Update of laboratories advances for OTC and clinical outcomes

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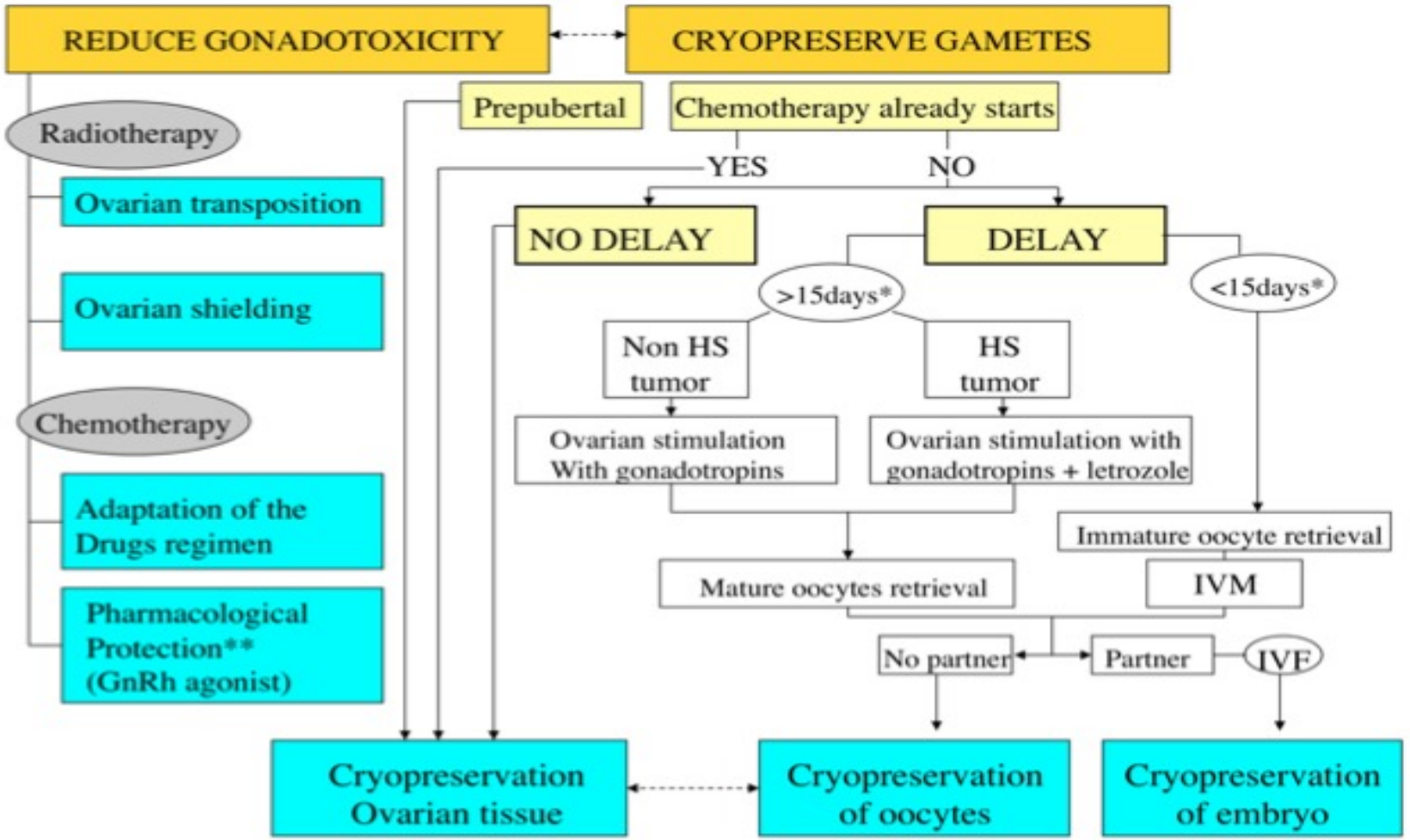
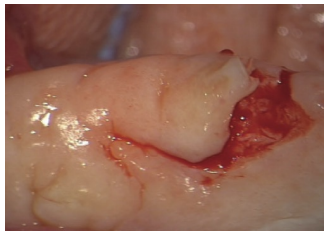
Fertility preservation Options



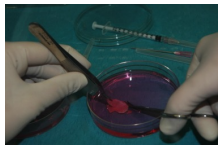
Cryopreservation of ovarian tissue procedure

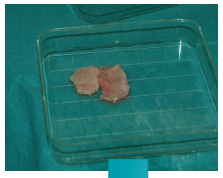
Ovarian biopsy
or oophorectomy



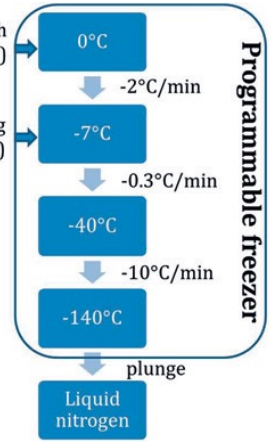
TRANSPORT
UP TO 24h

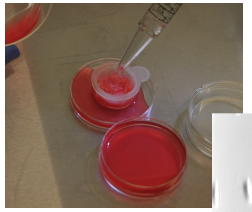
Slow-freezing

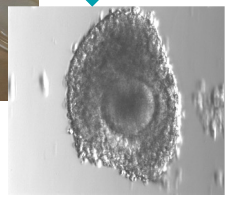




Long-term storage







IVM

To be considered as experimental

Leonel et al 2019

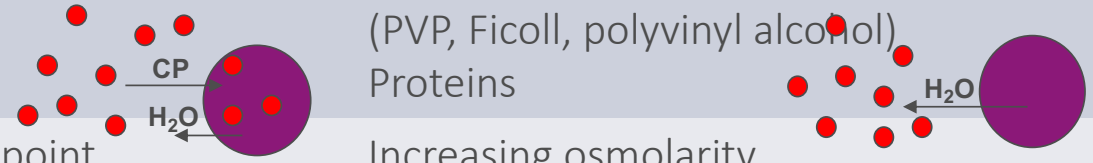
Challenges

Reduce Ice crystal formation
- Breaking cells membrane
- Increase intracellular volume

Reduce the osmotic stress

→ Reduce Follicular AND stromal damage

Permeable cryoprotectants	Non-permeable cryoprotectants
Dimethyl Sulphoxyde (DMSO) 1,2- Propanediol (PROH) Ethylene glycol (EG)	Disaccharides Macromolecules (PVP, Ficoll, polyvinyl alcohol) Proteins
Lowering the freezing point Stabilizing proteins and DNA Minimizing osmotic change	Increasing osmolarity Contributing to cell dehydration Reducing toxicity of permeable cryoprotectants (counteracts osmotic stress)



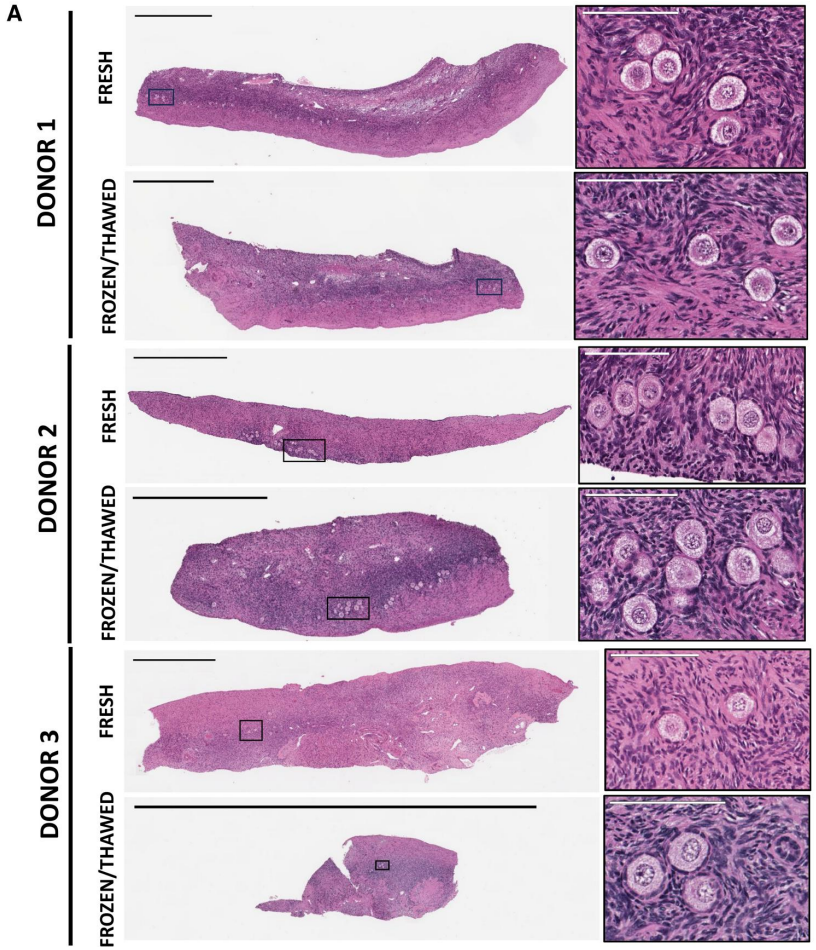
Slow Freezing Protocol

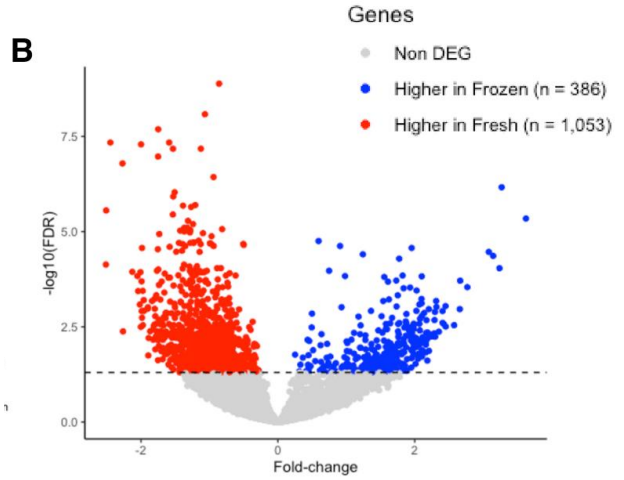
Table 1. Slow freezing methods used by the most prominent groups performing fertility preservation worldwide

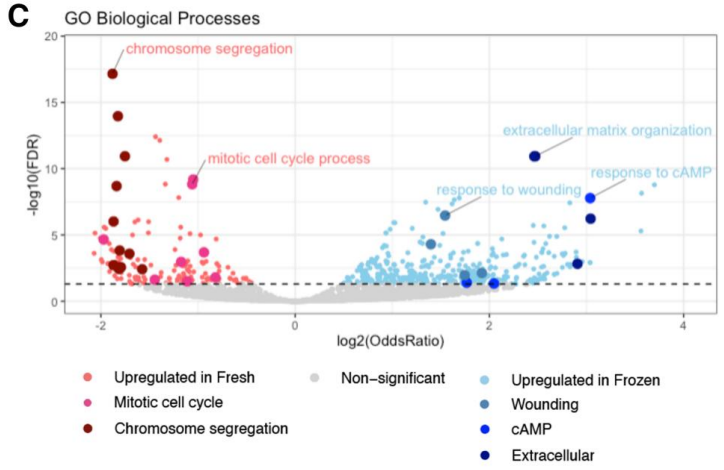
Country	Base medium and supplements	CPAs	Equilibrium	Cooling curve	Ref.
Belgium	Minimum essential medium (MEM) + 4 mg/mL HSA	10% DMSO	30 min at 0°C	−2°C/min to −8°C, manual seeding, −0.3°C/min to −40°C, and −30°C/min to −140°C	67
Belgium	Leibovitz L-15 medium	1.5 M DMSO 0.1 M sucrose	30 min at 4°C	−2°C/min to −7°C, manual seeding, −0.3°C/min to −40°C, and −10°C/min to −140°C	68–71
Denmark	PBS	1.5 M EG 0.1 M sucrose	30 min at 1°C	2°C/min to −9°C, manual seeding, −0.3°C/min to −40°C, and −10°C/min to −140°C	72–75
Portugal	RPMI 1640 medium + GlutaMAX and 15% FCS	10% DMSO	30 min at 4°C	Overnight in a freezer at −80°C	76
Australia	PBS + albumin	1.5 mol PROH 0.1 M sucrose	30 min at room temperature	−2°C/min to −8°C, manual seeding, −0.3°C/min to −30°C, and −50°C/min to −150°C	77–79
Australia	Dulbecco's PBS	1.5 M DMSO 0.1 M Sucrose			80
France	Leibovitz L-15 medium + 10% decomplemented patient serum	1.5 M DMSO 0.1 M sucrose			81
France	Leibovitz L-15 medium + 10% FCS	1.5 M DMSO	On ice for 15 min	−2°C/min to −7°C, seeding, −0.3°C/min to −40°C, and −10°C/min to −140°C	82
Germany	PBS	1.5 M DMSO + 1.5 M PROH	Increasing steps of 0.25 M up to 1.25 M of DMSO/PROH (7 min each) and then 1.5 M DMSO/PROH (30 min) at 37°C	−5°C/min to −3.8°C, −1°C/min to −5.3°C, −0.2°C/min to −6°C, hold for 20 min (autocrystallization), −0.3°C/min to −30°C, −0.1°C/min to −35°C, −0.3°C/min to −80°C, and −10°C/min to −110°C	83
Germany	Leibovitz L-15 GlutaMAX medium + serum substitute supplement	10% DMSO	30 min at 2°C	−2°C/min to −6°C, automatic seeding, −0.3°C/min to −40°C, −10°C/min to −140°C, stored at −150°C in MVE Vapor phase storage tanks	26
Spain	RPMI 1640 + 20% human serum	1.5 M DMSO	10 min at 4°C in 0.7 M DMSO and 10 min at 4°C in 1.5 M DMSO	−0.5°C/min to −7°C, automatic seeding, −0.5°C/min to −50°C, −5°C/min to −80°C, and −8°C/min to −120°C	84, 85
Spain	M199 + 5% human serum	12.5% DMSO	Not mentioned	−1.5°C/min to −12°C, seeding, −10°C/min to −30°C, −5°C/min to −20°C, stabilization at −20°C for 5 min, −0.5°C/min to −50°C, −5°C/min to −80°C, and −8°C/min to −120°C	86
Sweden	PBS	1.5 M PROH 0.1 M sucrose	Room temperature (time not mentioned)	Using the programmable freezing device CTE 920 with automatic seeding at an optimal temperature (detailed curve not described)	87
Sweden	Leibovitz L-15 medium + 10% FCS	1.5 M DMSO	On ice for 15 min	−2°C/min to −7°C, seeding, −0.3°C/min to −40°C, and −10°C/min to −140°C	88
Israel	Oocyte wash buffer + 15% synthetic serum	1.5 M DMSO 0.1 M sucrose	30 min	−1°C/min to −9°C, manual seeding, −0.3°C/min to −36°C, and −5°C/min to −140°C	89, 90
Israel	Leibovitz L-15 medium + 10% HSA	1.5 M DMSO 0.1 M sucrose	30 min at 4°C	−2°C/min to −9°C, manual seeding, −0.3°C/min to −40°C, and −10°C/min to −140°C	91
USA	HEPES-buffered Dulbecco's minimum essential medium (DMEM)-F12	1.5 M DMSO 0.1 M sucrose	30 min on ice	−2°C/min to −7°C, manual seeding, −0.3°C/min to −40°C, and −10°C/min to −140°C	92
USA	Leibovitz L-15 medium + 10% FCS	1.5 M DMSO	On ice for 15 min	−2°C/min to −7°C, seeding, −0.3°C/min to −40°C, and −10°C/min to −140°C	93
UK	Leibovitz	1.5 M DMSO 2.5% HSA	30 min at 4°C	−2°C/min to −9°C, manual seeding, −0.3°C/min to −40°C, and −10°C/min to −140°C	94
UK	Leibovitz L-15 medium + 10% FCS	1.5 M DMSO	On ice for 15 min	−2°C/min to −7°C, seeding, −0.3°C/min to −40°C, and −10°C/min to −140°C	95

CPAs, cryoprotectant agents; DMSO, dimethyl sulfoxide; EG, ethylene glycol; FCS, fetal calf serum; HSA, human serum albumin; PBS, phosphate-buffered saline; PROH, 1,2-propanediol.

Impact on follicular quality





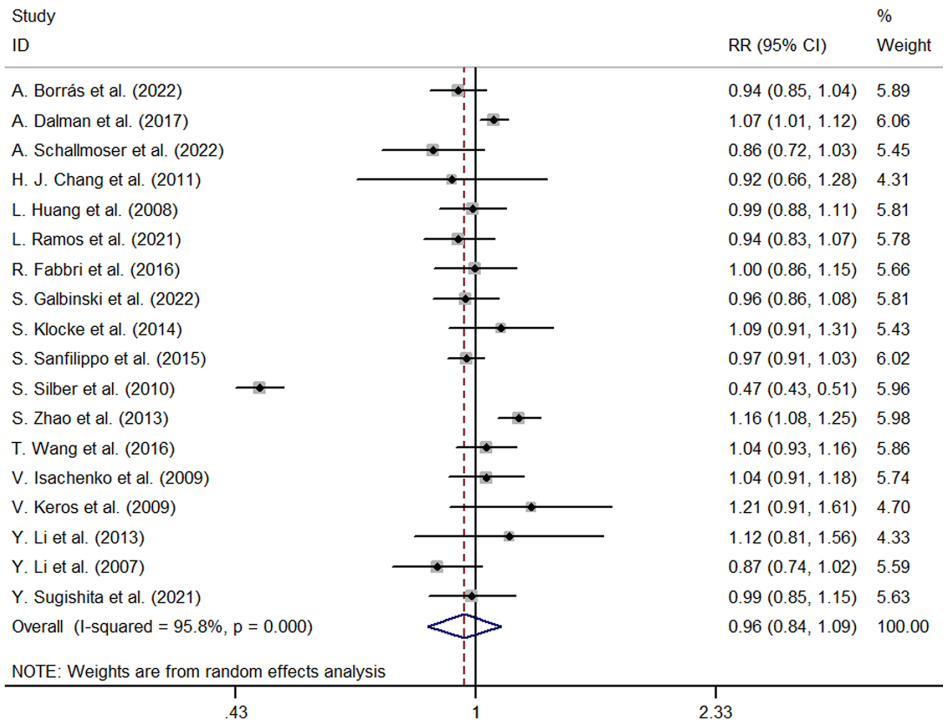


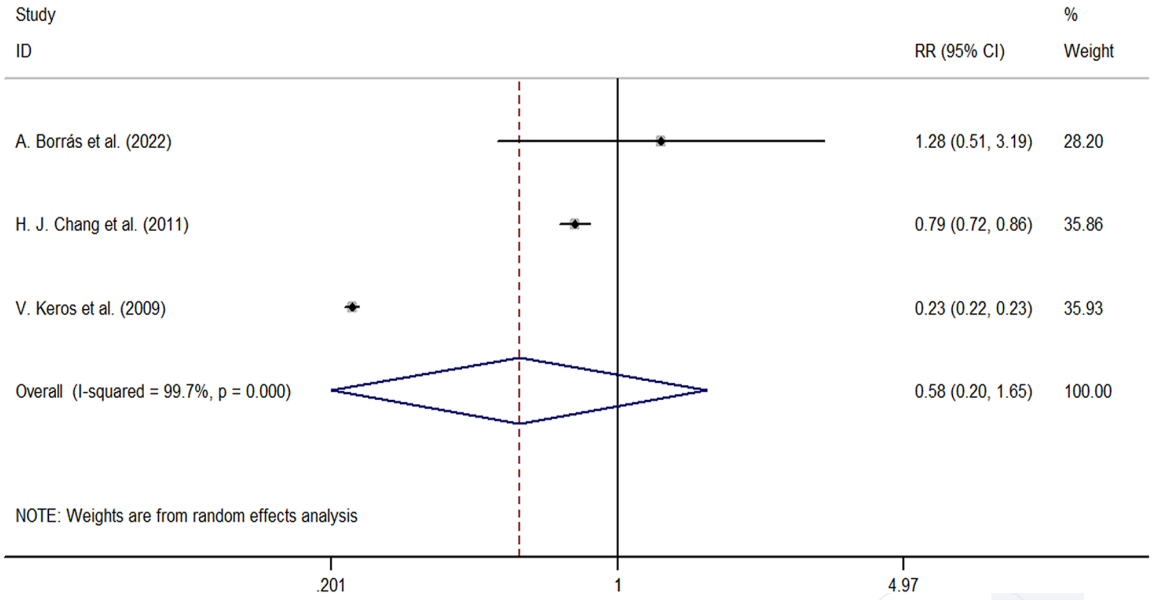
Machlin et al HR 2025

Slow freezing versus vitrification?

SLOW FREEZING	VITRIFICATION
Low concentration of cryoprotectants Slow dehydratation (equilibration) Slow cooling (0.1-0.3°min) Takes hours Required specific equipment or alternatives as « Mister frost » should be more investigated	High concentrations of cryoprotectants Very fast freezing (-23000°/min) Time-saving No special or expensive equipment required Technical training

Evidence: follicular survival





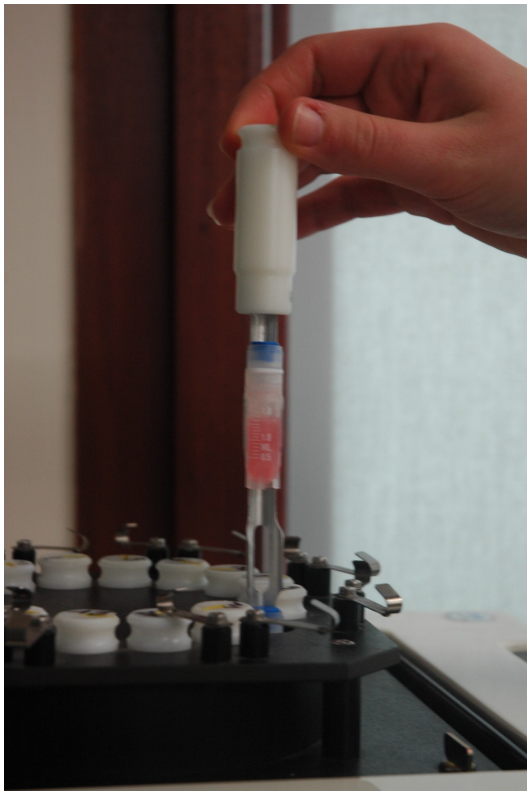
No difference in primordial follicular survival/ potentially better follicular environment

Large heterogeneity…

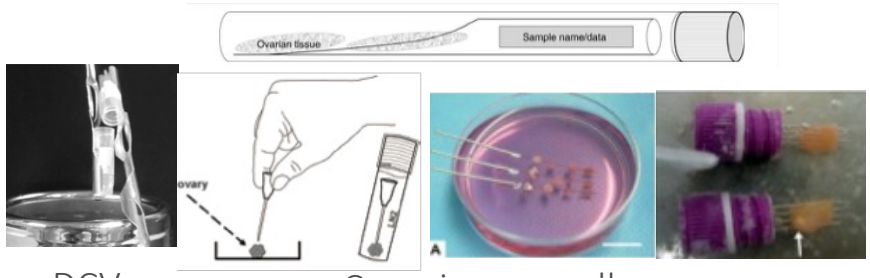
Kong et al 2025

Protocol standardisation?

SLOW FREZZING

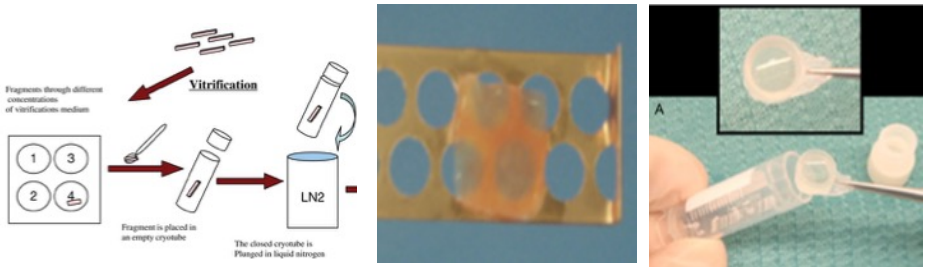


VITRIFICATION



DCV

Cryopin or needles



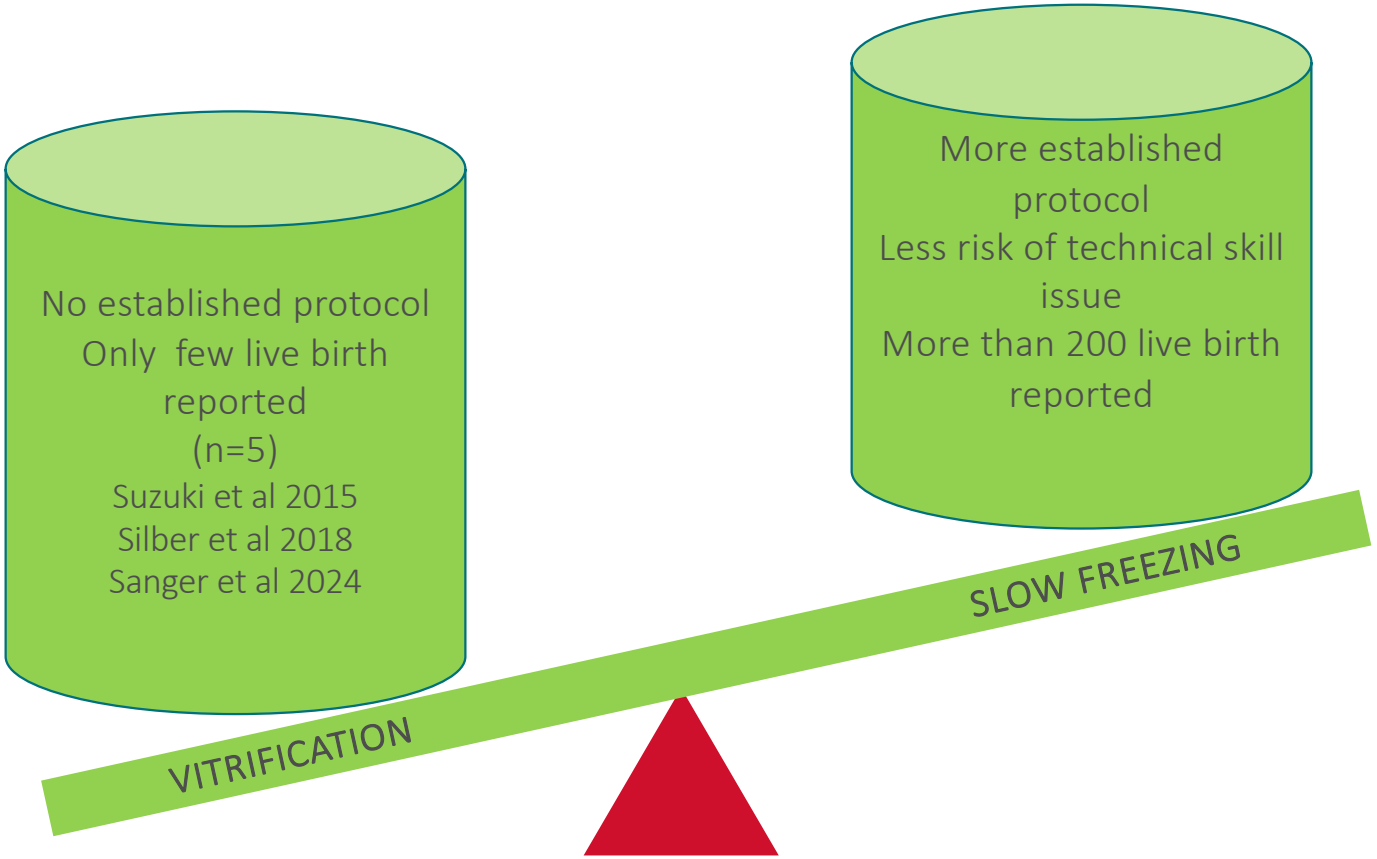
Closed-system

Open metalic/plastic support

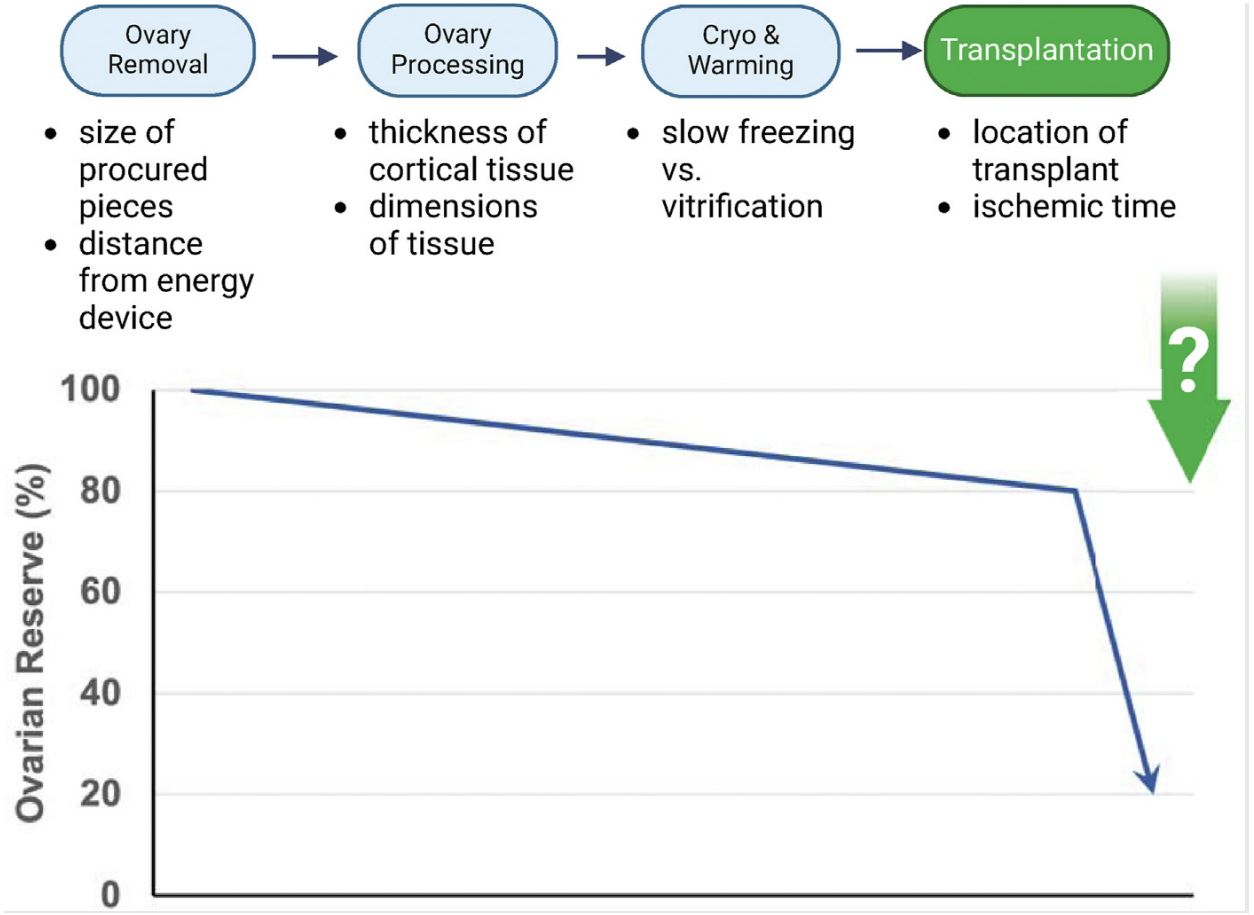
freezing solution	
TSC	VC
1.5M PrOH + 0.2M sucrose solution in 1.8 ml cryovials	20% ethylene glycol, 20% DMSO and 0.5M sucrose in 1.8 ml cryovials
0.1 mol/L sucrose + 1.5 mol/L ethylene glycol	15% ethylene glycol and DMSO
1.5M DMSO + 0.1M sucrose + 10% HSA	10% ethylene glycol+20% serum substitute supplement
1.5 M DMSO and 0.1 M sucrose solution in 1.8 ml cryovials	20% DMSO + 20% EG
0.1 M sucrose +1.5 M ethylene glycol	7.5% EG and 7.5%Dimethyl Sulfoxide
1.5 mol/L 1,2-propanediol and 0.1 mol/L sucrose	7.5% EG and 7.5%Dimethyl Sulfoxide
10% DMSO + 11% HSA	10% ethylene glycol+10% serum substitute supplement
10% DMSO + 10% EG + 6% PEG + 0. 5% BSA + 0. 5mol /L sucrose	15% DMSO + 15% EG + 6% PEG + 0. 2% BSA + 0. 5mol /L sucrose
1.5 M DMSO + 0.1 M sucrose + 10% SSS	2.62 M DMSO, 2.60 M acetamide, 1.31 M propylene glycol, and 0.0075 M polyethylene glycol
0.1 M sucrose +1.5 M ethylene glycol + 10% HSA	7.5% ethylene glycol+7.5% DMSO+20% HSA
1.5M DMSO + 0.1M sucrose + 10% HSA	7.5% ethylene glycol+7.5% DMSO+20% HSA
1.5M DMSO + 0.1M sucrose	2M DMSO + 0.1M sucrose
1.26 mol/L 1,2-propanediol and 0.175 mol/L sucrose and 30%HSA	2 M propylene glycol+3 M ethylene glycol+0.2 M sucrose+15% HSA
1.5M DMSO + 0.1M sucrose + 10% HSA	20% EG + 20% HSA
10% DMSO + 2% HSA	7.5% ethylene glycol+7.5% DMSO+20% HSA
1.5M DMSO + 0.1M sucrose + 10% HSA	7.5% ethylene glycol+7.5% DMSO+20% HSA
1.5 M PrOH and 0.025 M raffinose	1.5 M PrOH+1.5 M EG
1.5 M PrOH and 0.1 M sucrose	1.4 M DMSO, 1.5 M PrOH, 1.5 M EG

Sheikhi et al, 2011; Chen et al, 2006; Fathi et al, 2017; Fabbri et al, 2016; Keros et al, 2009 ; Silber, 2015; Wang et al, 2008; Suzuki et al, 2015

Vitrification versus slow freezing?



Limitation of the procedure

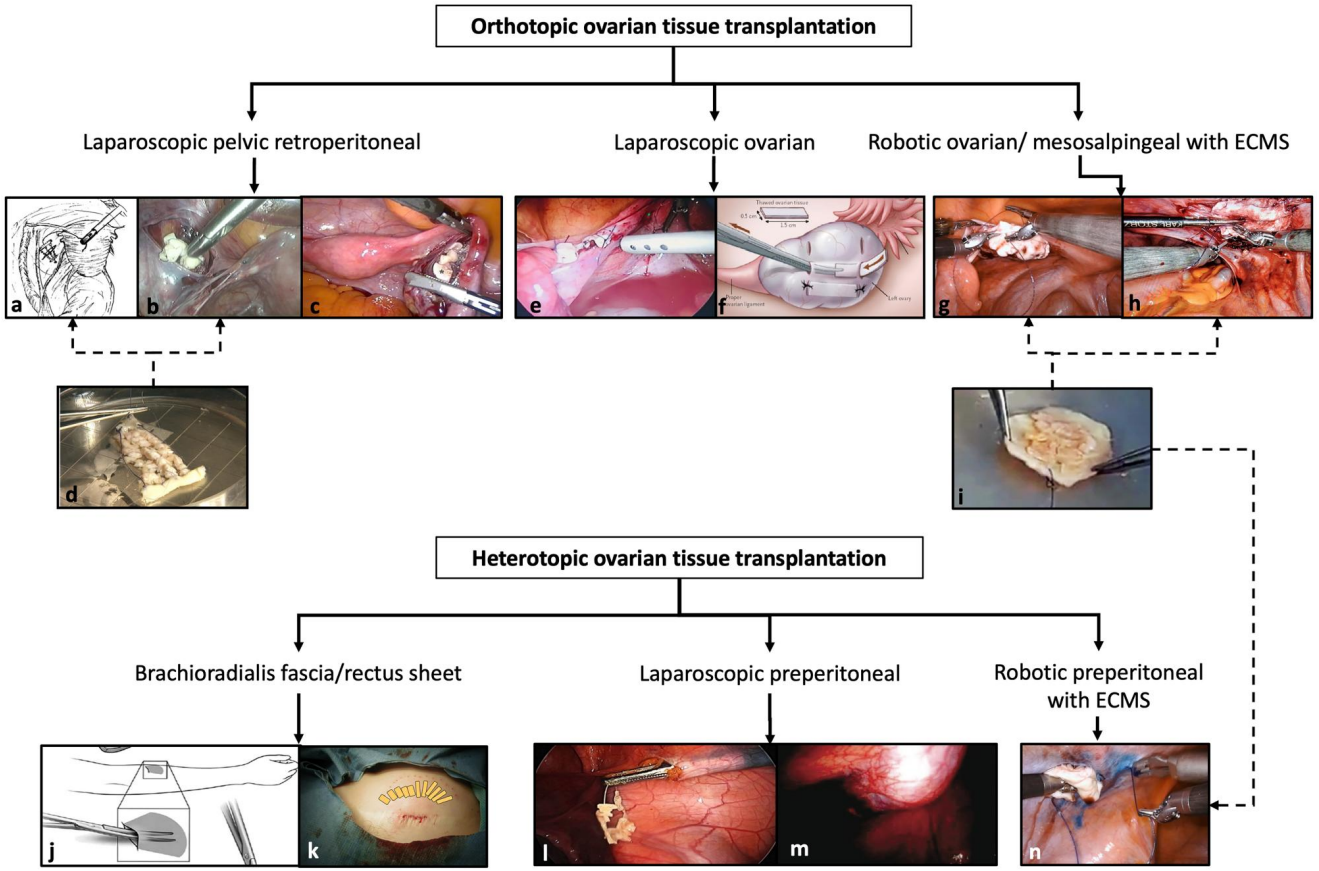


Gadek et al, 2024

Improve the transplantation procedure?

- Orthotopic transplantation site (ovarian sites) is favoured by all centers
- Subperitoneal pocket near the fallopean tubes favourated by many centers as alternatives site (primary site for FertiProtekt)
- Vascularisation can occur at both cortical and medulla sites (Kirstensen et al 2022)
- No data to estimate the optimal quantity of tissue (follicles) has to be transplantated (1/3 ovary?)
- Low success rate using ART after OTT (1-7% LBR/started cycle)(Colmorn et al Cancers 2022)
- No evidence in human for efficient treatment of the tissue or recipient to improve transplantation (Gadek et al 2024)

OTT techniques



The training is more important than the techniques…..

Sonmezer et al HRU 2025

Efficiency of the procedure

290 pregnancies and 189 deliveries published in 2022

Pooled pregnancy rate 37%

Pooled LBR 28% (23-41%)

Khattak et al 2022

Overall results are encourageing but return rate is low

Group	Number of patients	Population characteristics	Return rate	Reference
Belgium UCL	545 patients (1997-2013)	0-39 years at OTC mean (22.3 ± 8.8 years)	**24 return for autotransplantation (4.4%) and 21 performed the procedure** LB rate 33%	Jadoul et al, 2017
Sheba Medical Center	203/320 patients (2004-2015)	>20 years at the time of the follow-up	**18/320 patients performed autotransplantation (5%)**	Lantsberg et al, JARG 2019
Sweden	221 patients	18-39 years (mean age 28.1 years)	**Return rate of 5%**	Rodriguez-Wallberg et al, Acta Obst Scand. 2019
Denmark	1186 (1999-2020)	Age 0-43 years	**117 patients performed OTT (10%)** 106 for fertility restoration or improvement 10 for endocrine treatment 1 induced puberty	Kristensen S.G Fertil Steril 2021
FertiPROTEK	2475 patients (2000-2021)	Age 0-44 years at OTC	**124 patients performed autotransplantation (5%)** Pregnancy rate 34.5% LB rate 24.1%	Schallmoser et al, RBMO 2023 Emrich et al, Reprod Biol and endocrinology 2025
Melbourne IVF Australia	593 patients (1995-2022,)	>18 years old at the time of the study Age 9-44 years old at OTC (mean 27.2 (SD 7.3) years)	**48 patients performed autotransplantation (8.1%)** Pregnancy rate to term 25%	Finkelstein et al, 2025

TAKE HOME MESSAGE

- Oocytes cryopreservation is the first option to preserve fertility in adult women
- Slow freezing remains the recommended technique to freeze the ovarian cortex
- Although several approaches have been reported in experimental settings to improve transplantation, none are currently implemented in clinical practice.
- Ovarian tissue cryopreservation is an innovative and efficient fertility preservation technique offering several advantages in children, adolescent and probably young women but should not be offered to all…

OVA-NET

OvaNet

A Global Consortium for Ovarian Tissue Cryopreservation

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SIG Fertility Preservation

About OvaNet

OvaNet is a global consortium of centers specializing in ovarian tissue cryopreservation and transplantation (OTC & OTT), uniting international experts dedicated to advancing the field of female fertility preservation.

Why was OvaNet initiated?

OvaNet was established to promote best practices and coordinate clinical and research efforts aimed at preserving and restoring fertility in girls and young women at risk of fertility loss. Now is the time to come together—to share our collective knowledge and expertise for the benefit of patients worldwide.

To truly advance the field, we need a comprehensive overview of global practices and ongoing activities. Only through such collaboration can we begin to establish consensus guidelines and elevate standards of care in fertility preservation.

Stine Gry Kristensen, Founder of OvaNet

https://www.ovanet.org

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THANK YOU!

<u>Fertility Clinic</u>
Catherine Houba and all the team
Onco-fertilité
Margherita Condorelli
Agathe Rabattu
David Pening
<u>Fertility Lab</u>
Necati Findikli
Giovanna Fasano
Anne Sophie Vanin
Jamila Birame

Research Laboratory on Human Reproduction

All the patients and the oncologists!