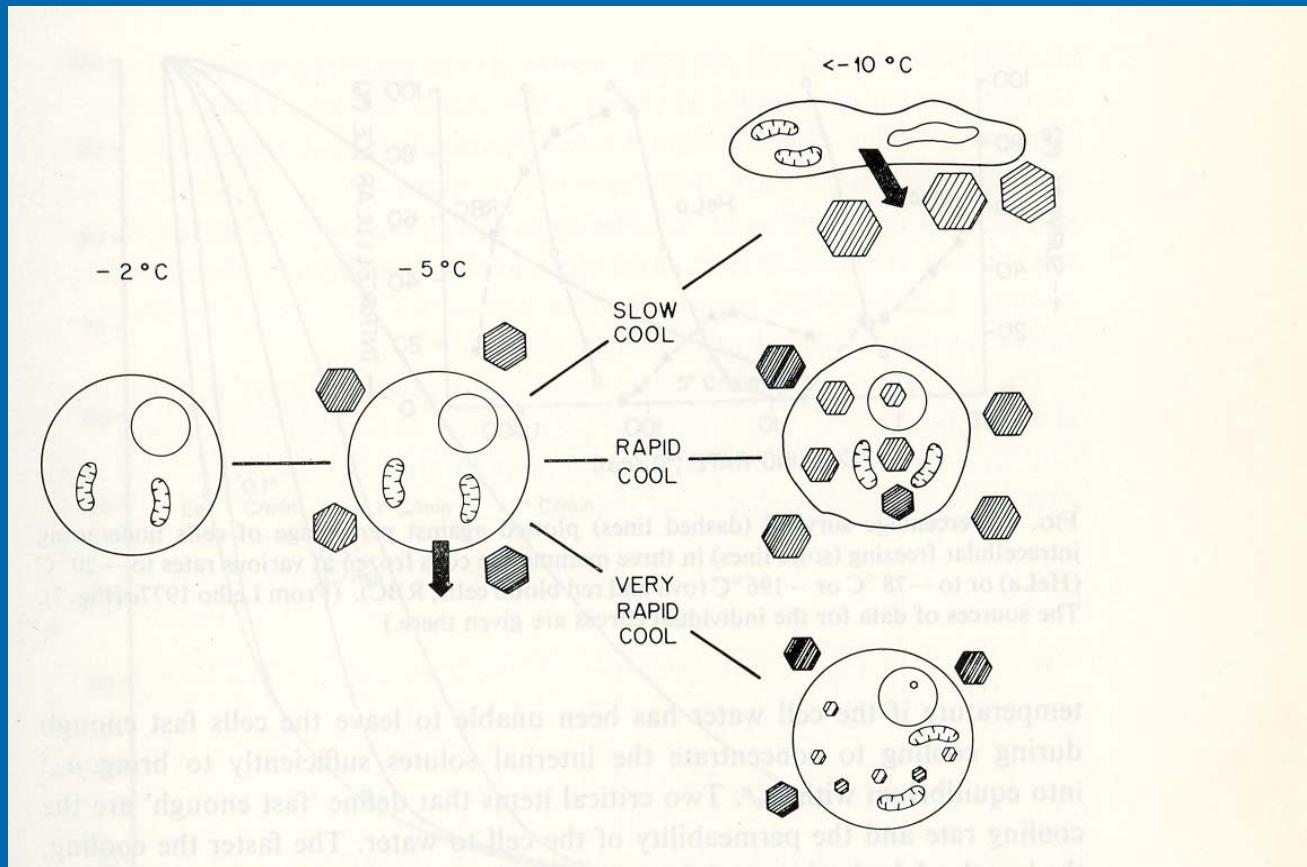


Cryopreserving whole organs

Jorge Sztein, DVM PhD
ARTiCS Unit
Comparative Medicine Branch
NIAID - NIH

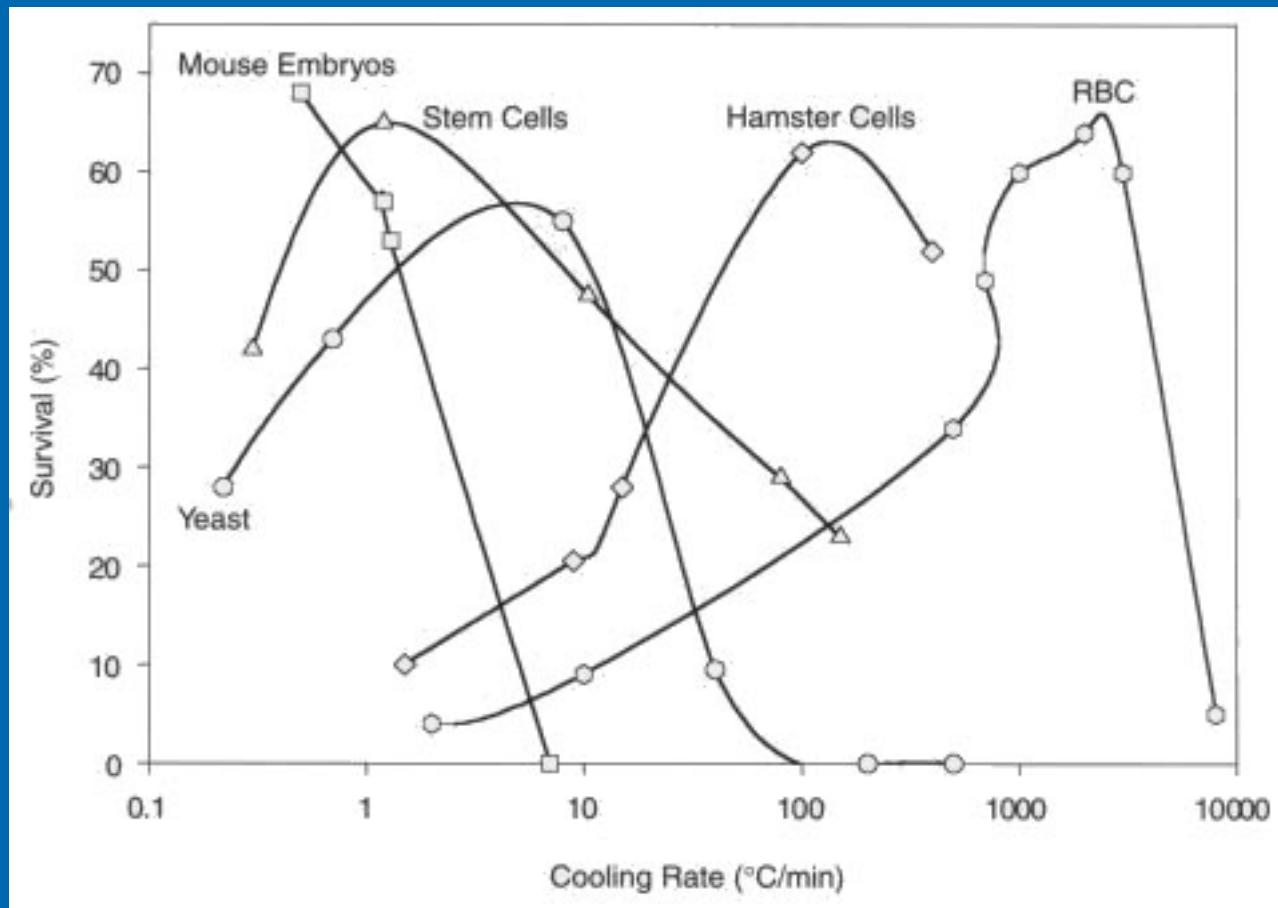


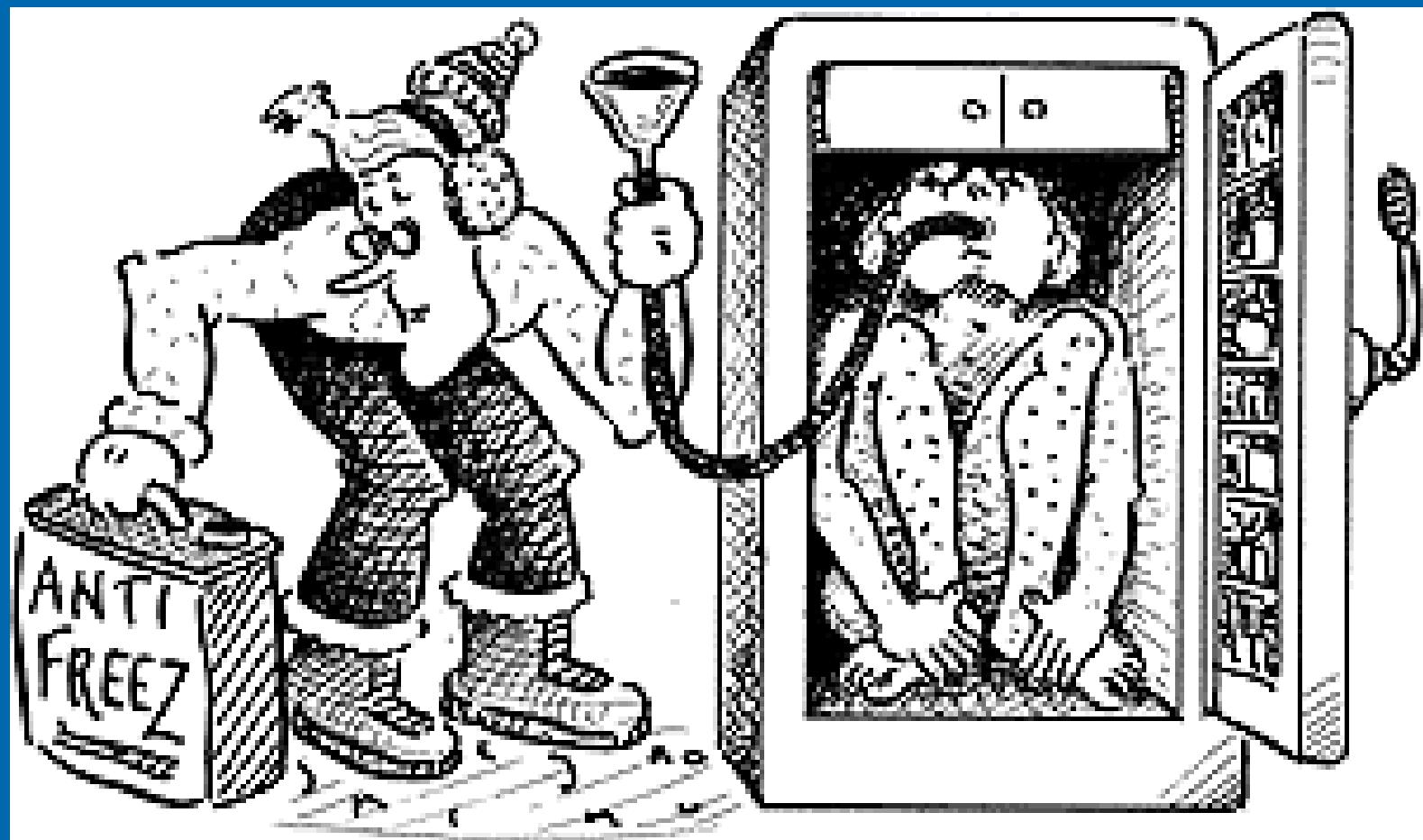
Peter's Principle



P. Mazur 1977

Stanley's Principle





Frozen Bodies



Frozen Bodies



Production of healthy cloned mice from bodies frozen at -20°C for 16 years



Sayaka Wakayamaa, Hiroshi Ohtaa, Takafusa Hikichia, Eiji Mizutania, Takamasa Iwakib, Osami Kanagawac,
and Teruhiko Wakayama
17318–17322 PNAS November 11, 2008 vol. 105 no. 45

Fertile offspring derived from mouse spermatogonial stem cells cryopreserved for more than 14 years

**Xin Wu¹, Shaun M. Goodyear¹, Lara K. Abramowitz²,
Marisa S. Bartolomei², John W. Tobias³, Mary R. Avarbock¹,
and Ralph L. Brinster^{1,*}**

¹Department of Animal Biology, School of Veterinary Medicine, University of Pennsylvania, 3850 Baltimore Avenue, Philadelphia, PA 19104, USA

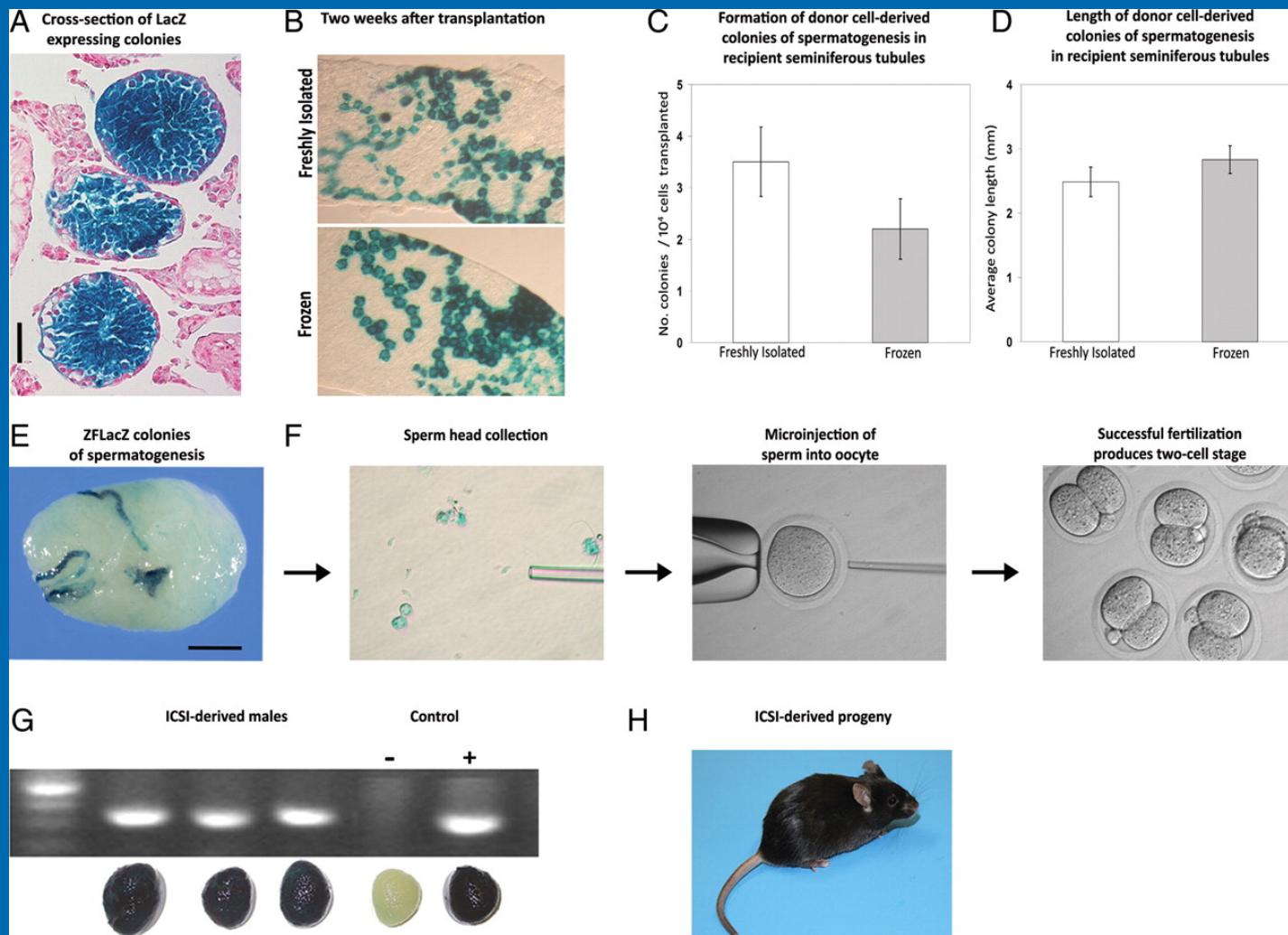
²Department of Cell and Developmental Biology, School of Medicine, University of Pennsylvania, Philadelphia, PA 19104, USA

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Submitted on December 15, 2011; resubmitted on January 18, 2012; accepted on February 14, 2012

Cryopreserved mouse testis cells re-establish spermatogenesis in recipient testes and produce viable progeny.



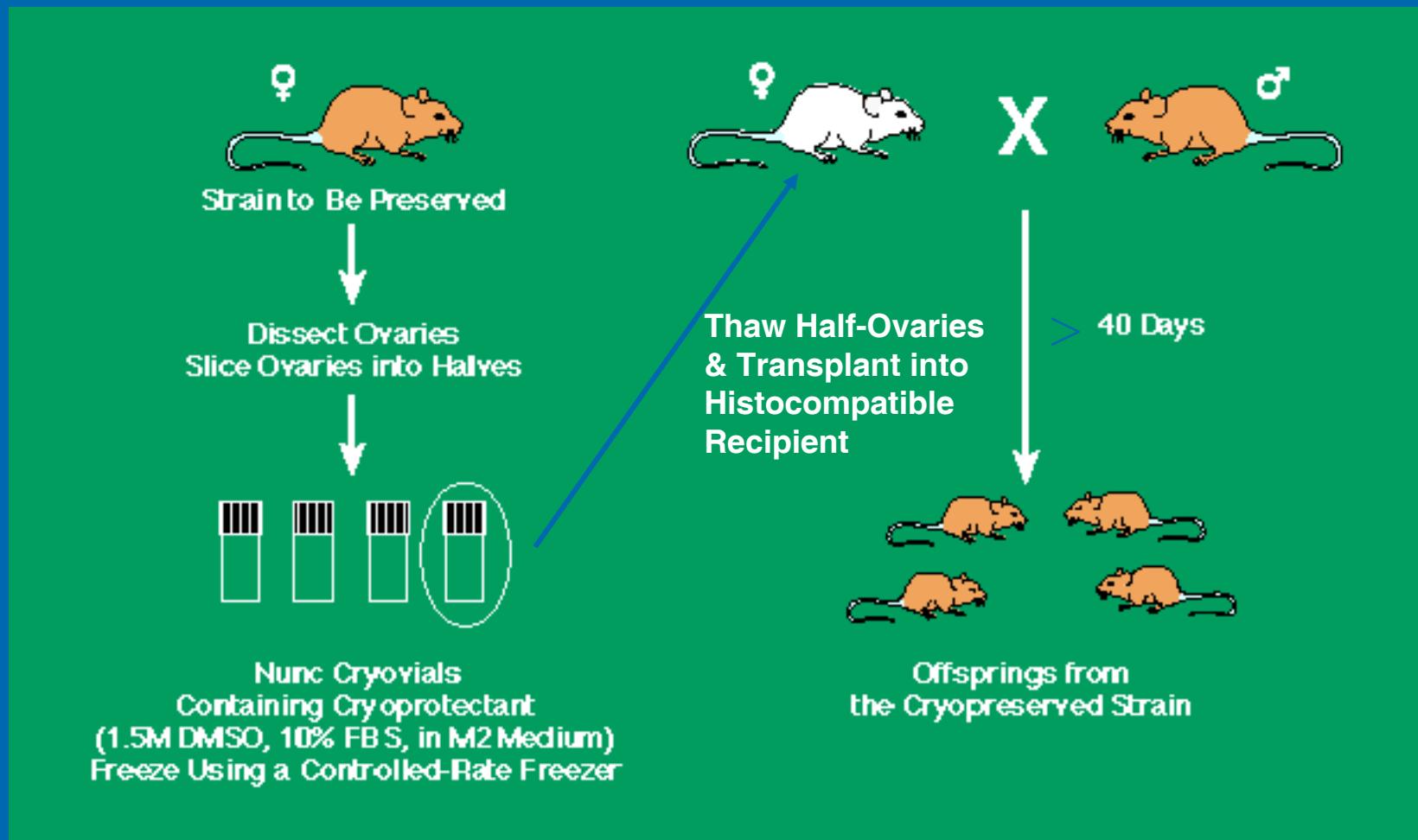
Wu X et al. *Hum. Reprod.* 2012;humrep.des077

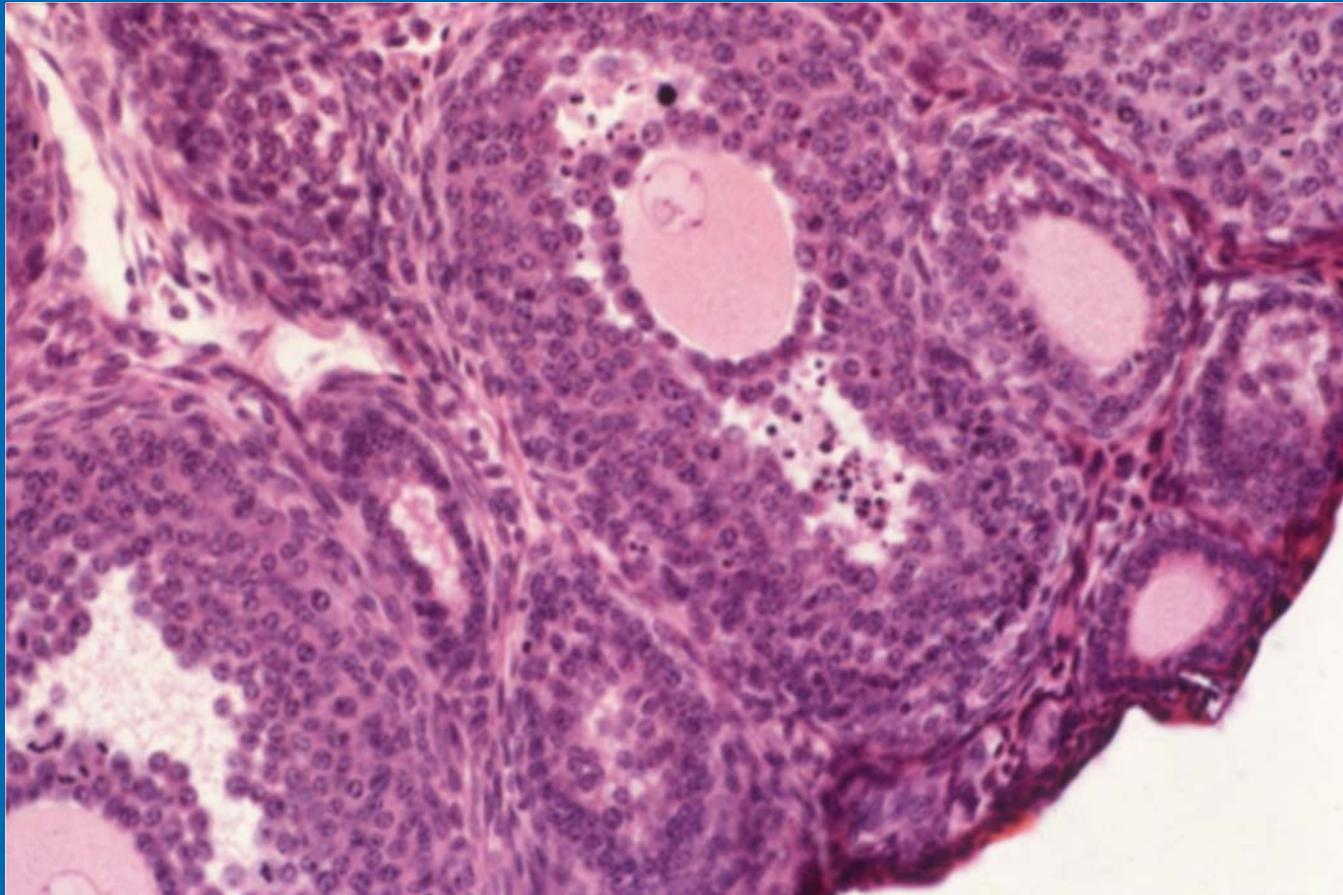
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human
reproduction



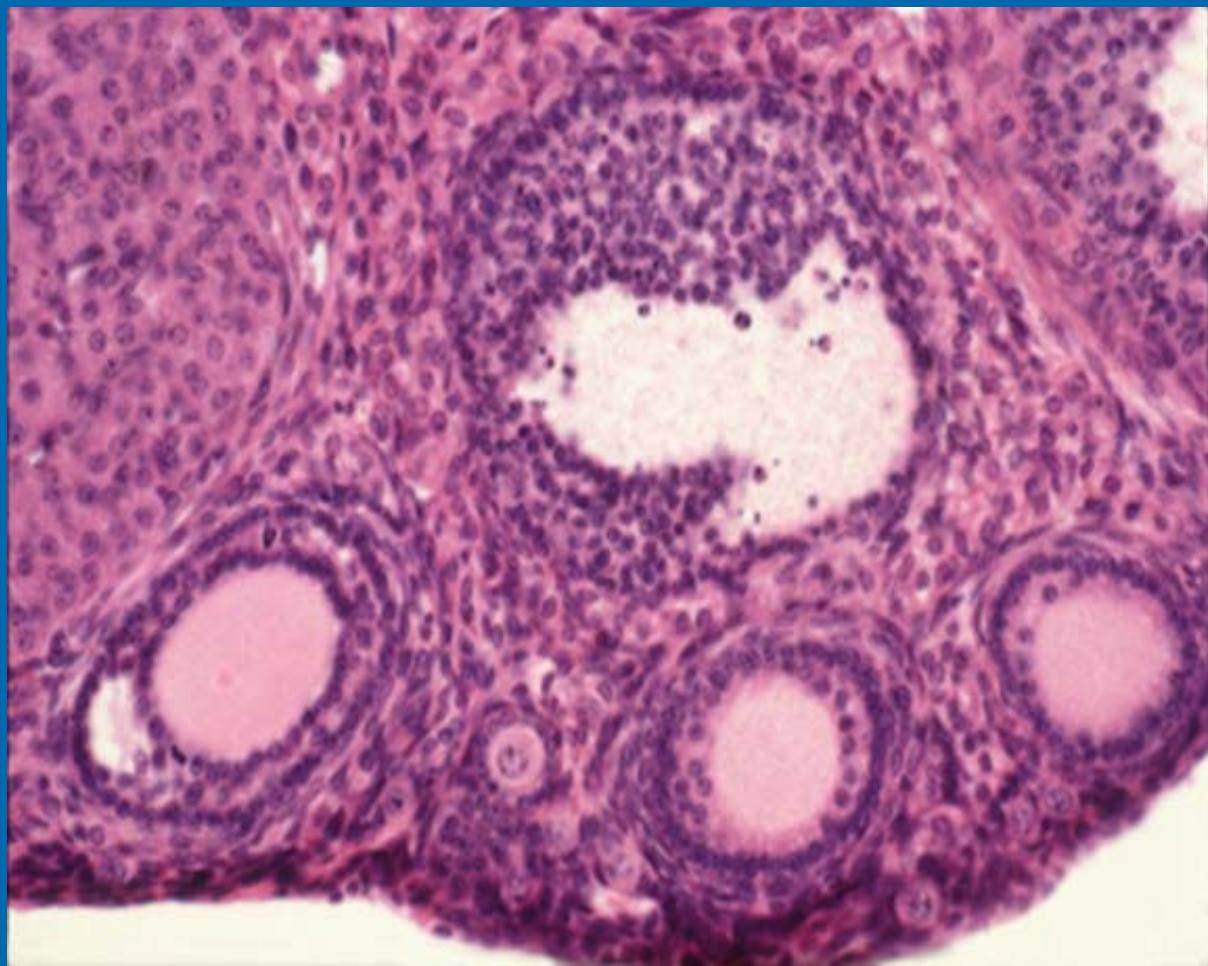
Ovary Cryopreservation





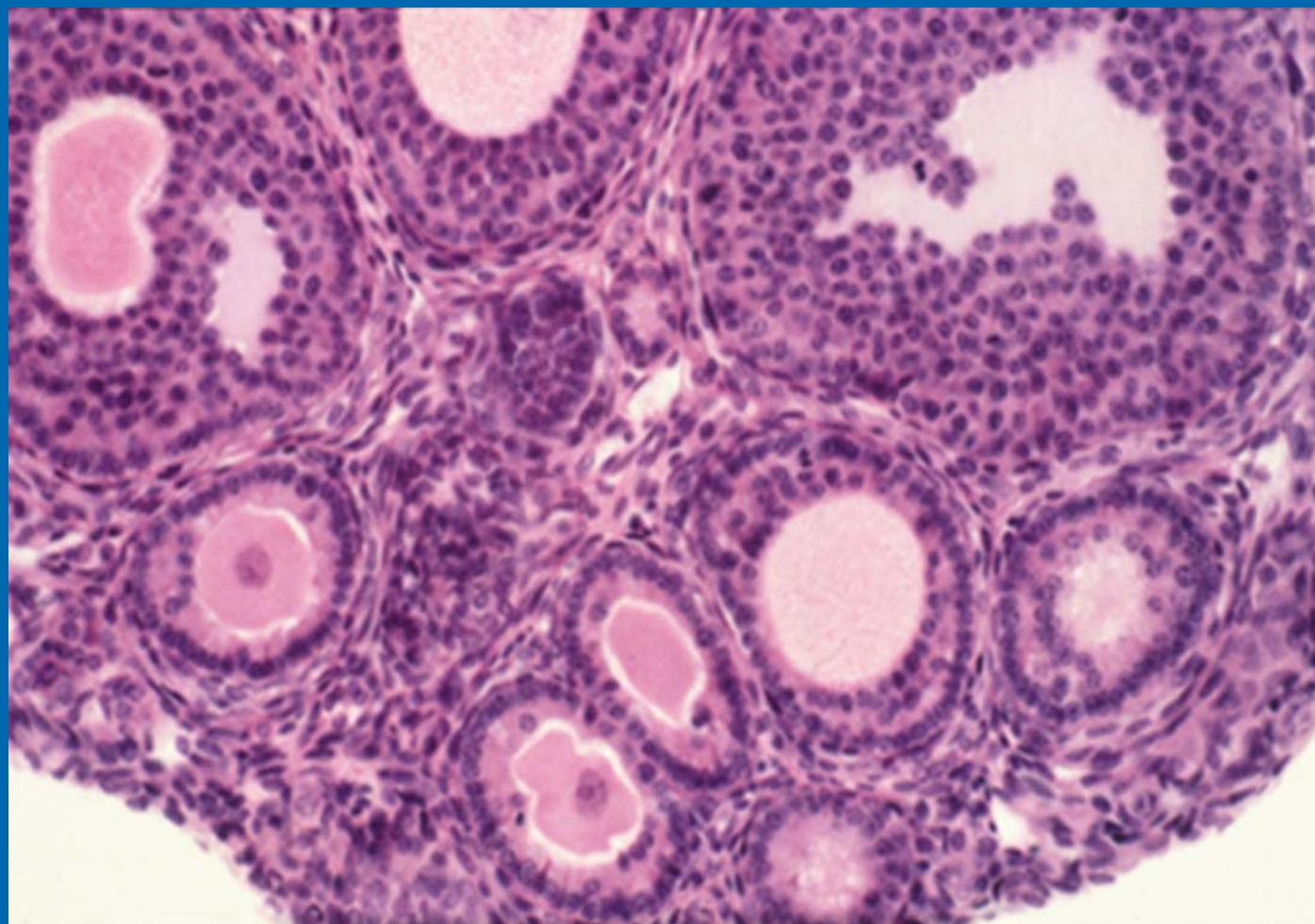
Fresh unprimed

Sztein et al, Biol rep 58,1998



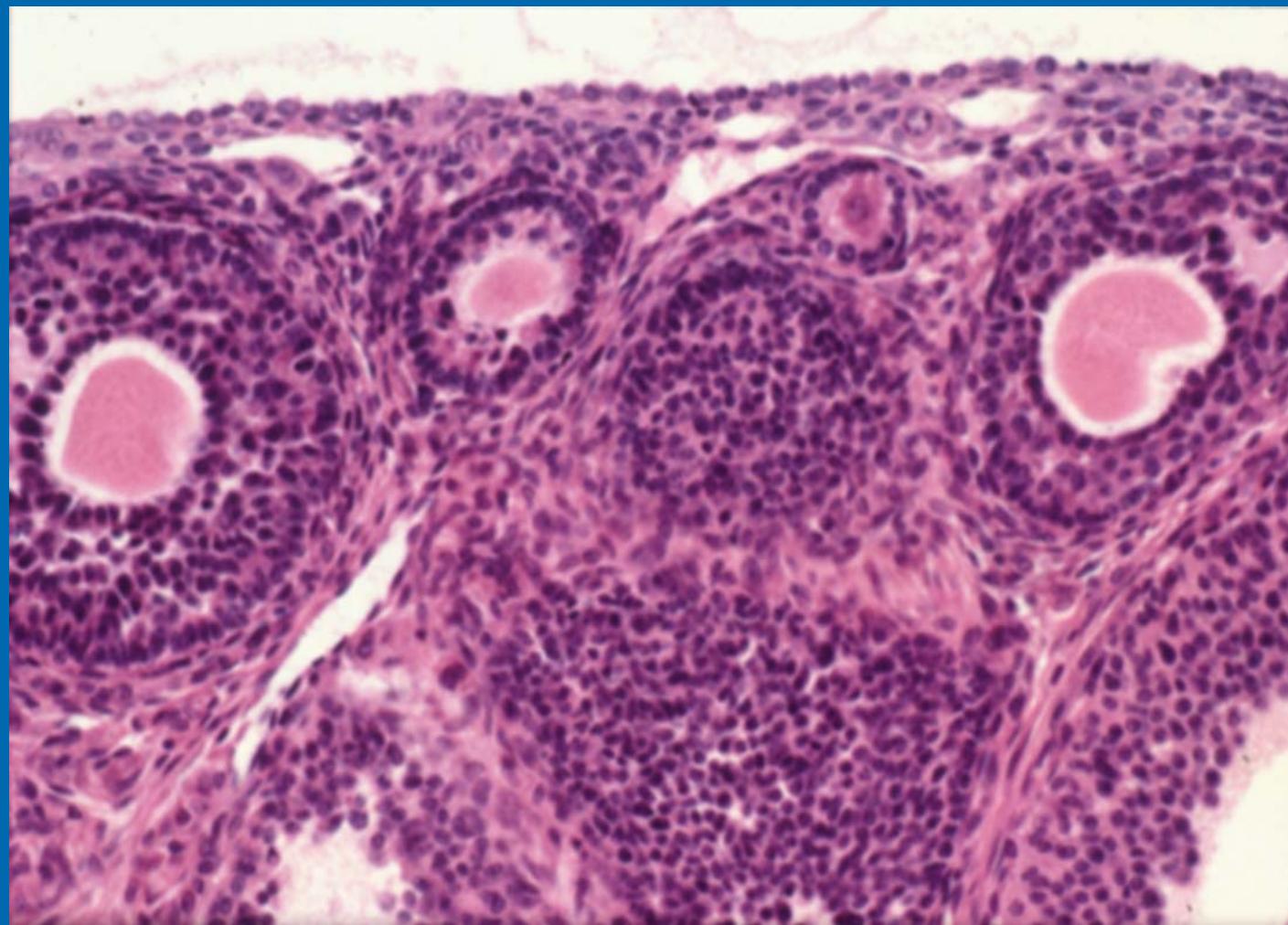
Fresh primed

Sztein et al, Biol rep 58,1998



Frozen non primed

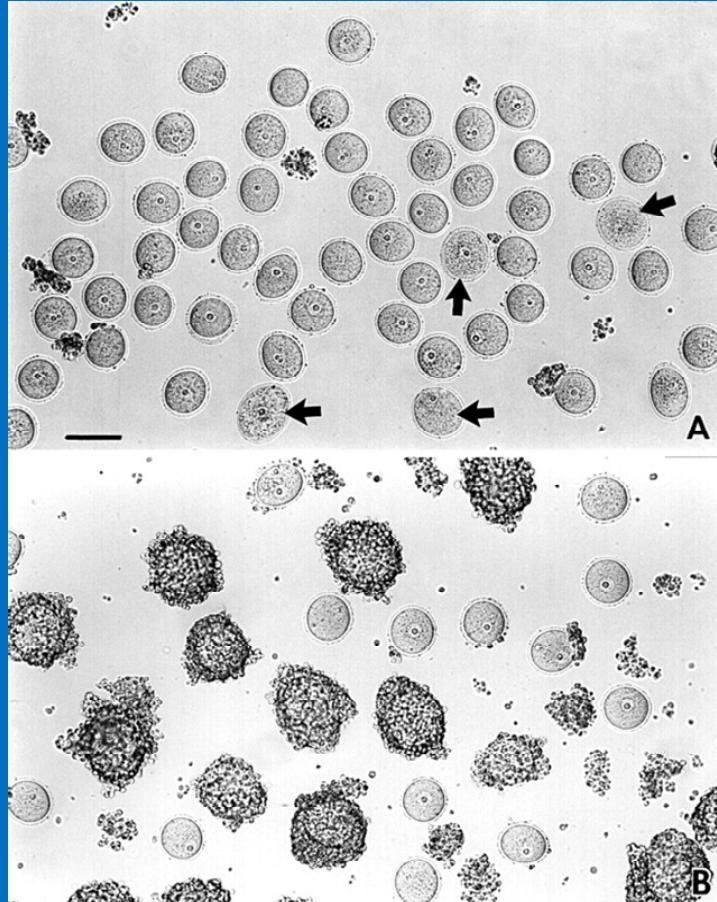
Sztein et al, Biol rep 58,1998



Frozen primed

Sztein et al, Biol rep 58,1998

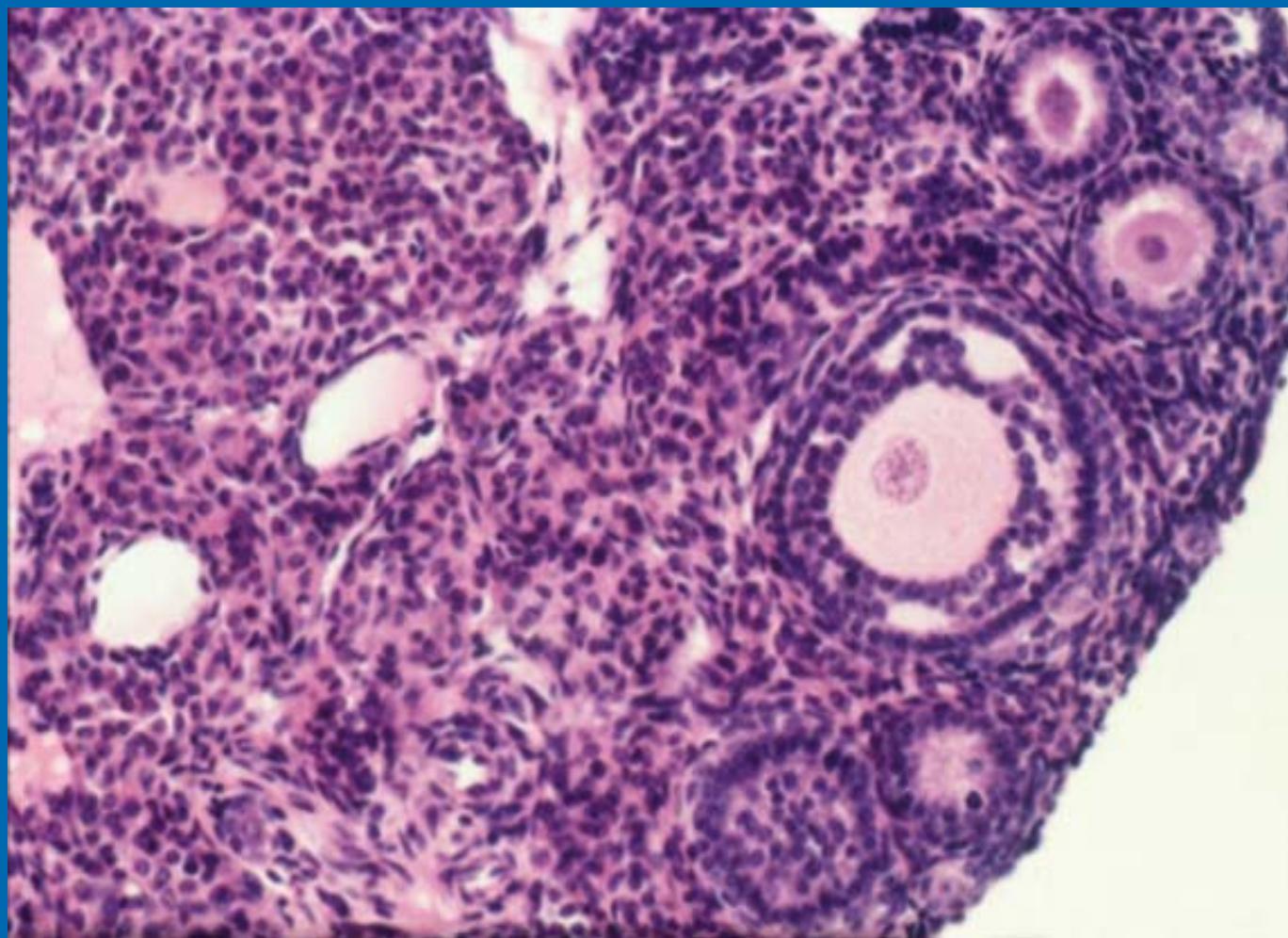
Representative sample of oocytes isolated from frozen–thawed ovaries.



Sztein J et al. Hum. Reprod. 2000;15:567-571

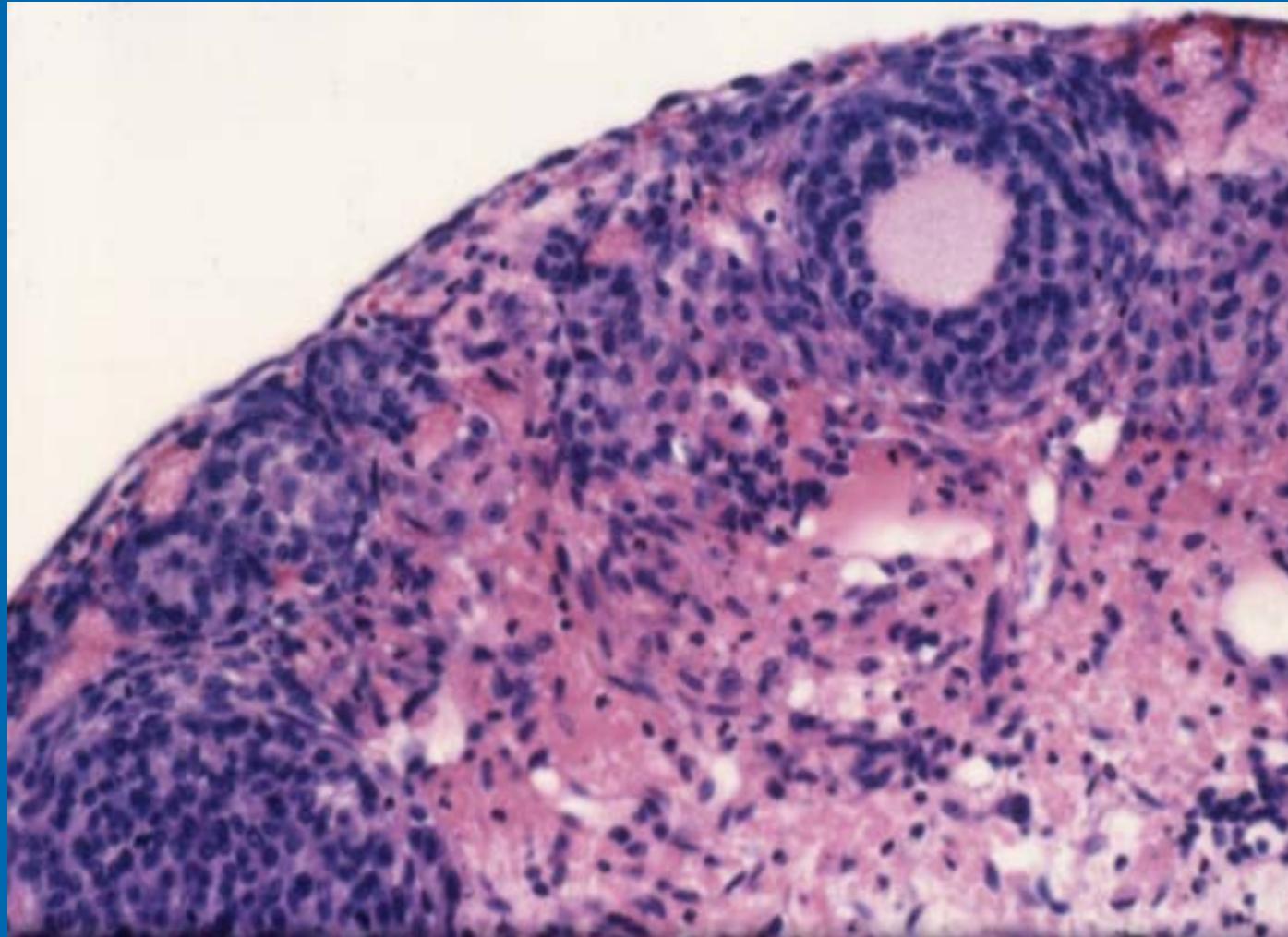
© European Society of Human Reproduction and Embryology

human
reproduction



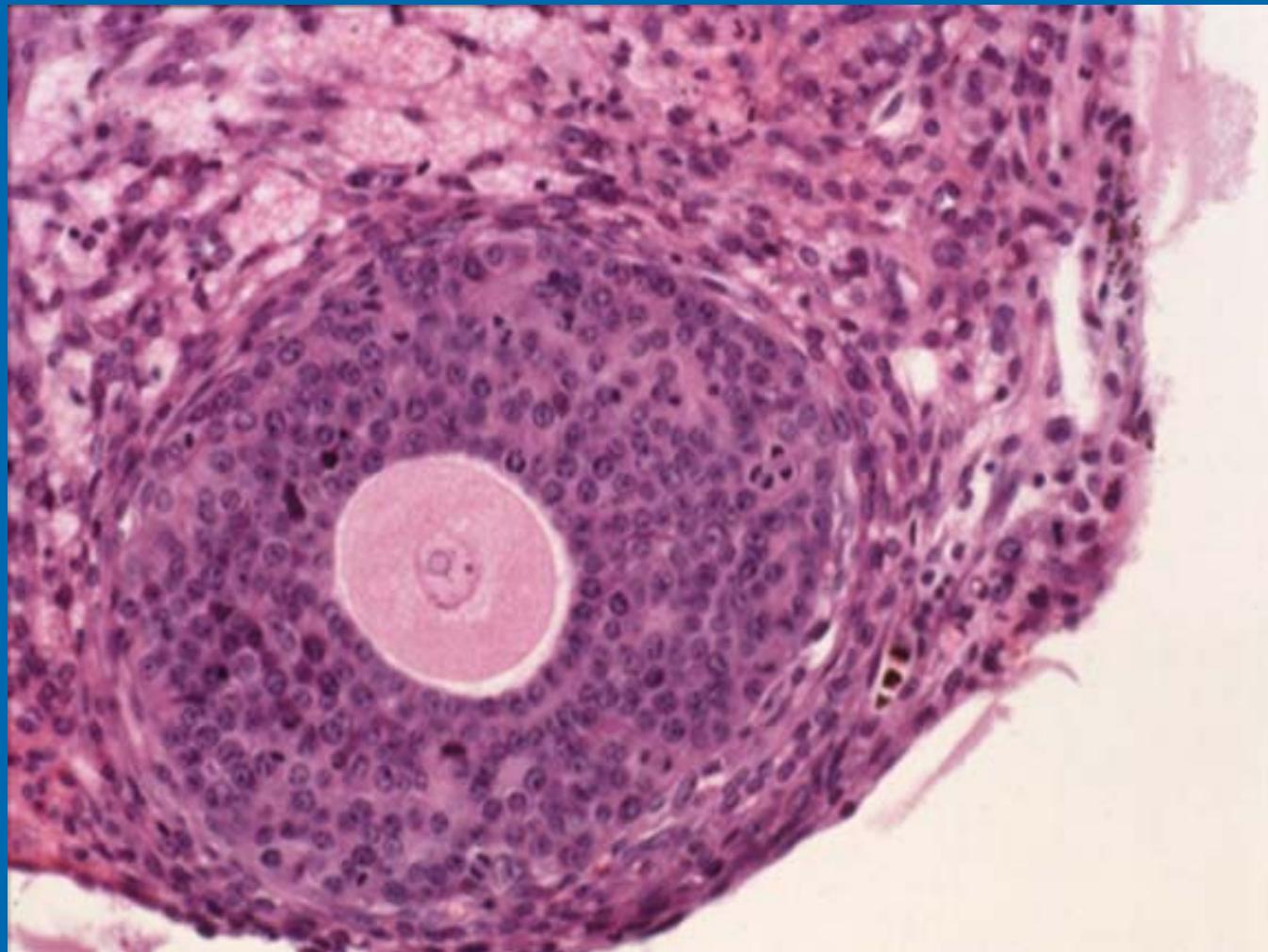
Thawed 10" X40

Sztein et al, Biol rep 58,1998



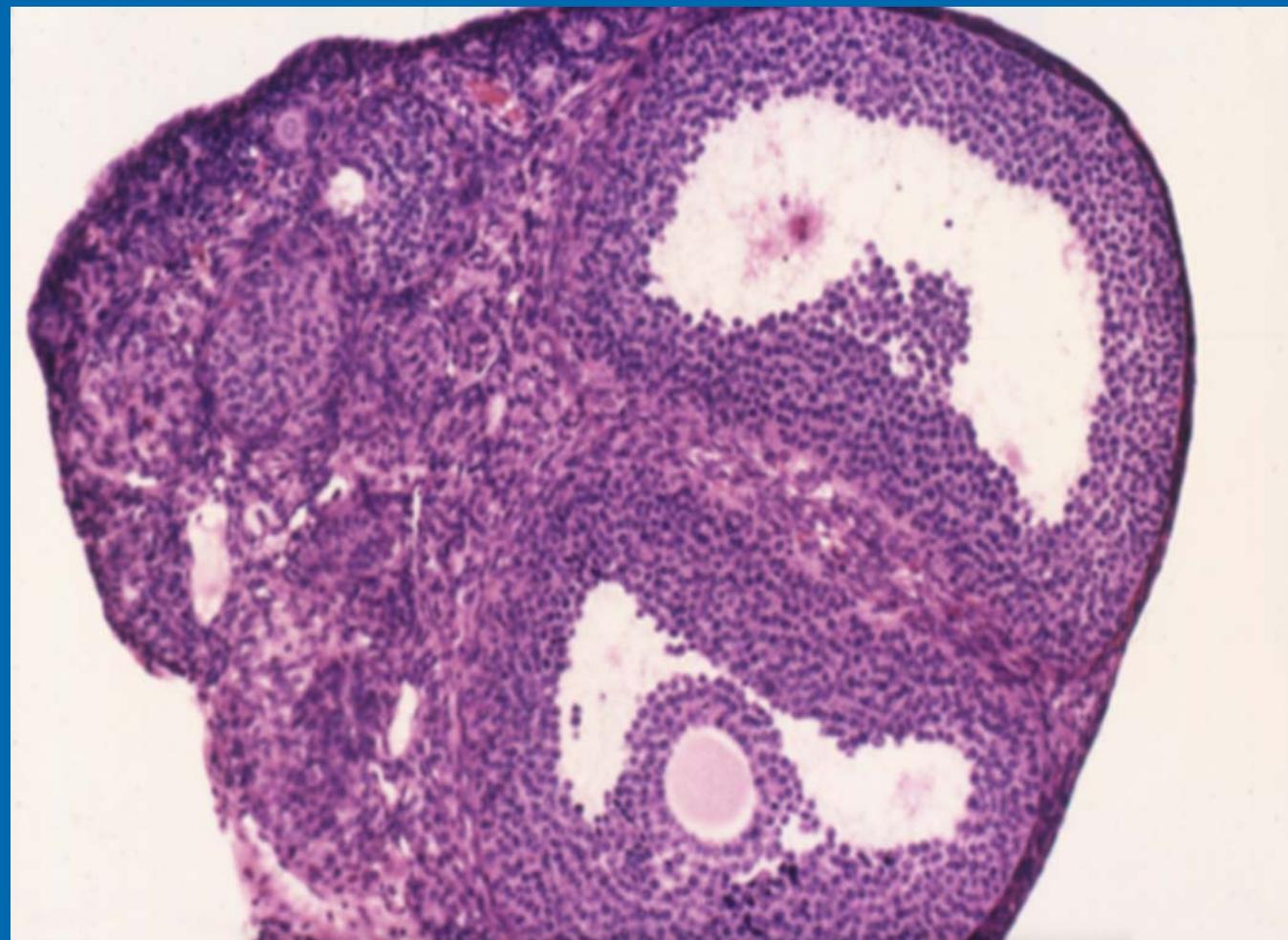
24 hours x40

Sztein et al, Biol rep 58,1998



3 days X40

Sztein et al, Biol rep 58,1998



4 months x20

Sztein et al, Biol rep 58,1998

Ovary Cryopreservation in Straws

- 0.5 cc French straw 100 µl CPA
(One step dilution)
- CPA 2 M PROH
- 10% FBS M2
medium
- Female age 21-60 days

Ovary Transfer

| | Vial | Straw |
|----------------------------------|------------|------------|
| Recipients | 23 | 11 |
| Implanted (+) | 13 (57%) | 7 (63%) |
| # positives pups | 3.2/female | 4.7/female |
| Pups/female | 1.5 | 6.5 |
| Interval first litter | 40.1 | 40 |



Peter Principle

$$\frac{dV}{dT} = -\frac{L_p A_c R T}{B v_w} \left[\ln \frac{(V_o - V_b - n_{cpa} v_{cpa})/v_w}{(V_o - V_b - n_{cpa} v_{cpa})/v_w + (\varphi_s n_s + n_{cpa})} - \frac{\Delta H_f v_w \rho}{R} \left(\frac{1}{T_R} - \frac{1}{T} \right) \right] \quad (1)$$

