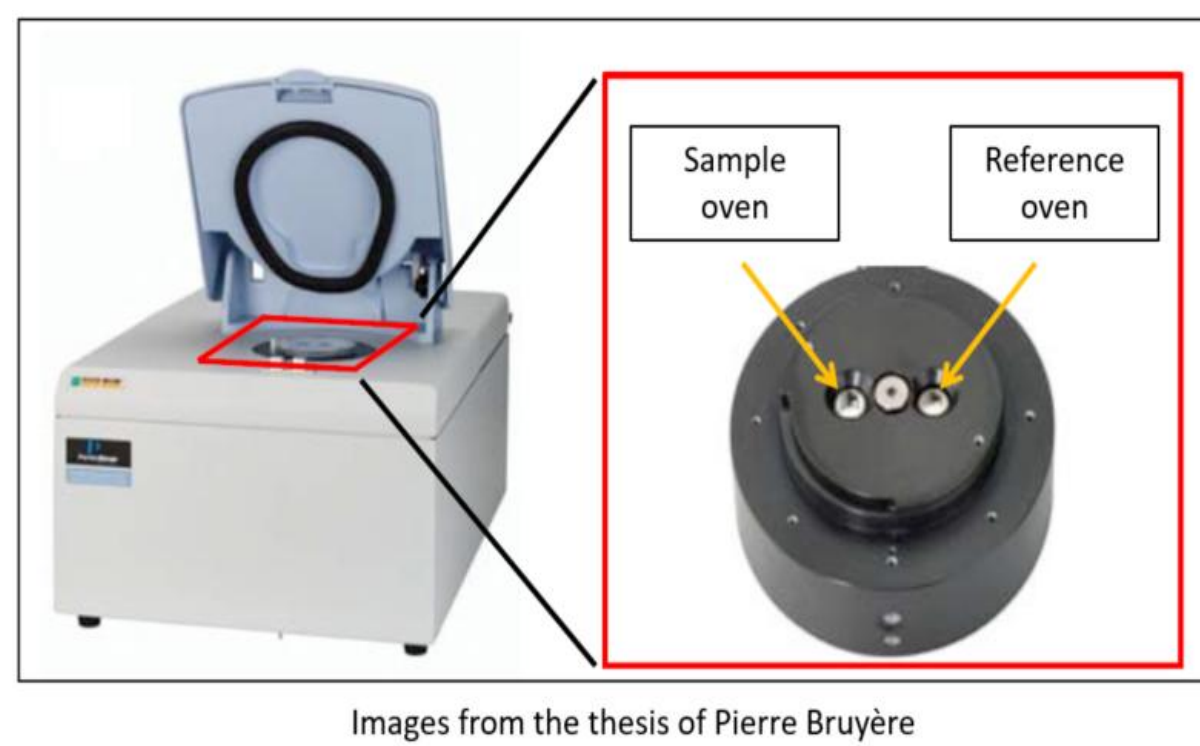
Aim of the study:
To perform an extensive thermodynamic characterisation of the most commonly and successfully used freezing medium for OTC using differential scanning calorimetry (DSC) to optimize freezing and thawing protocols.

2. Materials and Methods

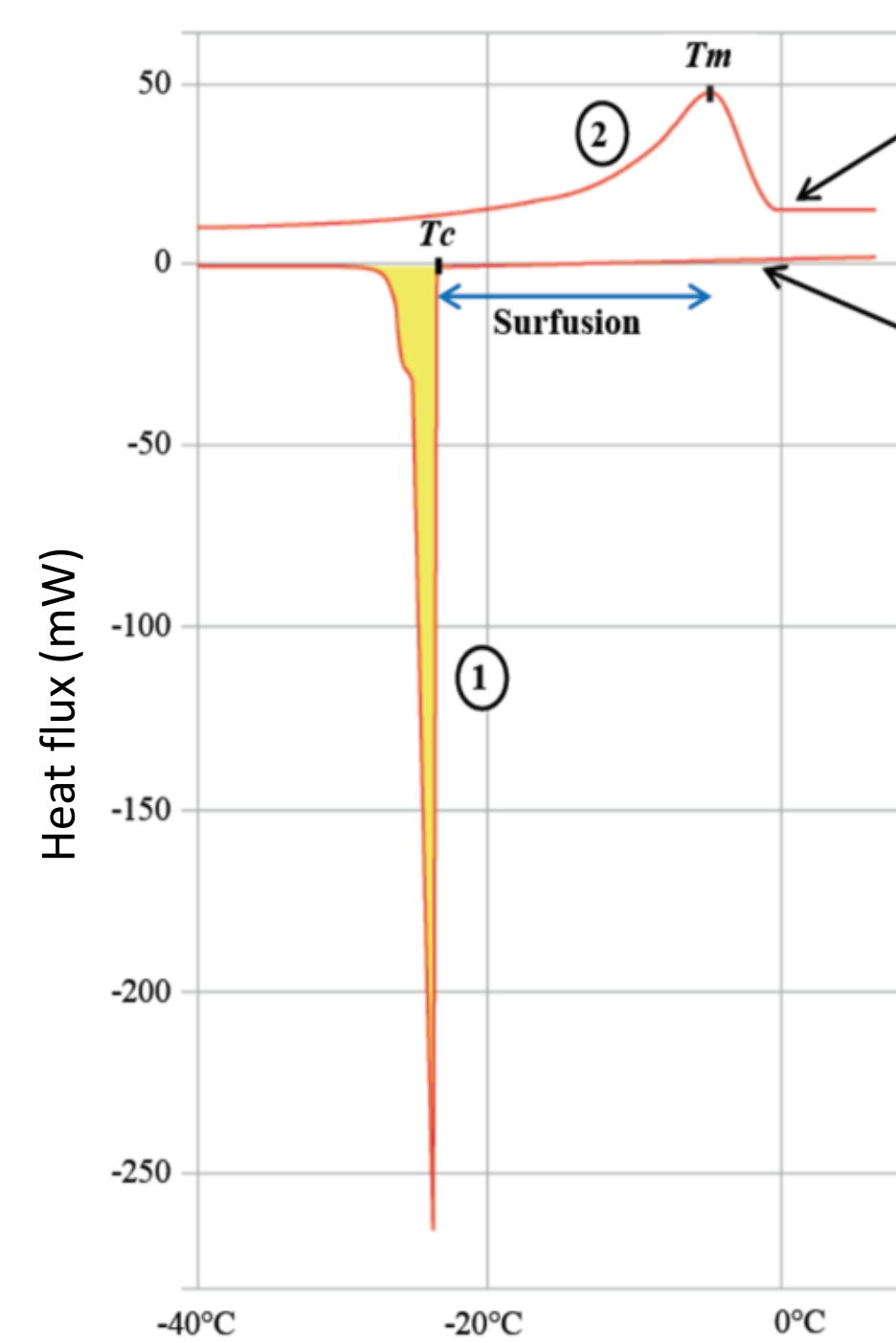
A. Thermodynamic properties of the freezing medium

Method: differential scanning calorimetry

Leibovitz
+ HSA
+ DMSO
+ Sucrose

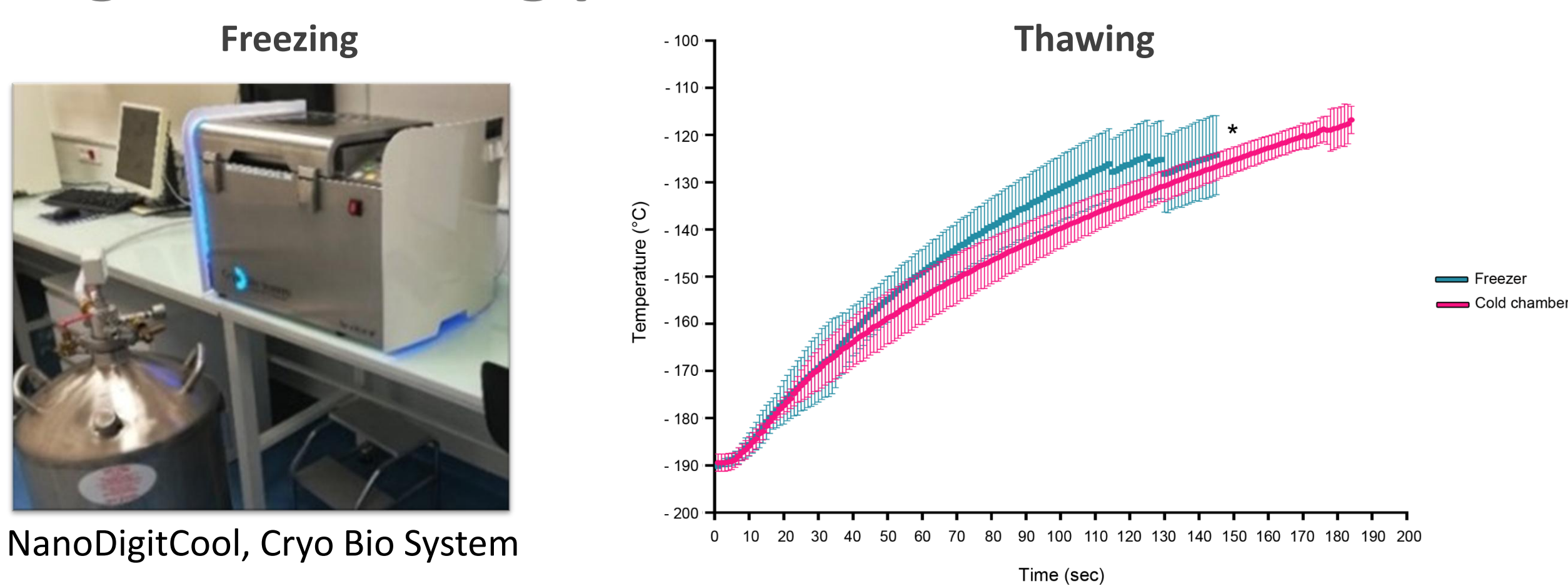


Images from the thesis of Pierre Bruyère



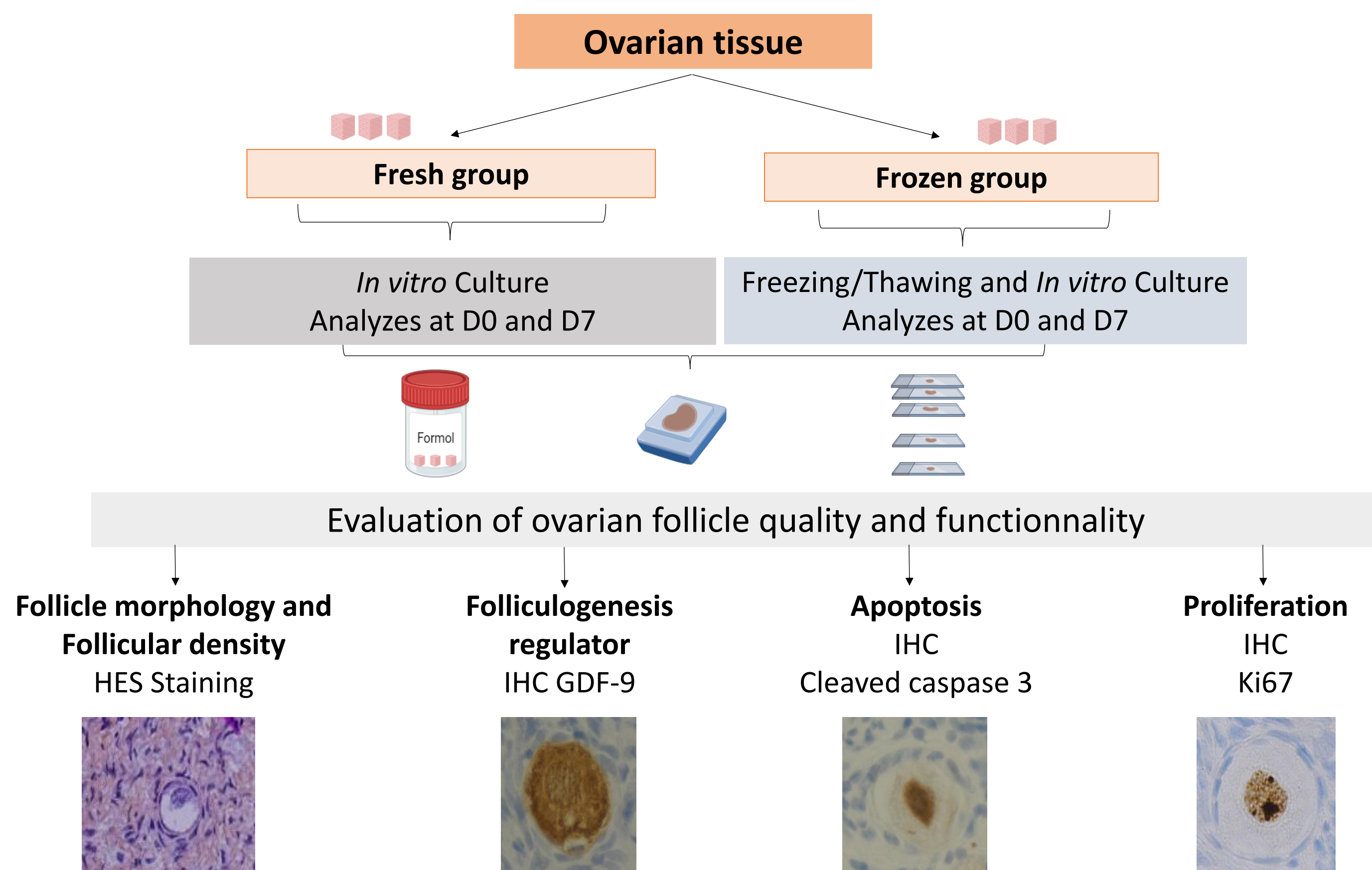
Tg': transition temperature
Tc: Crystallization temperature
Tm: end-of-melting temperature

B. Freezing and thawing protocols



Optimized according to thermodynamic properties of the freezing medium

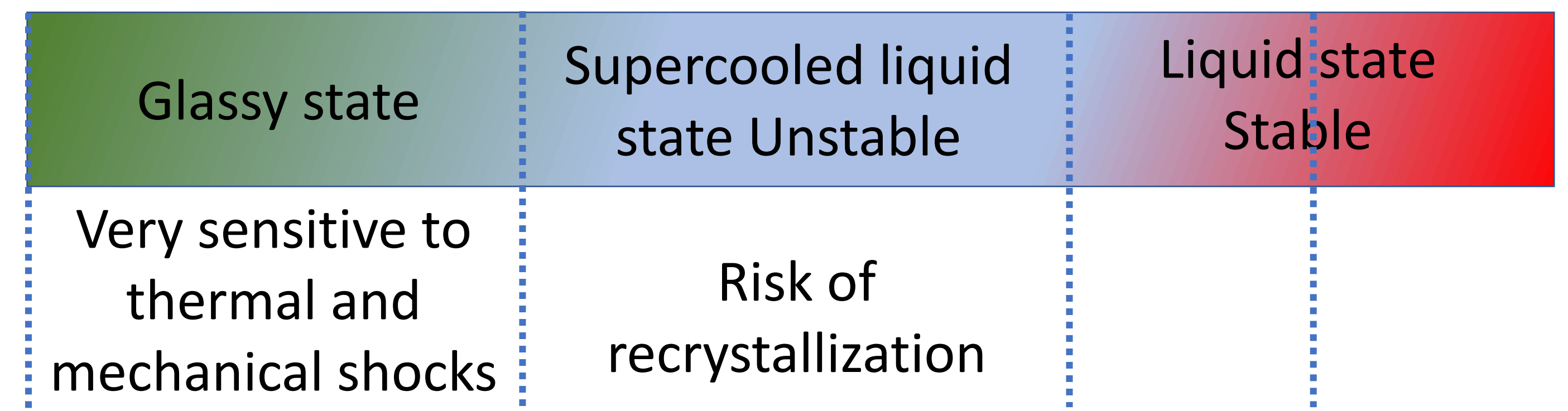
C. Quality of human ovarian tissue post freezing/thawing ?



3. Results

A. Thermodynamic properties

	Tg'	Tc	Tm
Liquid nitrogen	-120.49	-20	4.11
	-196°C	±3°C	±0.49°C

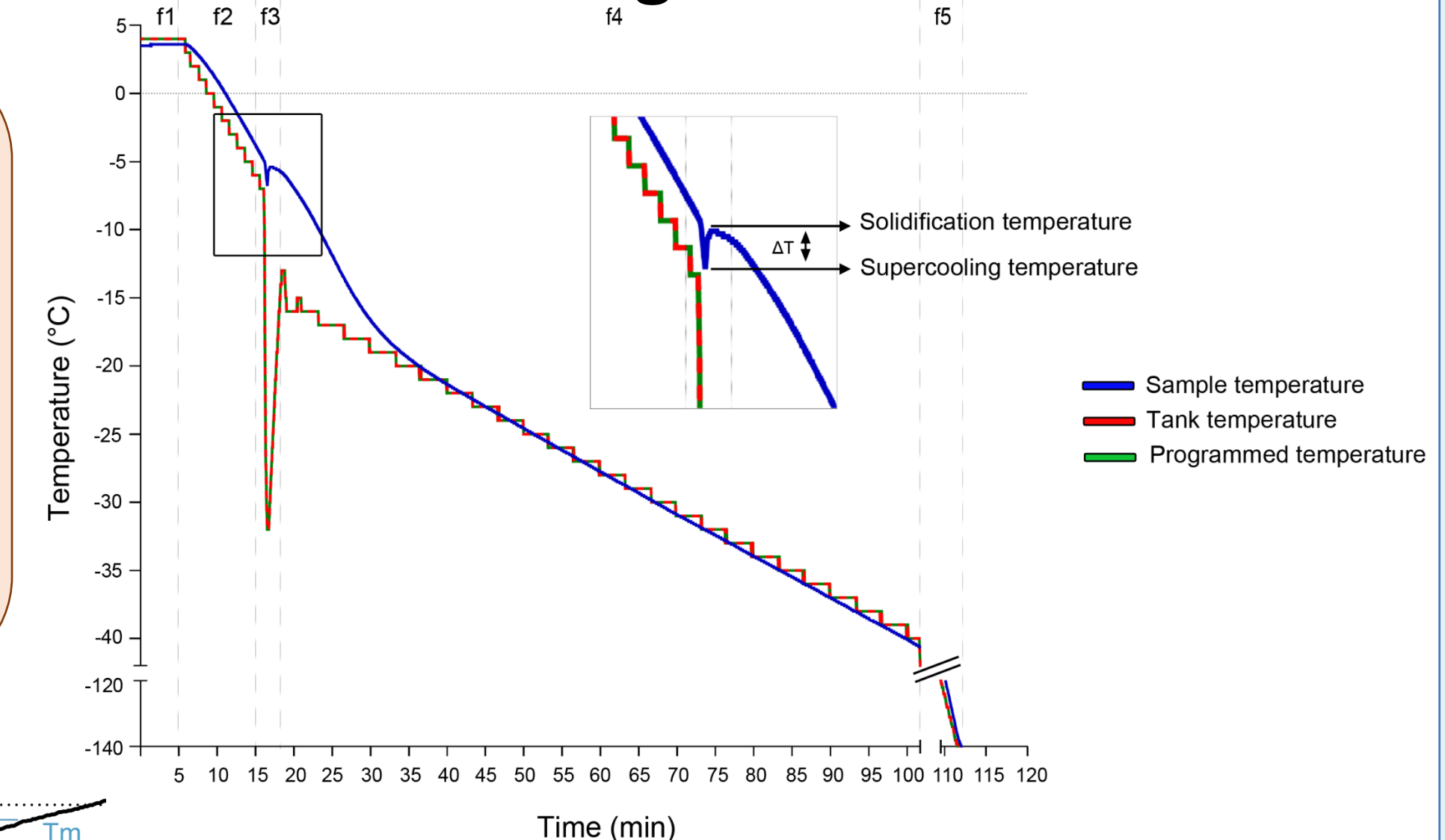


B. Freezing and thawing protocols

Freezing protocol

5 min at +4°C (f1)
1°C/min to -7°C (f2)
seeding: 60°C/min to -32°C to reach Tc
10°C/min to -15°C (f3)
0,3°C/min to -40°C (f4)
10°C/min to -140°C (f5)

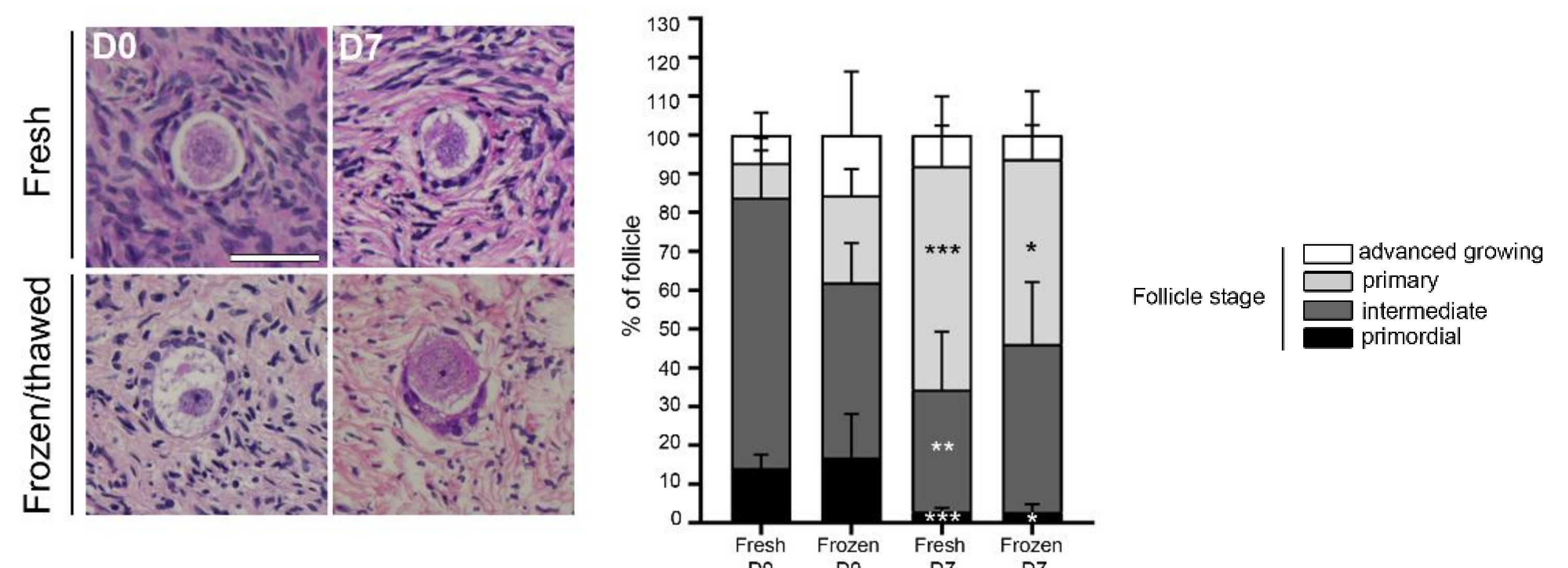
Freezing curve



Thawing protocol

3,5-minute step in a cold chamber to reach slowly Tg'
1,40-minute step at +37°C to quickly reach Tm

C. Quality of human ovarian tissue post freezing/thawing ?



4. Conclusions

- Freezing and thawing protocols for human ovarian tissue are optimized according to thermodynamic properties of the freezing medium
- Thawed ovarian tissues maintained a quality comparable to fresh tissue with evidence of follicle activation post-thawing
- Clinical perspectives: optimisation of cancer patient fertility preservation