Effects of zinc chloride supplementation during vitrification of ovarian tissue in pigs.

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Cryopreservation

- Four Major Steps:
  - Addition of cryoprotectant
  - Freezing at low temperatures and storing in liquid N_2
  - Warming tissue
  - Thawing cells and removing cryoprotectants

(Adedelahi et al., 2013)

- Ovarian tissue cryopreservation preserves large numbers of oocytes versus other techniques.

(Adedelahi et al., 2013)

- Cryopreservation of ovarian tissue is conducted to help preserve the fertility of biomedical models.

(Mouttham & Comizzoli, 2016)

Follicular Damage and Stress

- Ovarian tissue is susceptible to more damage during vitrification due to various cell types and water permeability.

(Adedelahi et al., 2013)

- Vitrification increases reactive oxygen species in oocytes, leading to decreased viability.

(Gupta et al., 2010)

- Cryoprotectant agents can induce oxidative stress thus causing structural and functional changes in tissue.

(Tian et al., 2015)

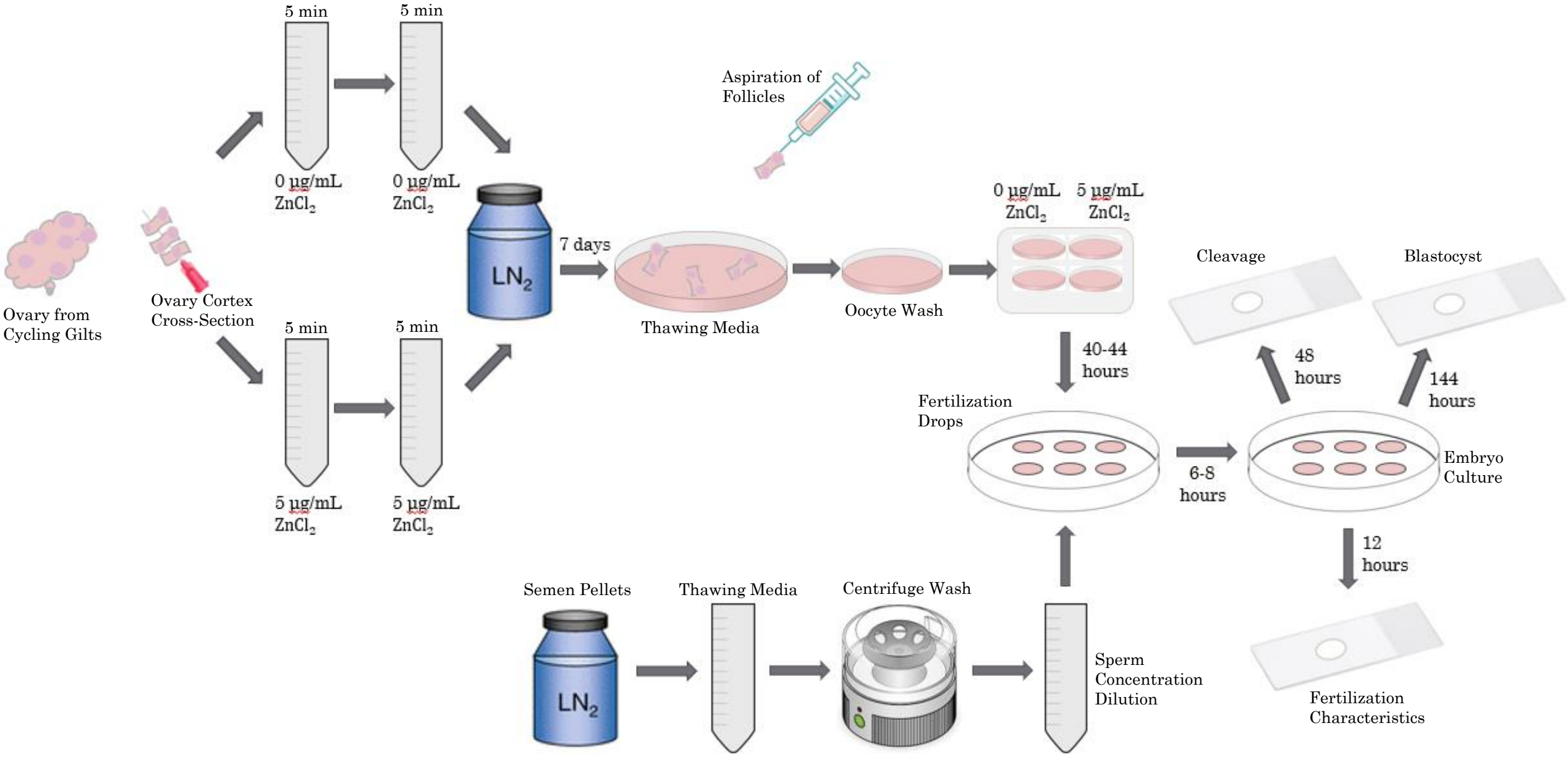
- Zinc reduces oxidative stress by synthesizing proteins that are effective in reducing reactive oxygen species.

(Marreiro et al., 2017)

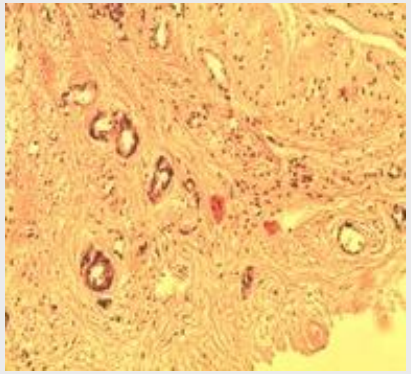
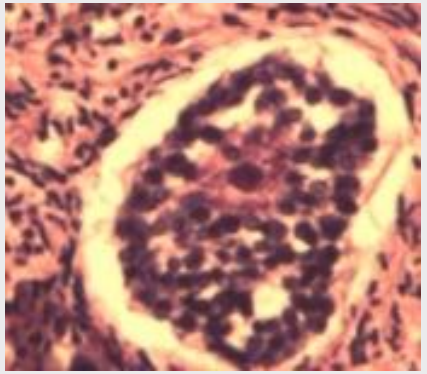
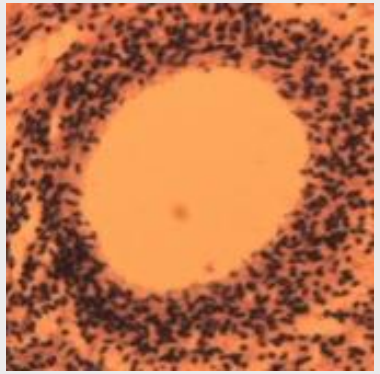
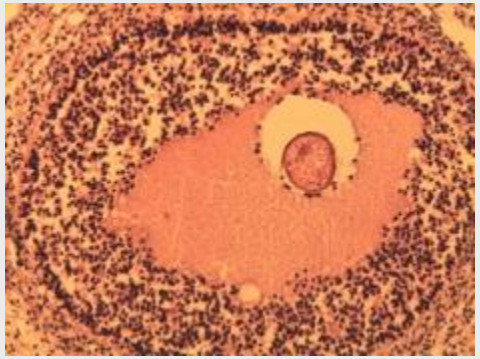
Objectives

- Determine the effects of adding 5 µg/mL $ZnCl_2$ during vitrification on:
  - *in vitro* follicle development
  - post-thawing fertilization success
  - embryonic development

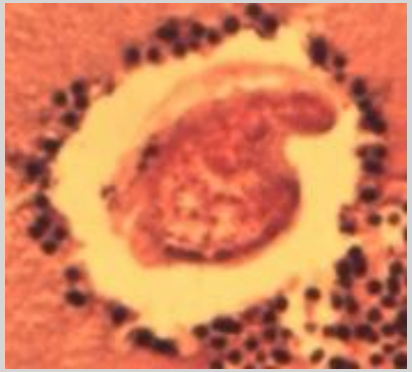
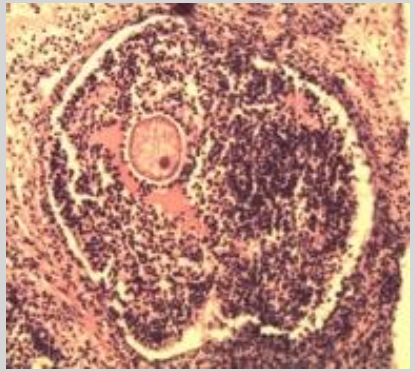
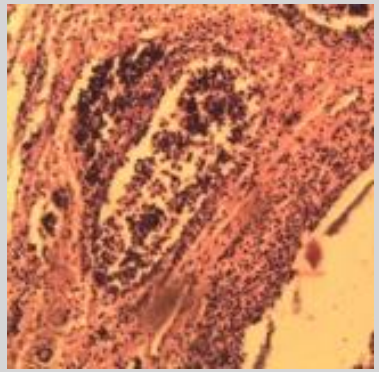
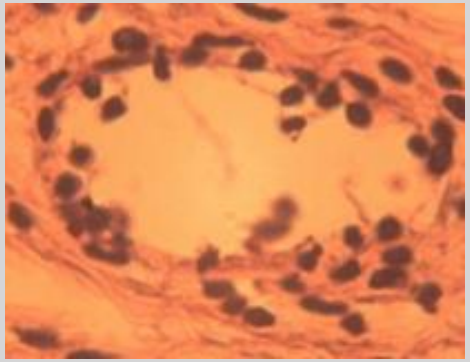
Experimental Design



Follicle Evaluation

Primordial	Primary	Secondary	Antral
• Single layer of squamous cells	• Single layer of cuboidal cells/stratified epithelium	• Theca interna cells • Granulosa cells • Zona pellucida	• Antrum • Cumulus cells
			

Damage Characteristics

Zona Pellucida Disruption	No Defined Antrum	Theca Interna and Granulosa Cell Disruption	Follicular Cells in Cytoplasm
			

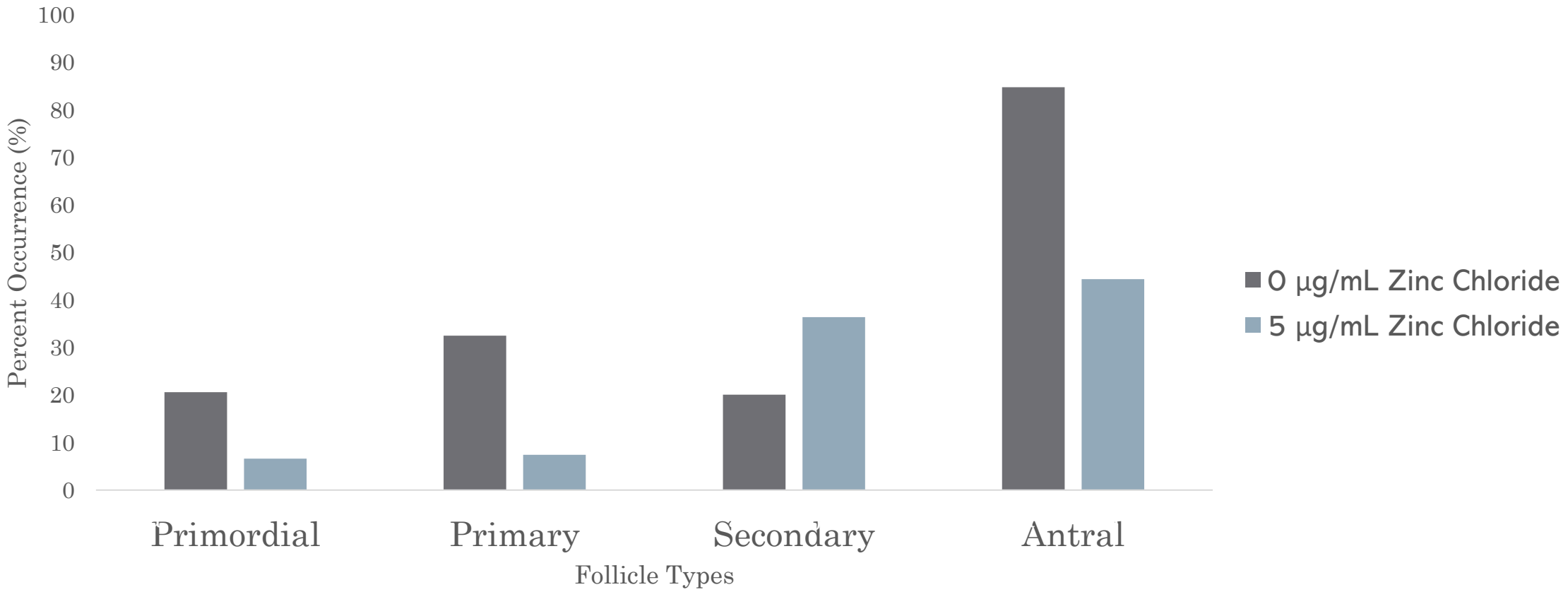
Follicle Counts

Treatment	Primordial (%)	Primary (%)	Secondary (%)	Antral (%)
0 µg/mL $ZnCl_2$	52.7[a]	31.0[a]	18.6[a]	25.6[a]
5 µg/mL $ZnCl_2$	39.4[b]	34.8[b]	14.2[b]	29.0[b]

Total Follicle Damage

Treatment	Total Follicles (%)	Damaged (%)
0 µg/mL $ZnCl_2$	100	46.5[a]
5 µg/mL $ZnCl_2$	100	23.2[b]

Type-Based Follicle Damage



[a,b] $p < 0.05$

Fertilization Characteristics

Sperm Penetration Rates (%)

Media Supplementation	% Penetrated
0 µg/mL $ZnCl_2$	78.60 ± 12.60
5 µg/mL $ZnCl_2$	54.50 ± 14.20

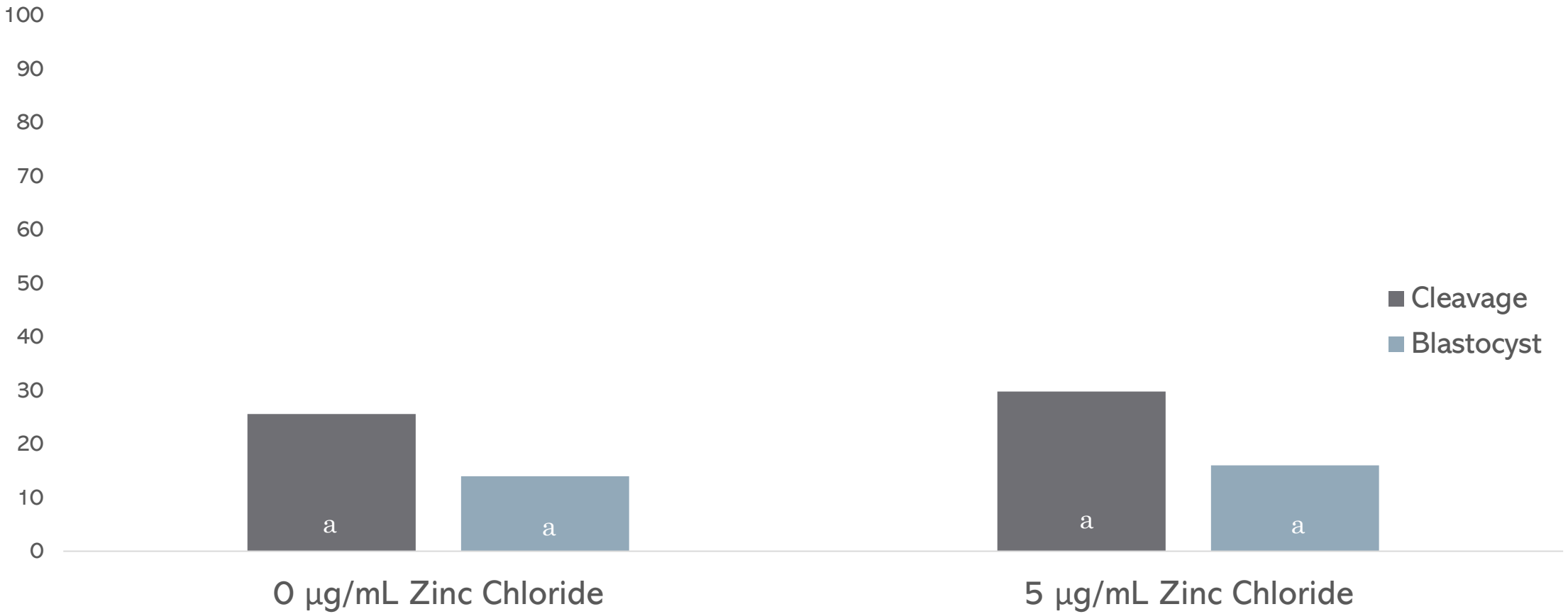
Polyspermy Rates (%)

Media Supplementation	% Polyspermic
0 µg/mL $ZnCl_2$	71.40 ± 12.50^{a}
5 µg/mL $ZnCl_2$	27.30 ± 22.40^{b}

MPN Rates (%)

Media Supplementation	% MPN Formation
0 µg/mL $ZnCl_2$	36.40 ± 13.70^{a}
5 µg/mL $ZnCl_2$	57.10 ± 11.10^{b}

Embryonic Development



[a,b] $p < 0.05$

Conclusion

- Supplementation of 5 mg/mL of $ZnCl_2$
  - Improves follicle development:
    - Reduces incidence of follicular damage from vitrification
    - Improves follicular integrity
    - Increases antral follicle development
  - Improves post-thawing fertilization:
    - Reduces incidence of polyspermy
    - Increases male pronucleus formation

Questions?