Spermatogonial stem cells: preservation of sperm production

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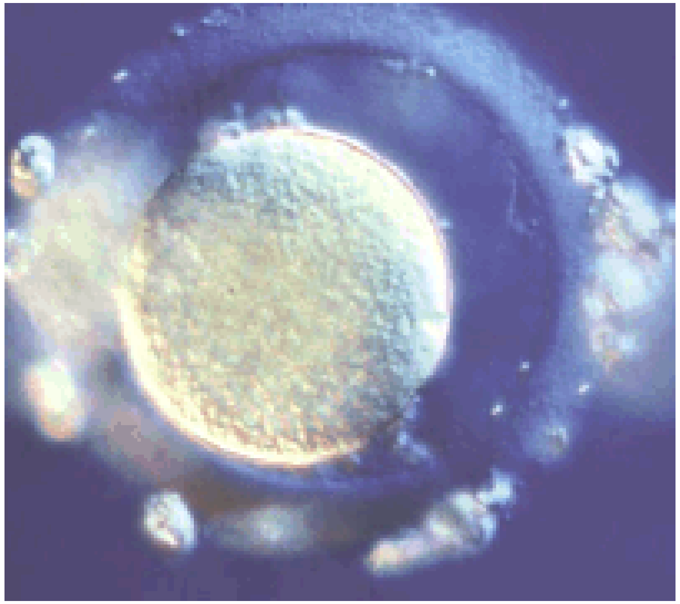
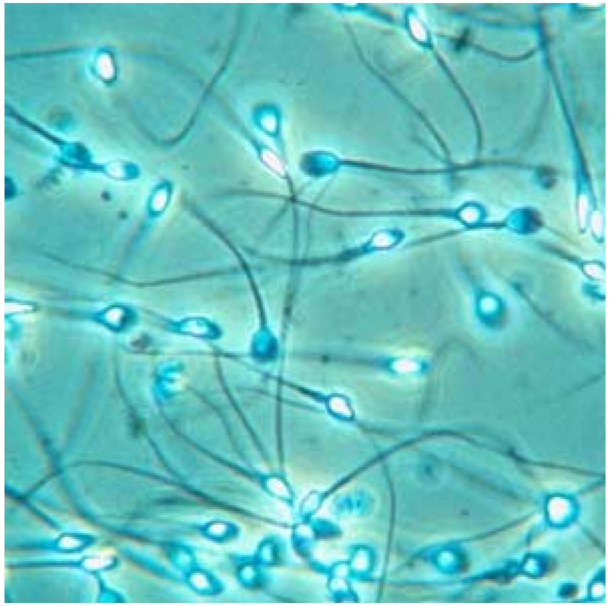
Gametes





Sperm production

Daily sperm production ± 50-100 10^6

Spermatogonial stem cells are necessary to maintain lifelong spermatogenesis

Delicate balance between self renewal and differentiation

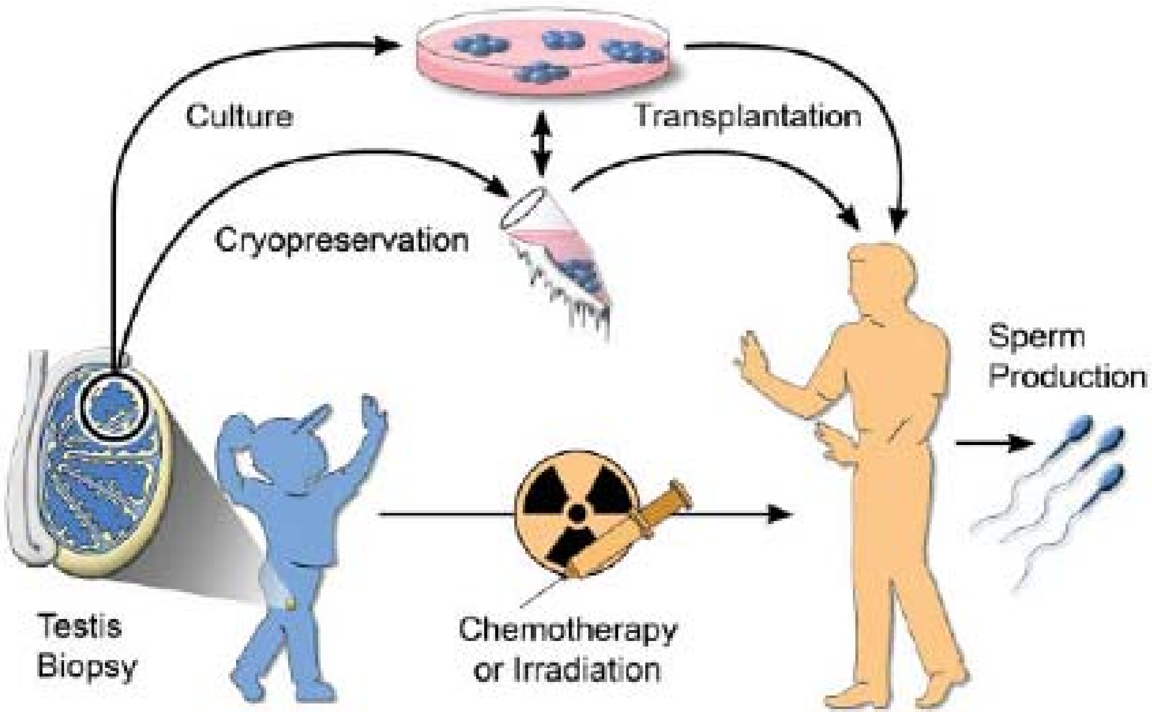
Male fertility problems

- Oligospermia (unknown)
- Azoospermia from maturatie arrest to SCO (unknown)
- Azoospermia after chemotherapy or irradiation (cell death stem cells)

- For prepubertal boys with cancer no means to preserve fertility
- Today, one in 250 young adults is a childhood cancer survivor

Theoretical solution

Cryopreservation of spermatogonial stem cells and autotransplantation



Brinster, www.sciencemag.org (2007)

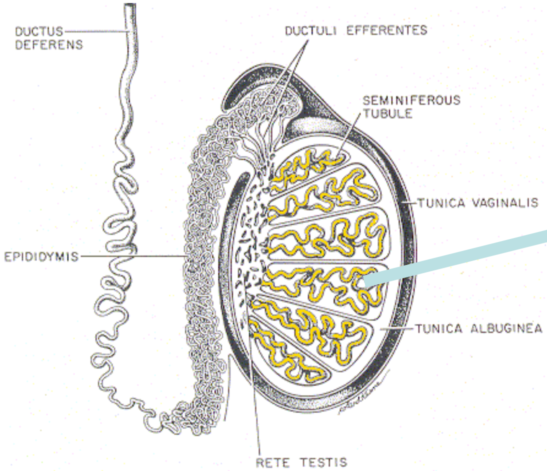
Is it important?

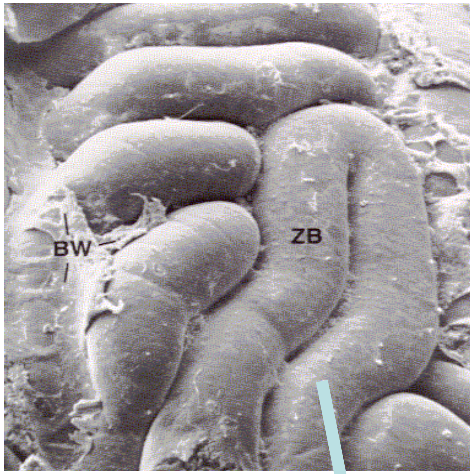
Survey among parents

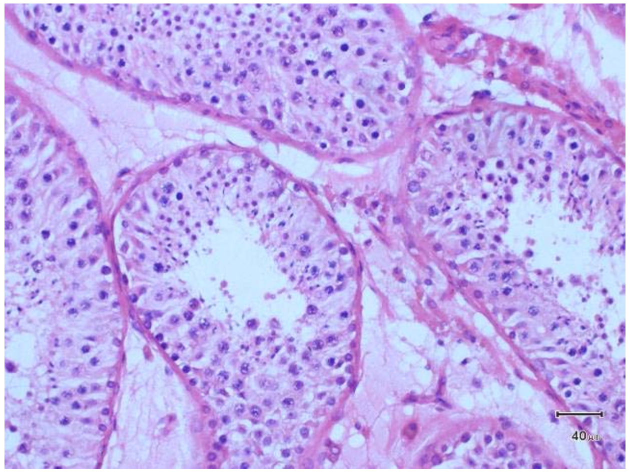
- AMC (Van den Berg et al., Hum Rep 2007)
  - Retrospective 162 parents (median 7 years post-diagnosis)
  - 62% would want to store testicular tissue

- USA (Ginsberg, et al., Hum Rep 2009)
  - Prospective 21 parents
  - 76% stored tissue

Niche


DUCTUS DEFERENS
DUCTULI EFFERENTES
SEMINIFEROUS TUBULE
TUNICA VAGINALIS
EPIDIDYMIS
TUNICA ALBUGINEA
RETE TESTIS


BW
ZB




St
Ep
ZB
Ep
St

Molecular characteristics of spermatogonia

A_s and A_{pr}	GFRalpha1
A_s, A_{pr} and A_{al}	PLZF, OCT4, NGN3, NOTCH-1, SOX3, c-RET
A spermatogonia	RBM
Spermatogonia	EP-CAM
Pre-meiotic germ cells	STRA8, EE2
Cells on basal membrane and interstitium	CD9
Spermatogonia, spermatocytes and round spermatids	GCNA1, Hsp90α
Spermatogonia and spermatids	TAF4B

Aponte et al., APMIS 113, 727-742, 2005

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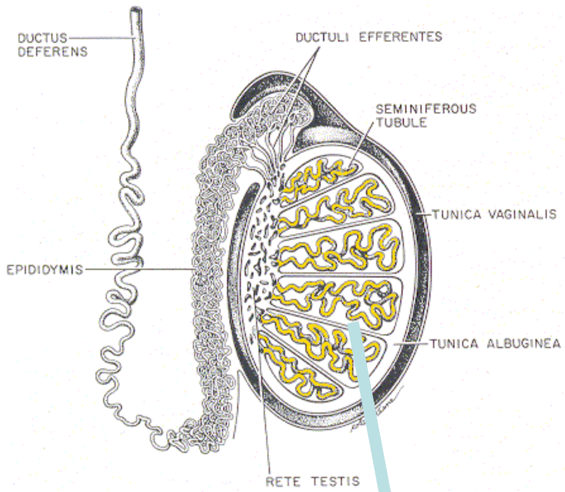
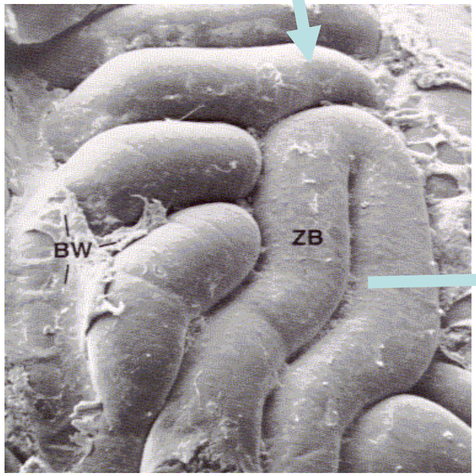
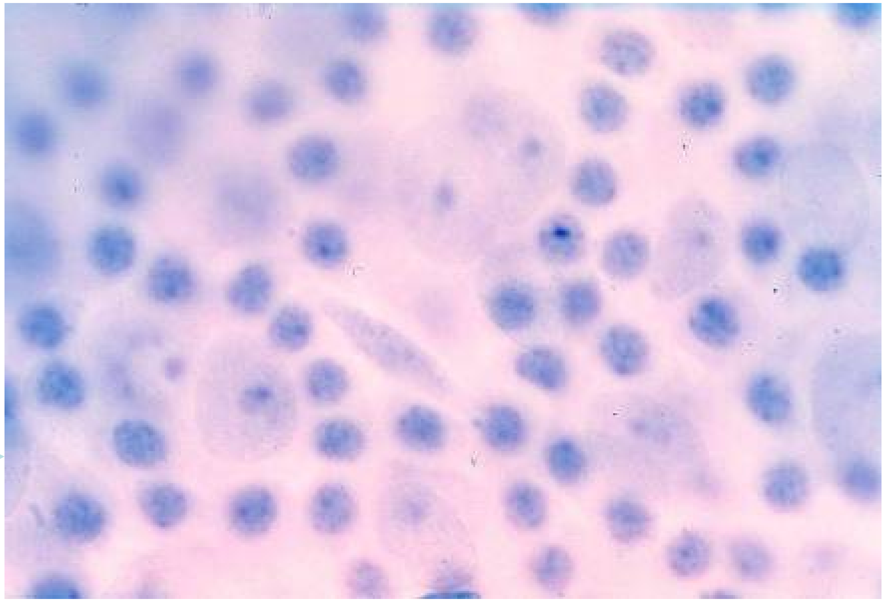
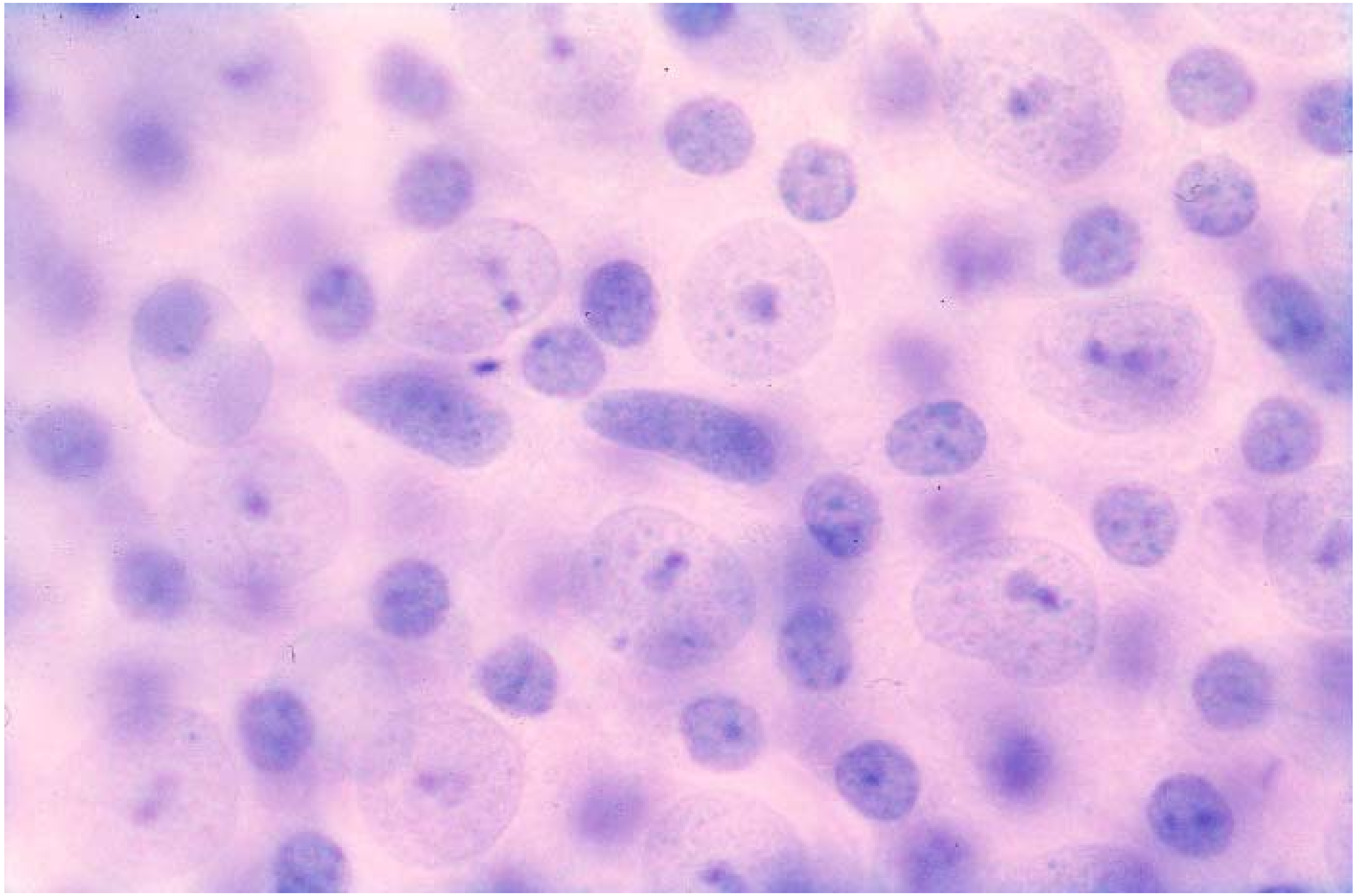
Spermatogonial stem cells in the testis



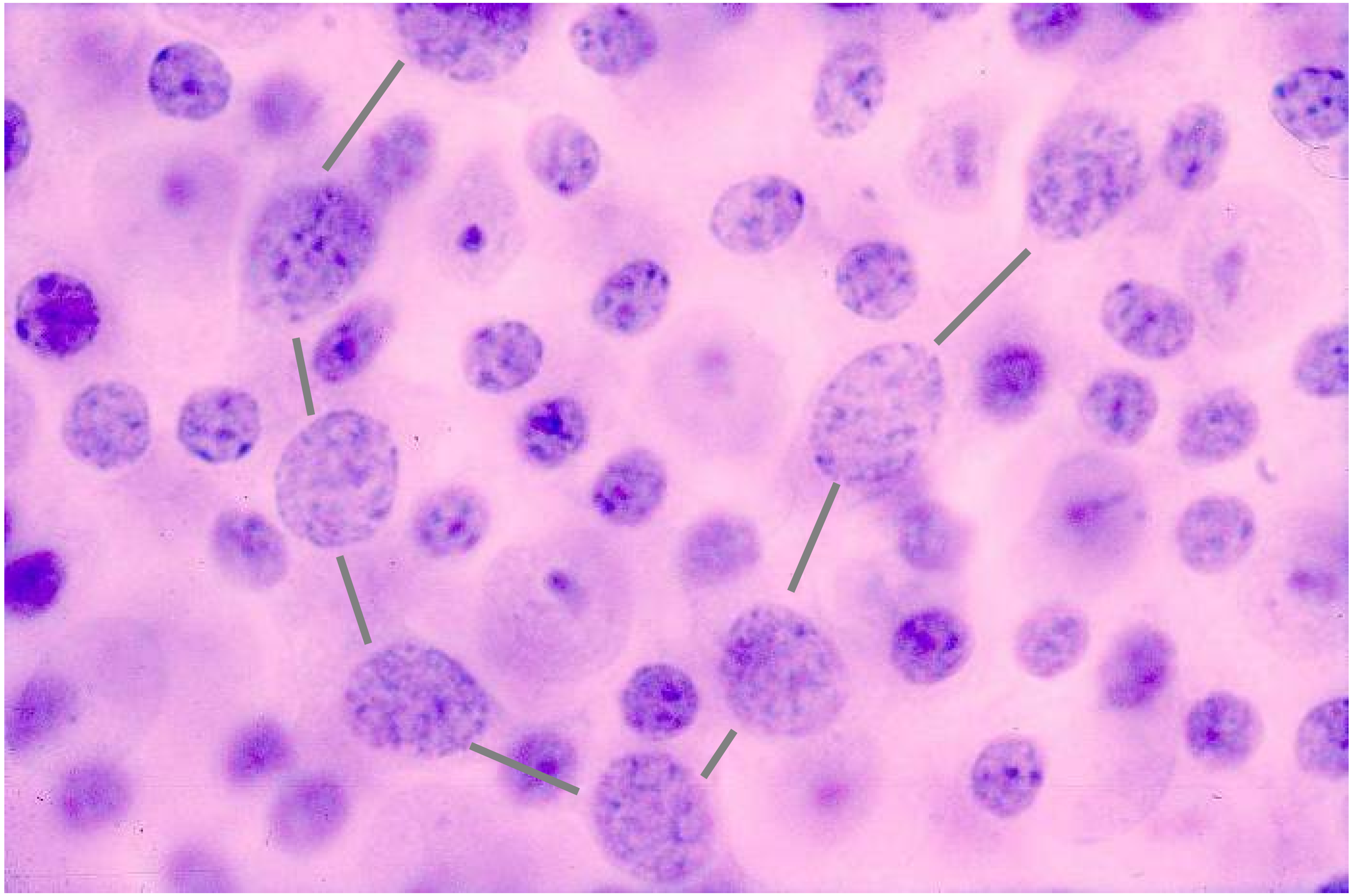




Paired spermatogonia - first step towards differentiation



Chain of spermatogonia



Relative numbers of spermatogonial stem cells in mouse testis

- 0.03 % of all germ cells
- 1.3 % of all spermatogonia
- 3.3 % of all A spermatogonia
- 10.6 % of all A_s, A_{pr} and A_{al} spermatogonia

35,000 stem cells per mouse testis

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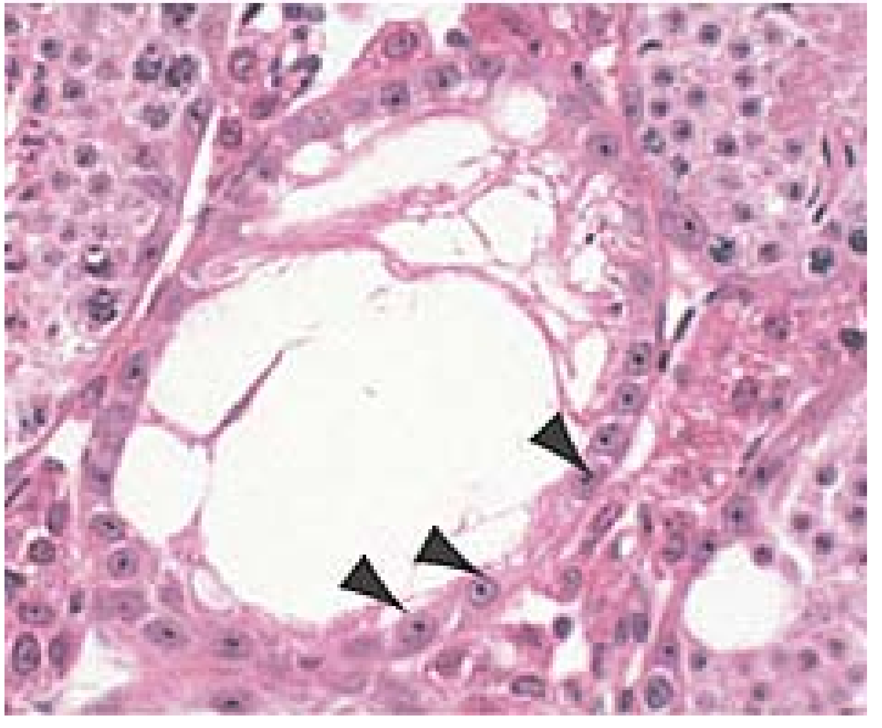
Tegelenbosch & de Rooij, Mut Res 290, 193-200,1993

Selfrenewal and differentiation

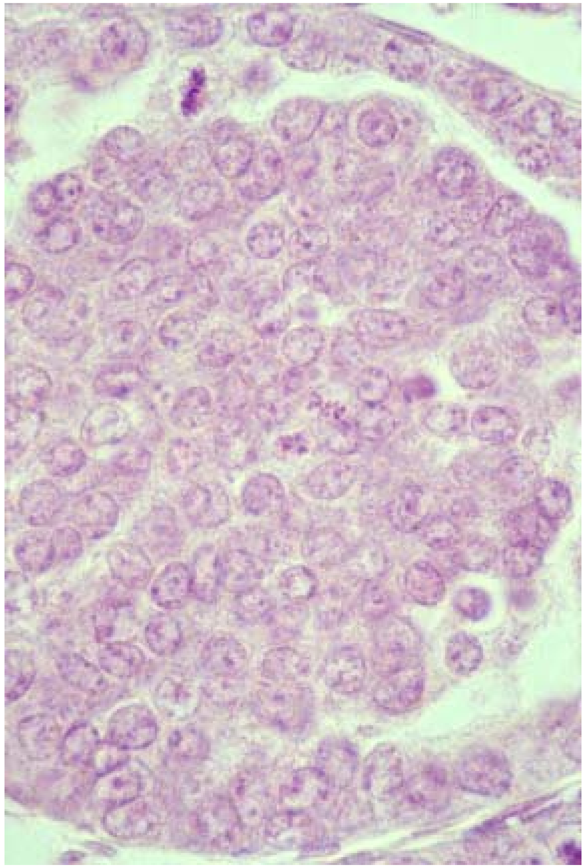
PLZF mutant



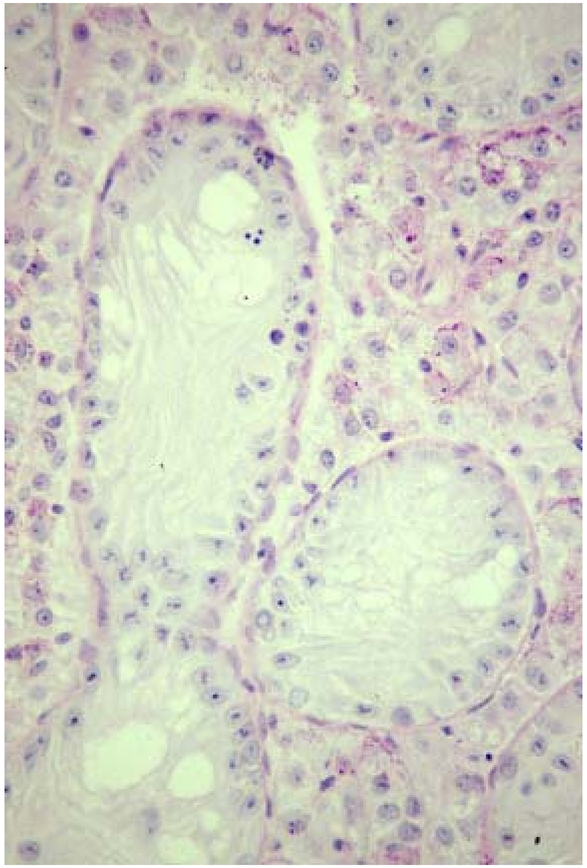
Buaas et al., Nat Genet 36, 647-652, 2004

Selfrenewal and differentiation

GDNF overexpressie muis



GDNF +/- muis



Meng et al., Science 287, 1489-1493, 2000

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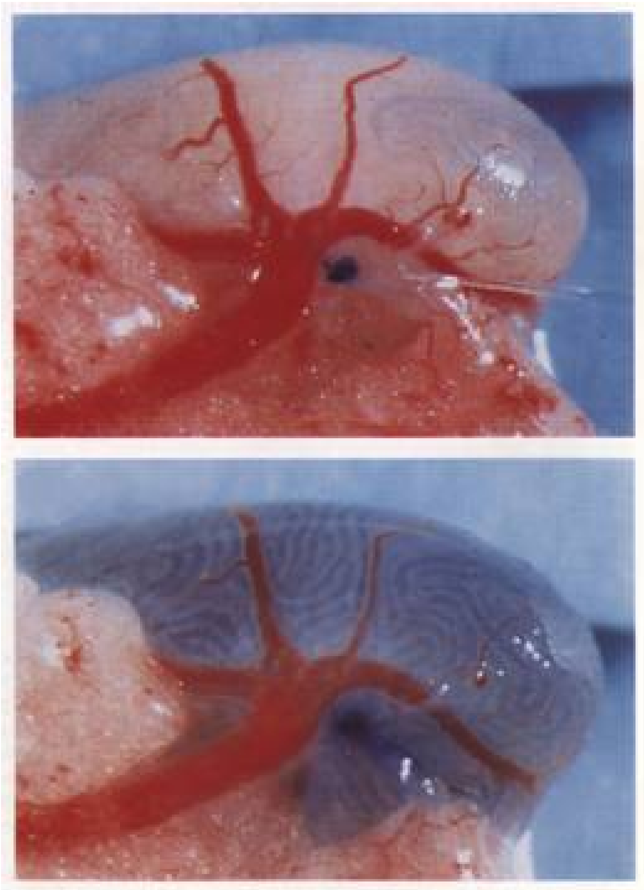
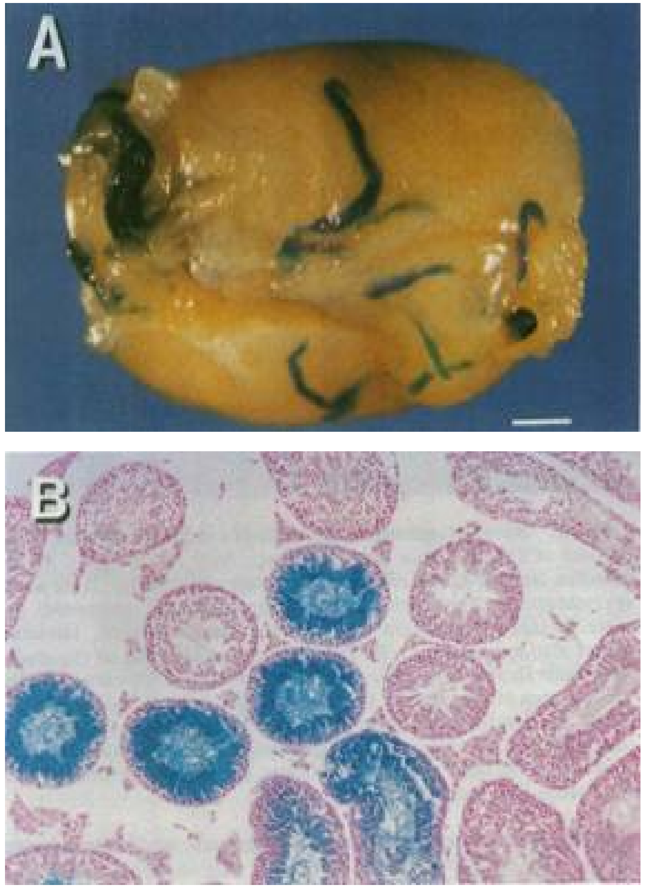
Breaktrough I: Spermatogonial stem cell transplantation



Brinster & Averbock, PNAS 91, 11303, 1994

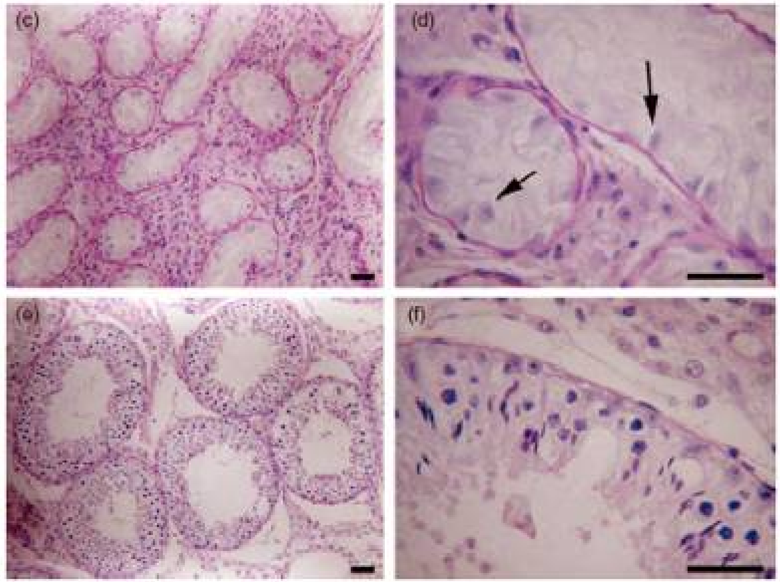


Nagano et al., Int J Dev Biol 41, 111-122, 1997

Spermatogonial stem cell transplantation

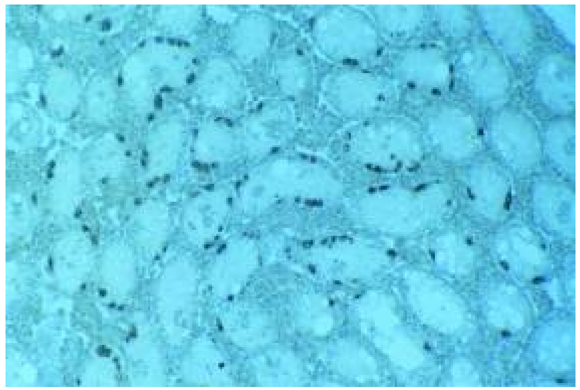
Autotransplantation:

Mouse to mouse
Bull to bull (Izadyar et al., Reproduction 2003) →
Goat to goat (Honaramooz et al., Mol Reprod Dev 2003)
Rat to rat (Hamra et al., PNAS 2005)
Ram to ram (Ridriguez-sosa et al., Theriogenology 2006)
Dog to dog (Kim et al., Reproduction 2008)

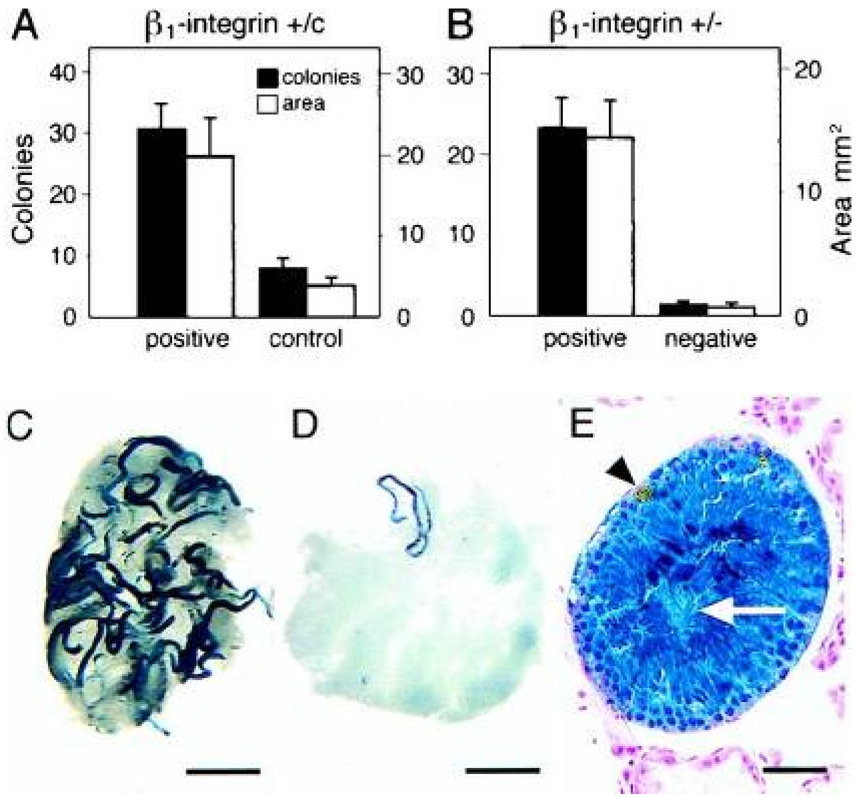


Xenotransplantation:

Rat to mouse (Cloutier et al., Nature 1996)
Hamster to mouse (Ogawa et al., Biol Reprod 1999)
Rabbit/dog to mouse (Dobrinski et al., Biol Reprod 1999)
Baboon to mouse (Nagano et al., Biol Reprod 2001)
Bull to mouse (Izadyar et al., Reproduction 2002 →
Human to mouse (Nagano et al., Fert Steril 2002)



Transplantation to prove enrichment of mouse spermatogonial stem cells during isolation



Shinohara et al. PNAS 96, 5504-5509, 1999

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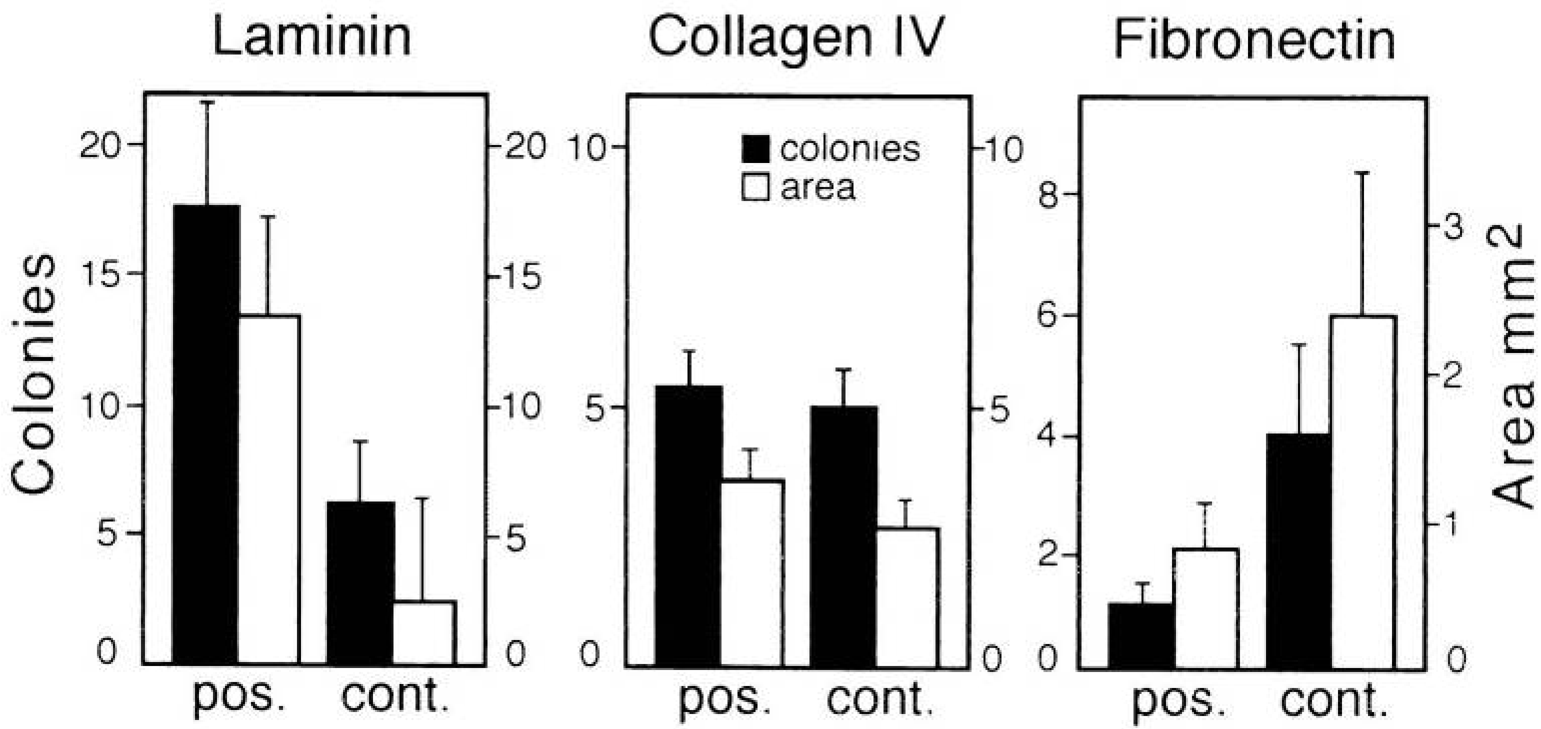
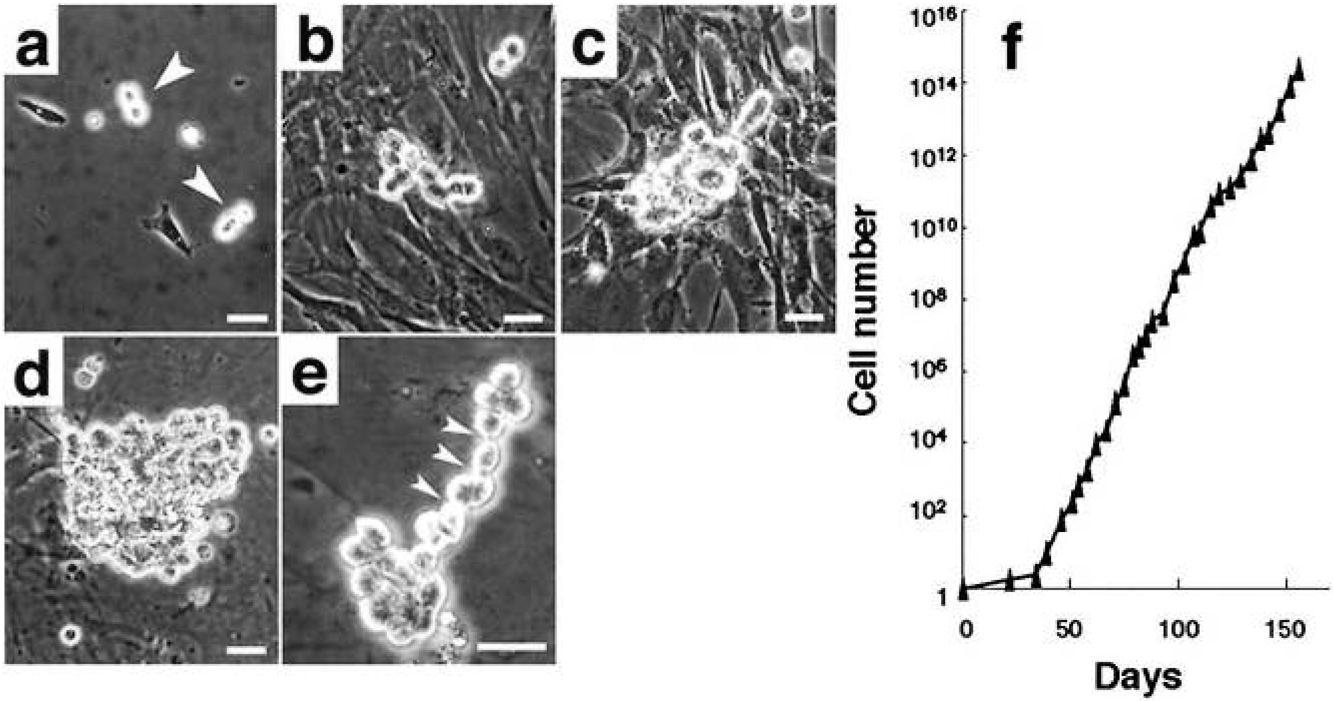
Transplantation to prove mouse spermatogonial stem cell proliferation in vitro



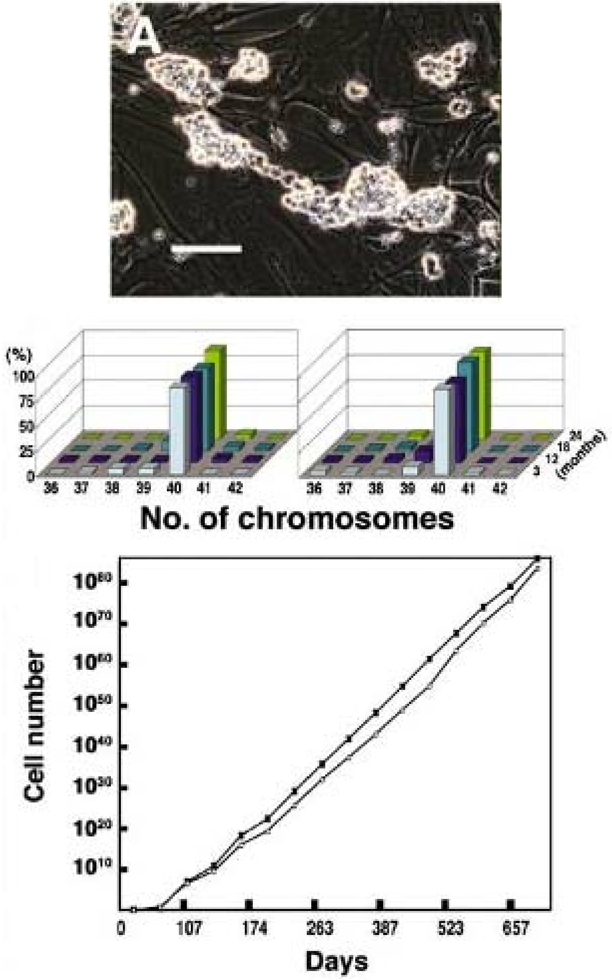
Shinohara et al. PNAS 96, 5504-5509, 1999

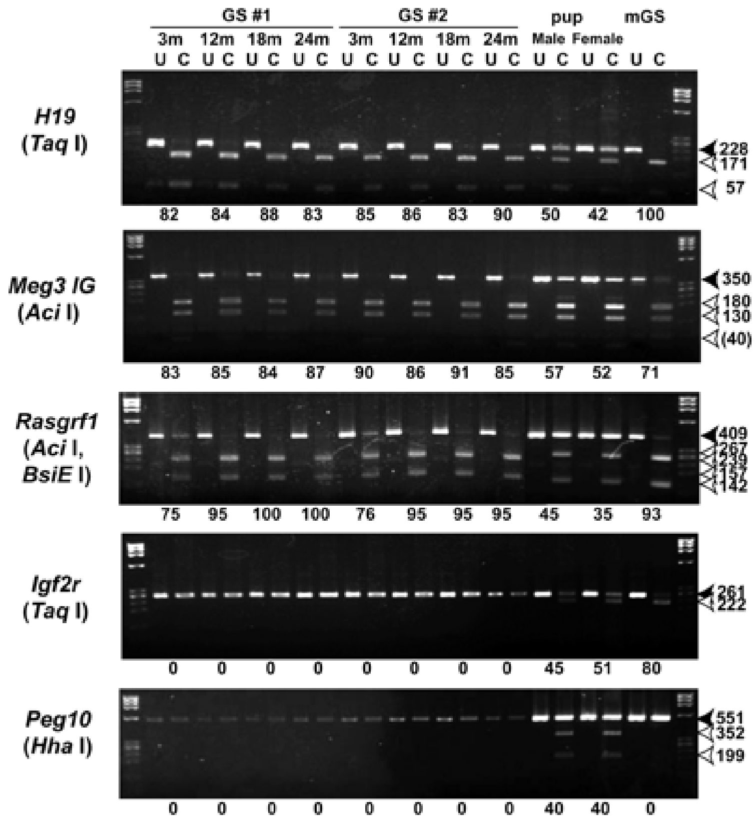
Breakthrough II: Long term culture



Kanatsu-Shinohara, Biol Reprod 69, 612-616, 2003

Long term culture and propagation of mouse SSCs





Kanatsu-Shinohara, M. et al. Development 2005;132:4155-4163

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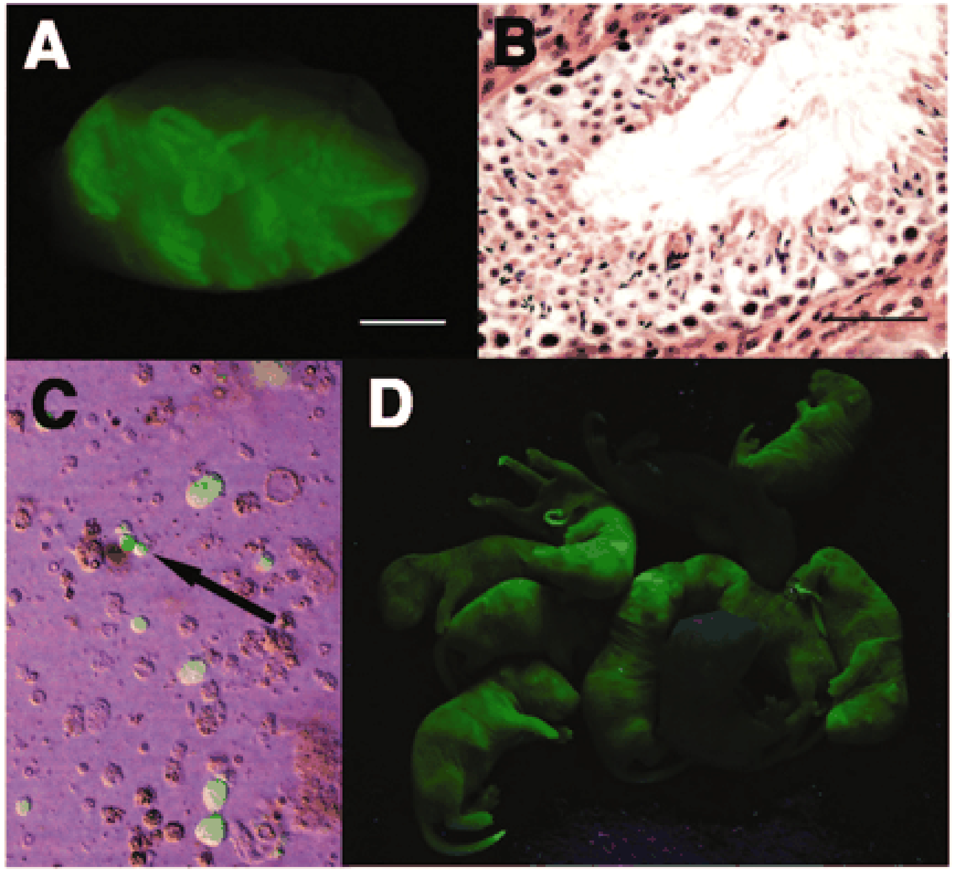
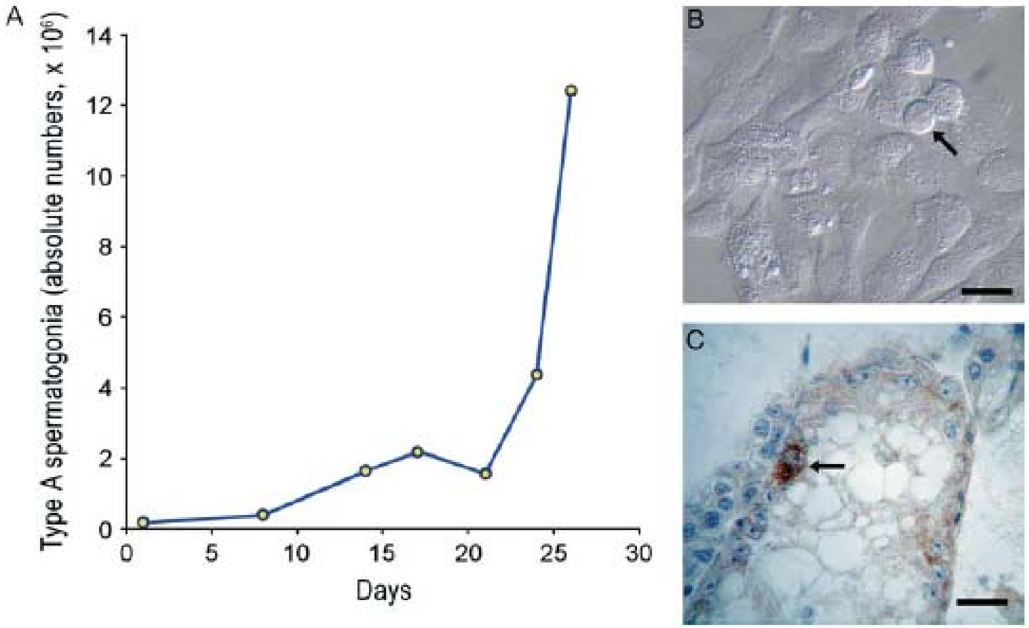
Spermatogenesis and generation of offspring of long term cultured SSCs



Kanatsu-Shinohara, M. et al. Development 2005;132:4155-4163

Long term culture of SSCs from several species

- Mouse
- Rat (Hamra et al., PNAS 2005)
- Hamster (Kanatsu-Shinohara et al., Biol Reprod 2008)
- Bull (Aponte et al., Reproduction 2008)



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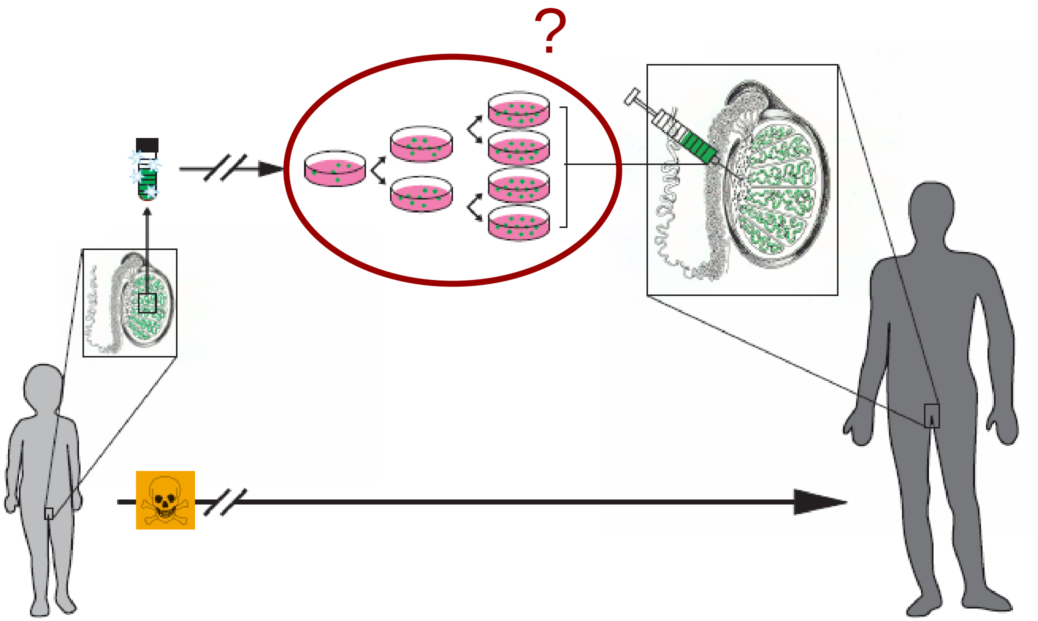
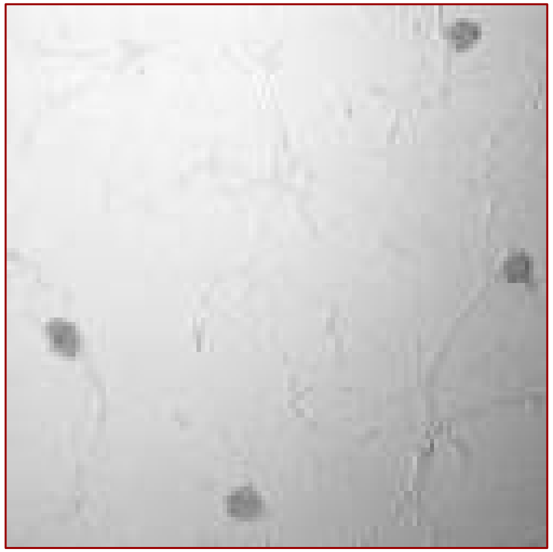
Aim

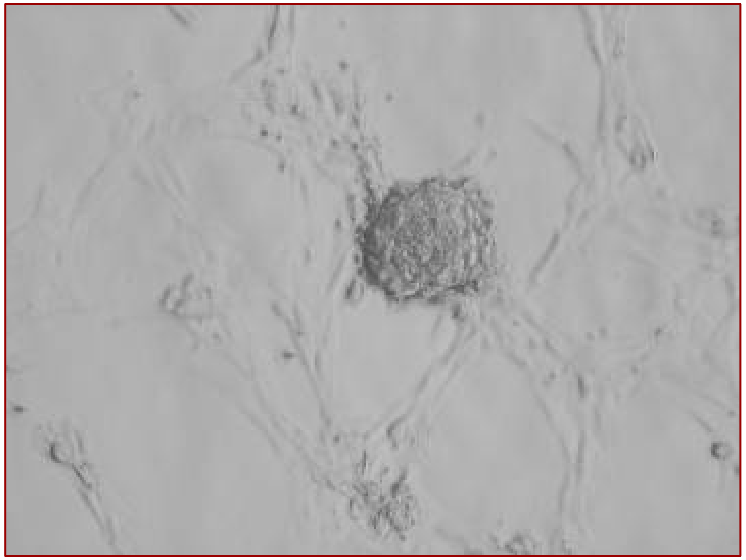
SSC cryopreservation and transplantation to preserve fertility in boys diagnosed with cancer



Adult human testicular cells in culture

Human SSC clusters





Sadri Ardekani et al., JAMA 302, 2127-2134, 2009

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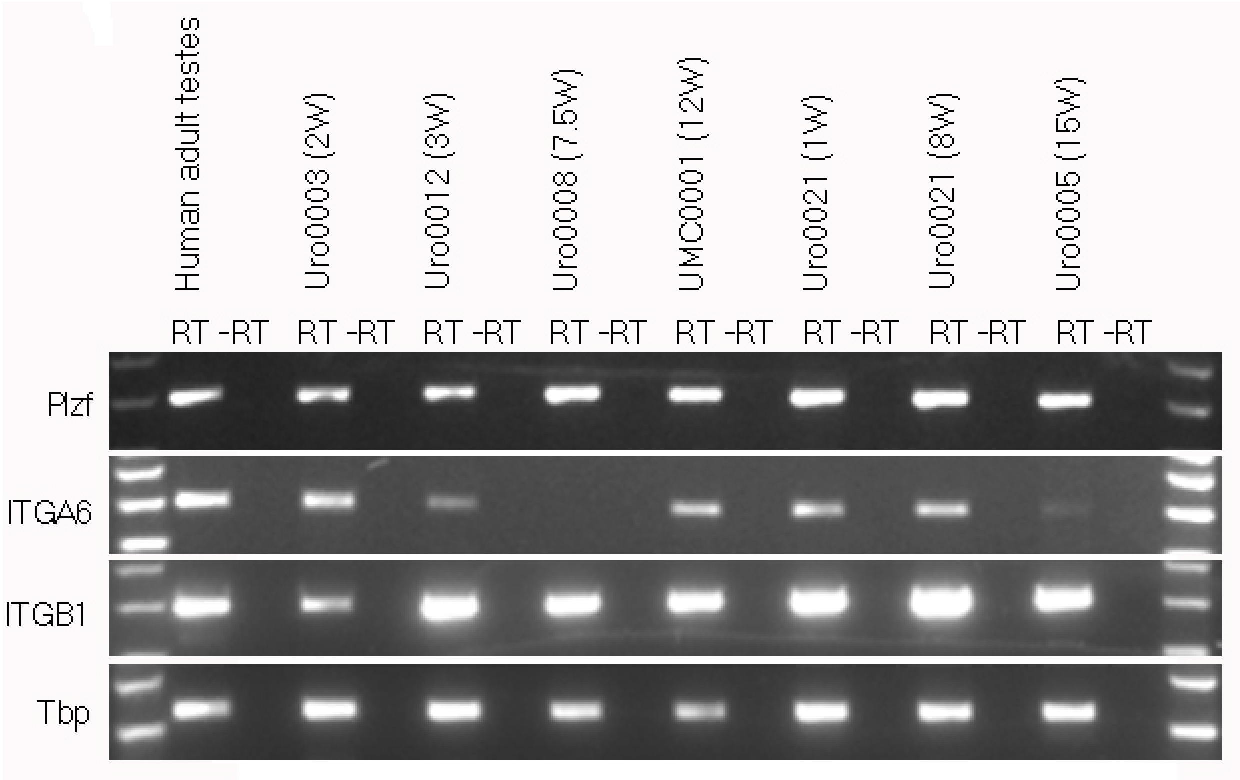
Expression spermatogonial markers



Sadri Ardekani et al., JAMA 302, 2127-2134, 2009

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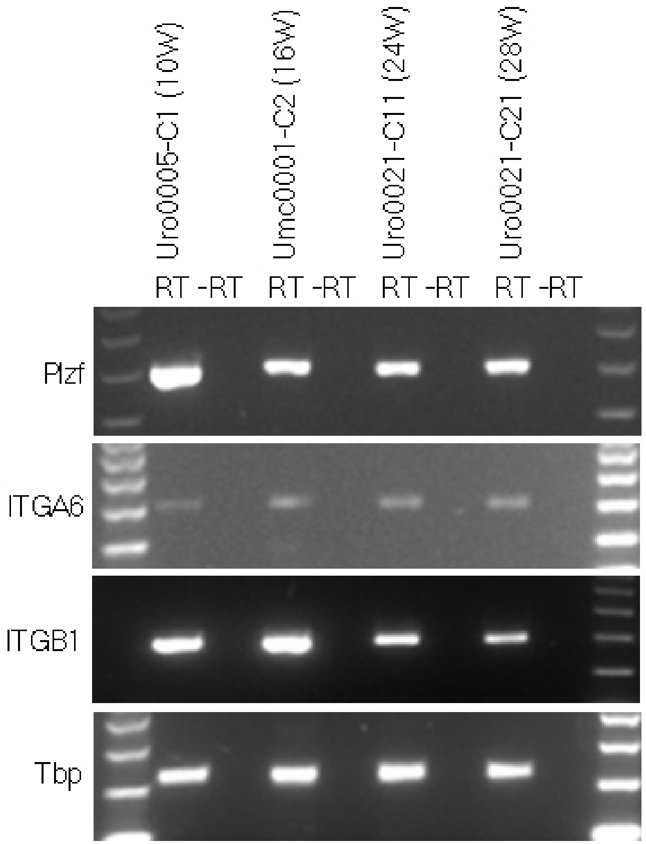
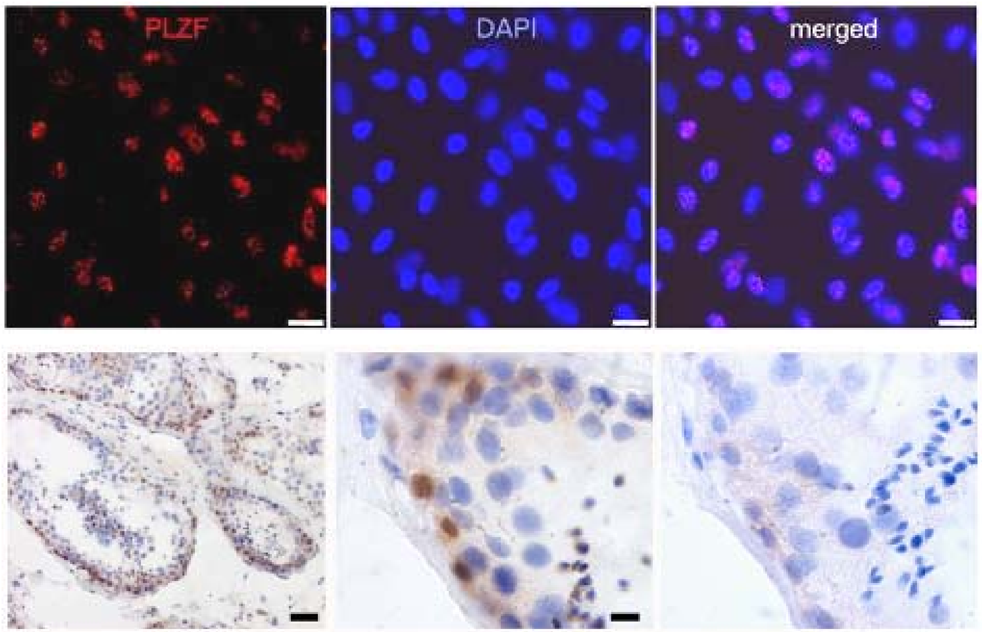
Human Germline stem cell subculture: expression spermatogonial markers





Sadri Ardekani et al., JAMA 302, 2127-2134, 2009

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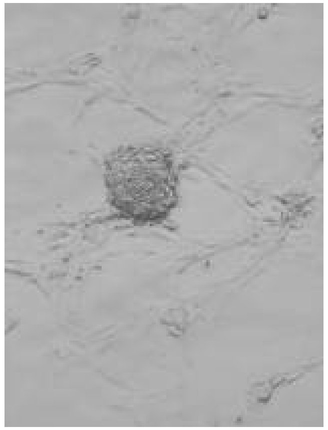
Human testicular cell culture: Xenotransplantation

cultured cells



Nude mouse
38 mg/Kg Busulfan treatment
4-6 weeks before transplantation

Colonization?

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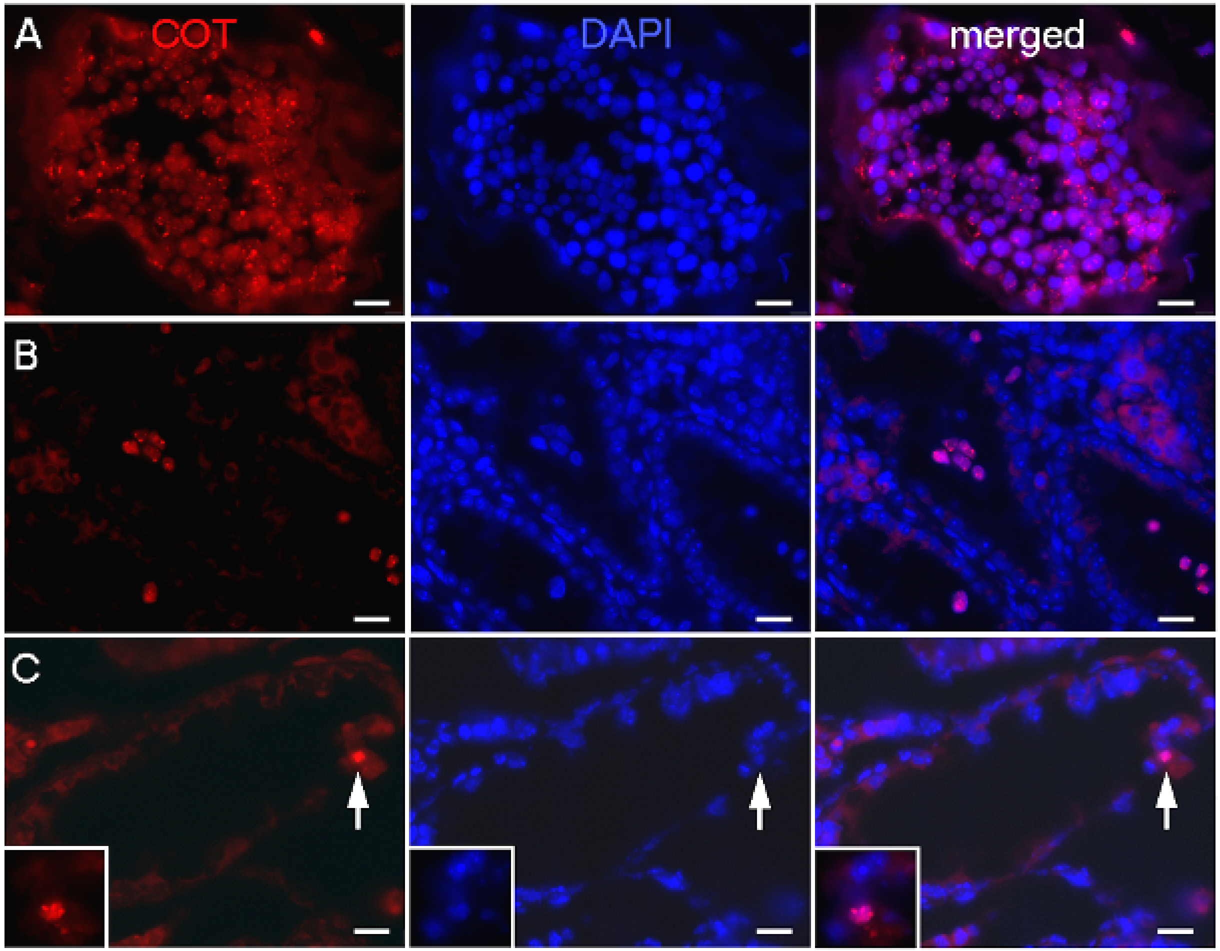
Transplantation to testis of immunodeficient mouse

Human
adult testis

2 hours
after transplantation

10 weeks
after transplantation



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Sadri Ardekani et al., JAMA 302, 2127-2134, 2009

Transplantation of adult human SSCs to mouse

Human Sample	Culture days	passage number	Number of injected cells(10^5)	Number of colonies /10^5 cells	Dilution factor	Human SSCs fold increase
Testicular cells culture						
UMC0001	63	4	1.3	0		
URO0003	14	1	3.5	0.7		
	14	1	0.2	12.5		
URO0005	14	1	2.7	0.9		
	42	3	0.3	0		
URO0008	28	3	0.7	3.6		
URO0012	21	1	0.6	0		
URO0021	28	3	0.1	0		
	56	7	0.4	0		
	28	2	2.55	2	↓ 133	53
	47	5	3.1	0.8		
GSCs subculture						
URO0005	91	6	2.5	0		
URO0021	77	7	2	1.25	↓ 8,870	18,450
	84	8	0.5	5		
	141	12	1.9	2.6		

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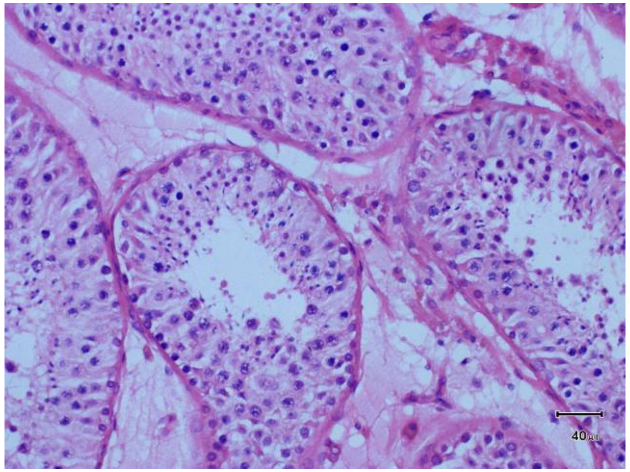
Sadri Ardekani et al., JAMA 302, 2127-2134, 2009

Conclusion I

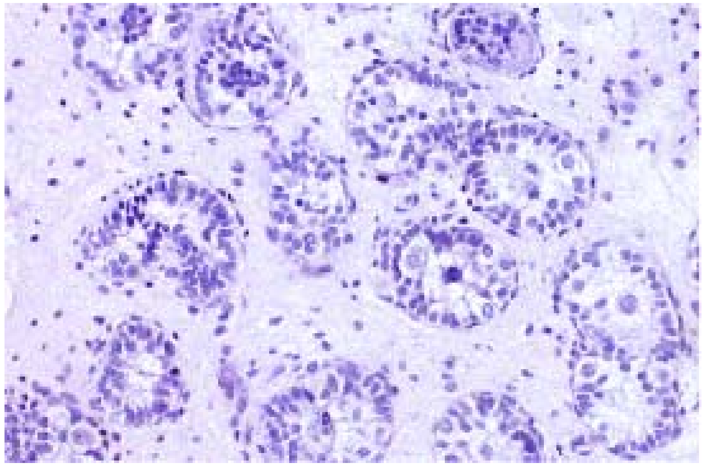
- Adults spermatogonial stem cells can be maintained in culture for a long period
- Adult spermatogonial stem cells can be propagated in culture
- Adult spermatogonial stem cells maintain their stem cell capacity to migrate to their niche upon transplantation

Adult vs prepubertal testis

Adult



prepubertal



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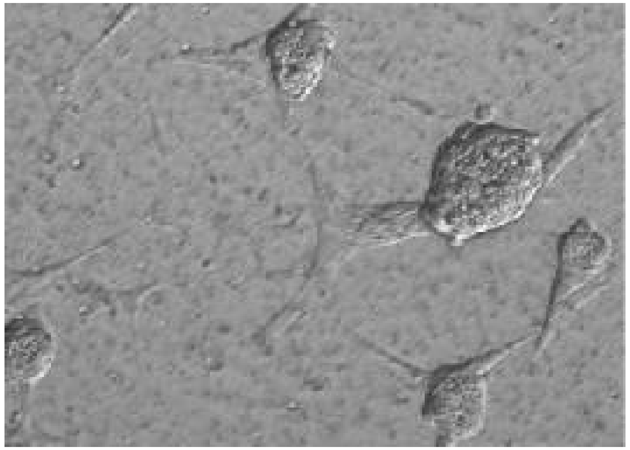
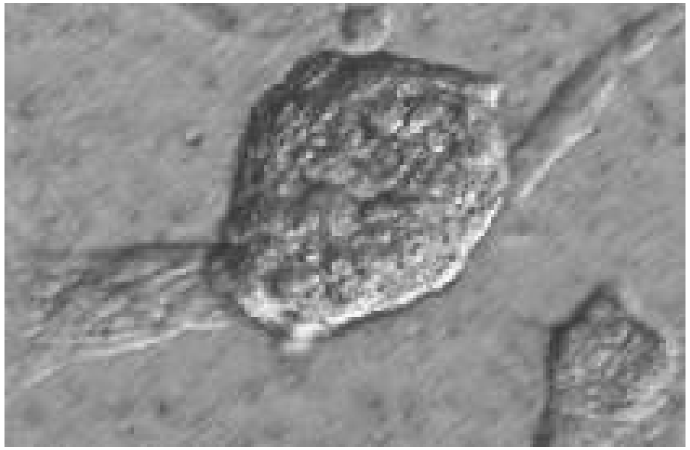
Prepubertal testicular cell culture





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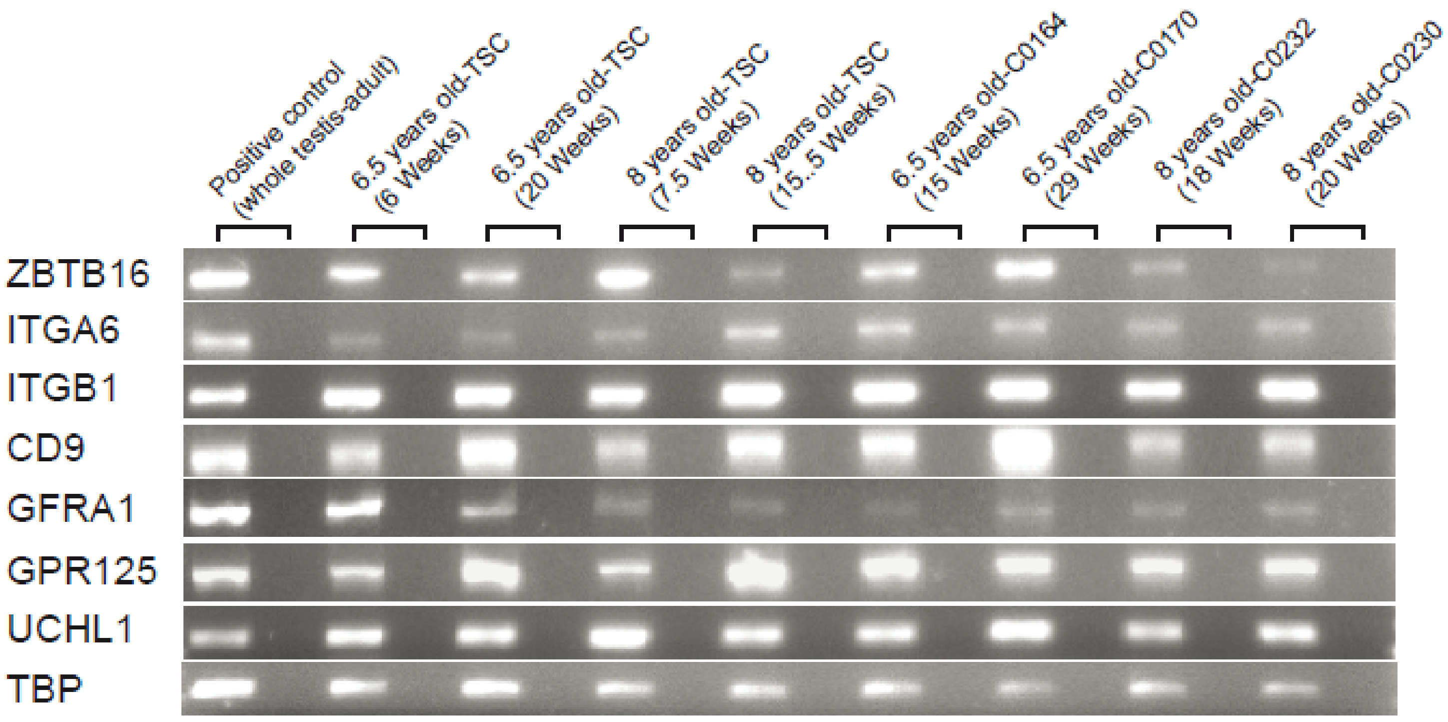
Expression spermatogonial markers



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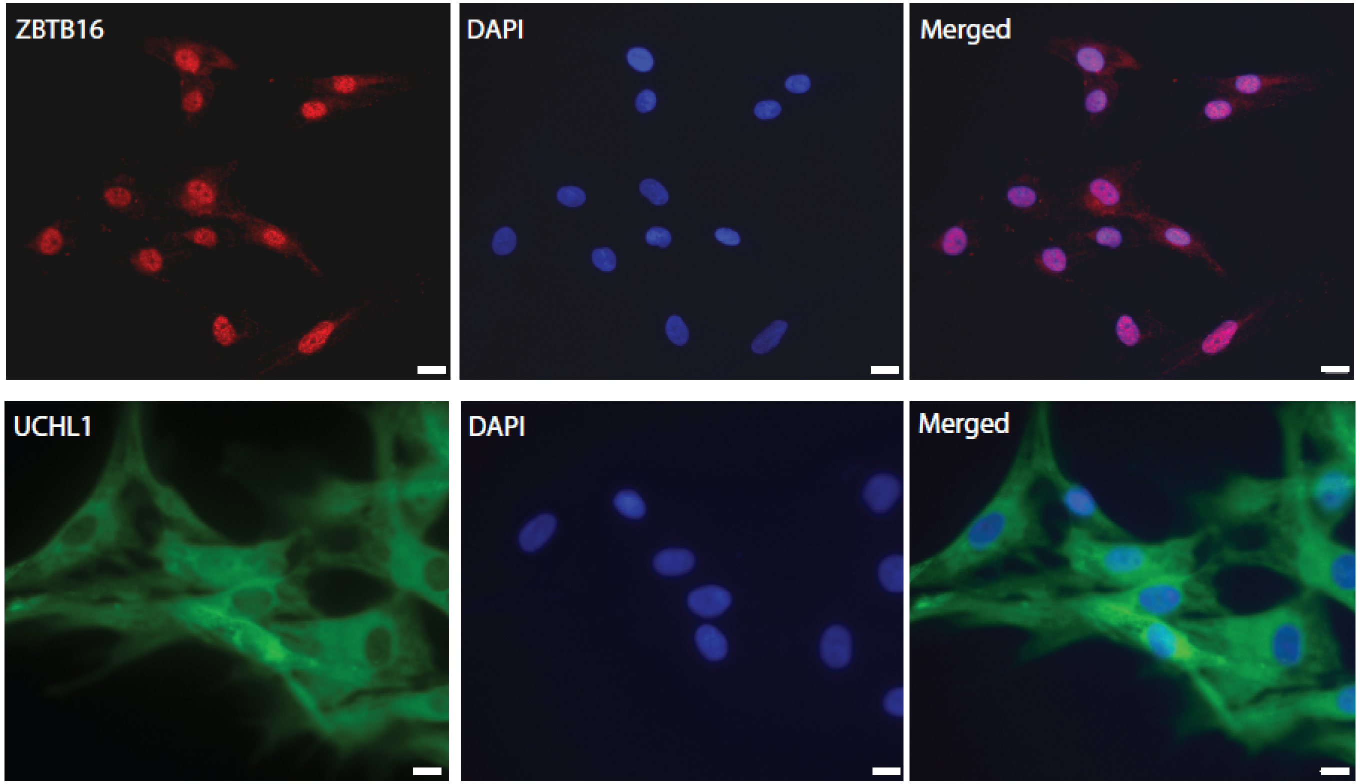
Expression spermatogonial markers



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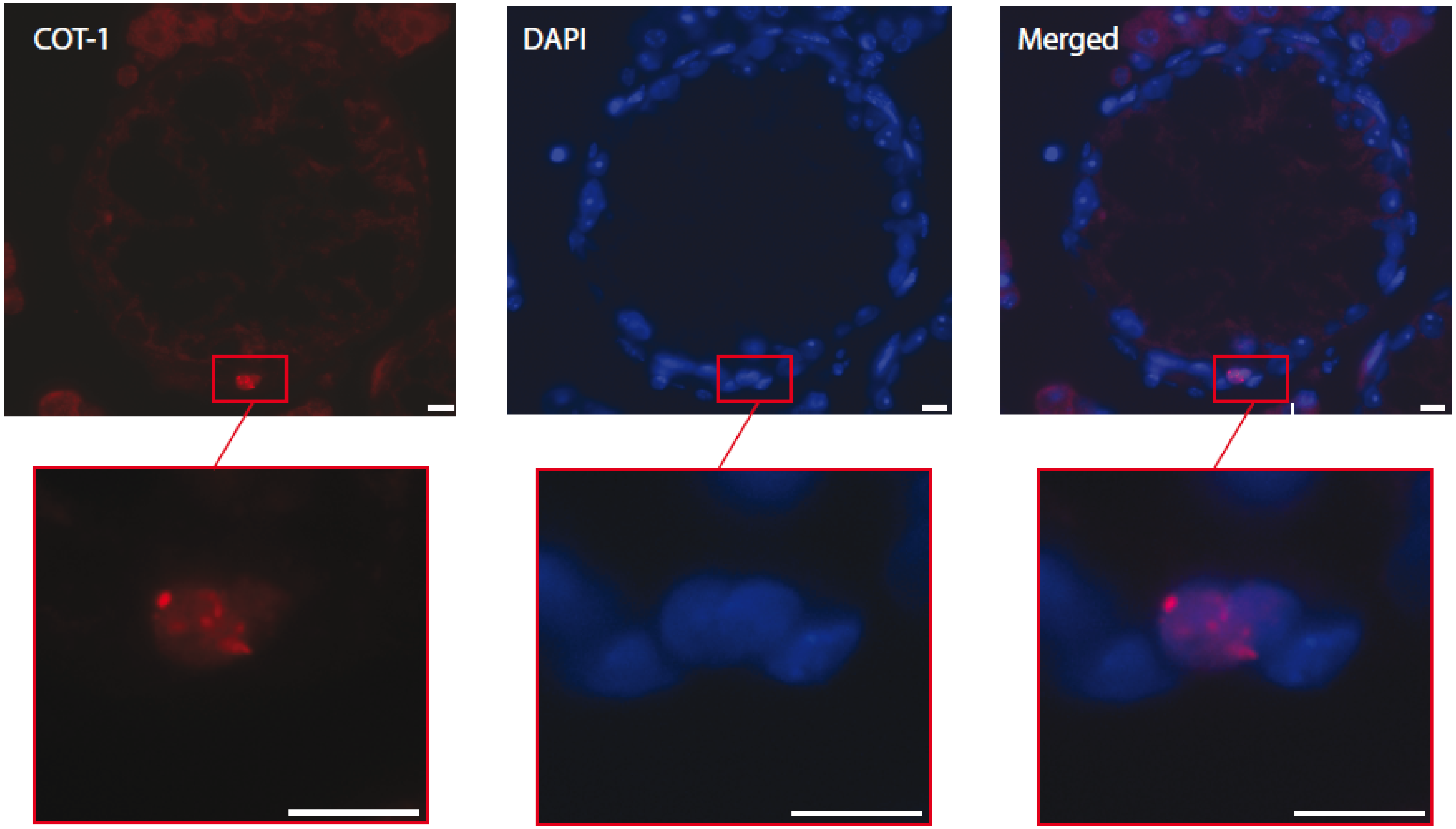
Transplantation to testis of immunodeficient mouse



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Transplantation of prepubertal human SSCs to mouse

Patient I.D	Culture days (passage number)	Number of injected cells (10^5)	Number of colonies /10^5 cells	Dilution factor	Human SSCs fold increase
Testicular cells culture					
6.5 year old	70(5)	2.4	1		
	98(9)	3.6	0		
8 year old	46 (4)	2	0		
	63 (6)	5.1	0.5	↓ 1.2	9.6
	74 (7)	1.9	4		
GSC subculture					
6.5 year old-C164	84(6)	0.7	3.6	↓ 8	6.2
6.5 year old-C164	105(7)	0.9	2.8		
8 year old-C233	84 (6)	2.3	0		
8 year old-C233	84 (6)	0.4	0		
8 year old-C234	84 (6)	2.6	1	↓ 1.33	5.6
8 year old-C234	98 (7)	0.6	4.2		

Sadri Ardekani et al., submitted

Conclusion II

- Prepubertal spermatogonial stem cells can be maintained for long time in similar culture conditions as adult spermatogonial stem cells
- Prepubertal spermatogonial stem cells can also be propagated in culture
- Prepubertal spermatogonial stem cells maintain their stem cell capacity to migrate to their niche upon transplantation

Summary

• No specific spermatogonial stem cell markers are available to date and spermatogonial stem cell transplantation is only (bio)assay

• Human adult and prepubertal spermatogonial stem cells can survive and proliferate in long term culture

• Cultured human adult and prepubertal spermatogonial stem cells maintain their stem cell characteristics to migrate to their niche upon transplantation

This culture system is an important step in the future clinical application to preserve fertility in childhood cancer patients.

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Acknowledgements

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