

TESTIS TISSUE CRYOPRESERVATION MAY BE CONSIDERED FOR BOYS WITH CRYPTORCHIDISM

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No disclosures.

The first fertility preservation programme specifically for pre-pubertal boys was initiated in Brussels (Belgium) in 2002 offering cryo-preservation of testicular tissue.

Setting Up a Cryopreservation Programme for Immature Testicular Tissue: Lessons Learned After More Than 15 Years of Experience

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Based on positive experience with cryo-preservation of female ovarian tissue prior to gonadotoxic treatment.

Results of basic research on culturing testicular tissue from rats (Steinberger et al. 1964) and adult men (Steinberger et al. 1970 and Tesarik et al. 1998)

Long-term cultures of testicular biopsies from boys with cryptorchidism: effect of FSH and LH on the number of germ cells.

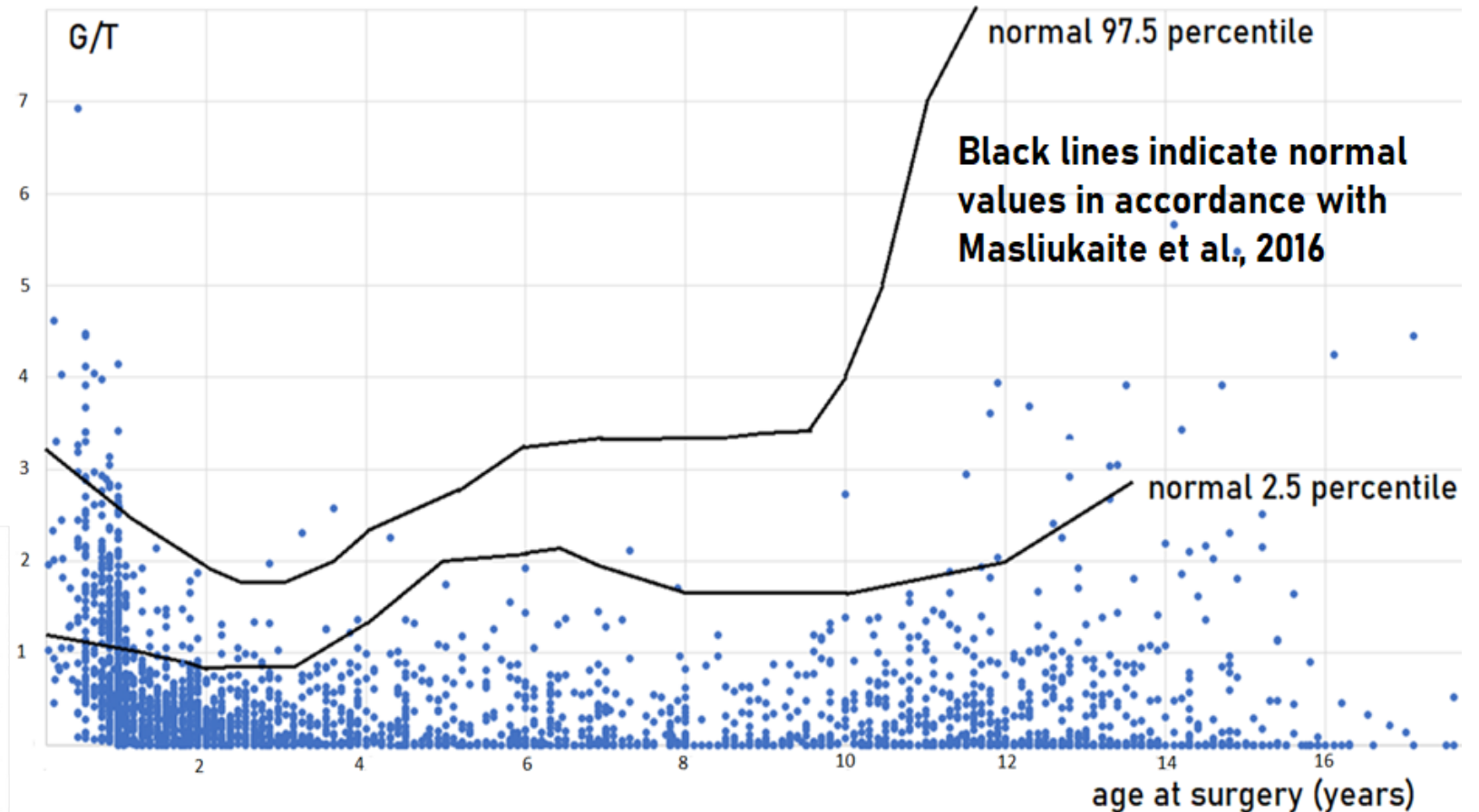
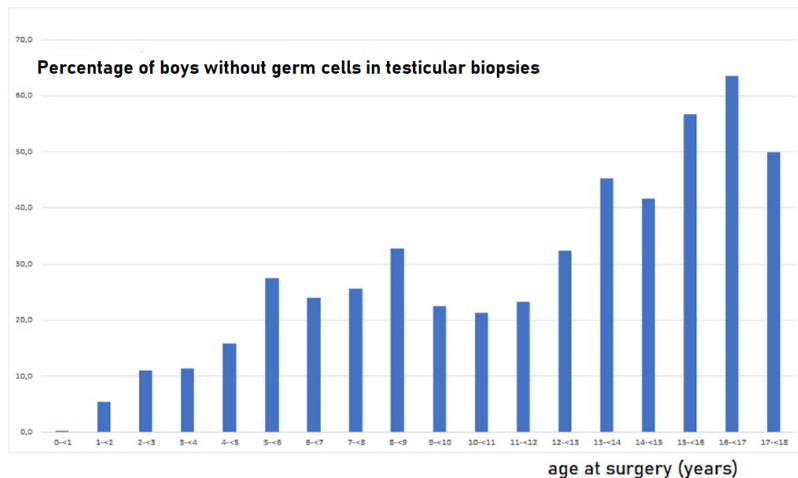
Larsen et al. Human Reproduction 2002.

Cryptorchidism

Infertility

27% of men with azoospermia have a history of cryptorchidism

(Fedder et al. Fertil Steril; 2004)



Germ cell number per tubular cross section (G/T) in testicular biopsies of 2410 boys operated for cryptorchidism in relation to age at surgery and normal values.

38 Infertile formerly bilateral cryptorchids

Cortes et al. Br J Urol 2003 (follow-up data from study published Br J Urol 1991)

N = 23 patients <i>HYPERGONADOTROPIC HYPOGONADISM</i> Primary Testicular Damage	N = 15 patients <i>HYPOGONADOTROPIC HYPOGONADISM</i> (Deficiency of FSH and LH?)
Elevated FSH. 16.0 (8.4-20.4) IU/L	Normal FSH. 5.3 (2.6-7.4) IU/L
Germ cells absent in childhood	Number of germ cells severely reduced in childhood. Median G/T = 0.3
In adulthood (Sertoli cells only).	In adulthood Spermatogenic arrest and Sertoli cells only
<i>Sperm conc. (10⁶/ml)</i> <i>Median (range): 0.0 (0 – 2.0)</i>	<i>Sperm conc. (10⁶/ml)</i> <i>Median (range): 1.5 (0 – 4.5)</i>

Long-term cultures of testicular biopsies from boys with cryptorchidism: effect of FSH and LH on the number of germ cells.

Larsen HP, Thorup J, Skovgaard LT, Cortes D, Byskov AG.

Hum Reprod. 2002;17:383-9

Age group	Uncultured	Weeks of culture	TCM	TCM+FSH (50 IU/l)	TCM+LH (5 IU/l)	TCM+FSH+LH (50 IU/l+ 5 IU/l)
Group I (n = 12)	0.105 ± 0.023	1	0.163 ± 0.052	0.131 ± 0.036	0.102 ± 0.032	0.087 ± 0.026
		3	0.126 ± 0.044	0.083 ± 0.035	0.088 ± 0.045	0.083 ± 0.031
Group II (n = 13)	0.113 ± 0.024	1	0.132 ± 0.039	0.158 ± 0.038	0.148 ± 0.034	0.127 ± 0.031
		3	0.104 ± 0.026	0.193 ± 0.062	0.123 ± 0.036	0.133 ± 0.041
Group I+II (n = 25)	<u>0.109 ± 0.016</u>	1	<u>0.147 ± 0.032</u>	<u>0.145 ± 0.026</u>	<u>0.125 ± 0.023</u>	<u>0.108 ± 0.021</u>
		3	<u>0.115 ± 0.024</u>	<u>0.138 ± 0.037</u>	<u>0.106 ± 0.028</u>	<u>0.109 ± 0.026</u>

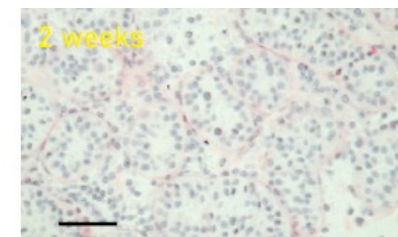
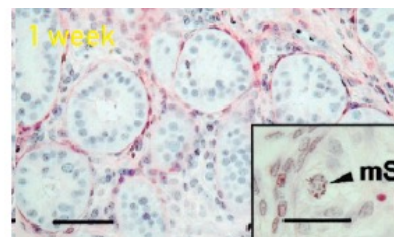
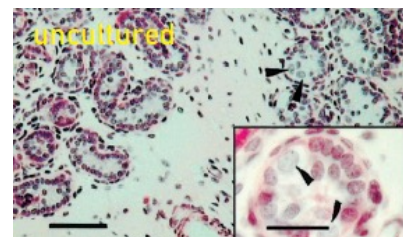
Age 1-10 yrs, median

4

Mean Germ cell number per tubular transverse section G/T ± SD

n = number of biopsies in the group

The TCM contained Dulbecco's modified eagle medium/F-12, supplemented with 5µg/ml Transferrin, 20µg/ml insulin, 30 mg/ml L-Glutamin plus 50 IU/ml penicillin and 50µg/ml Streptomycin.



Cryopreservation of intact testicular tissue from boys with cryptorchidism

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¹Laboratory of Reproductive Biology, Section 5712, ²Department of Paediatric Surgery, Section 4072, University Hospital of Copenhagen, Rigshospitalet, DK-2100 Copenhagen and ³Department of Medical Anatomy, University of Copenhagen, DK-2200 Copenhagen, Denmark

⁴To whom correspondence should be addressed. E-mail: yding@rh.dk

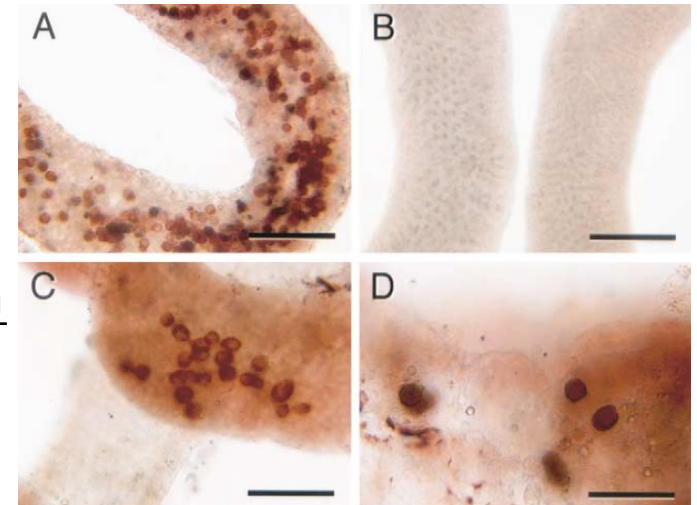
BACKGROUND: Boys with cryptorchidism often face fertility problems in adult life despite having orchiopexy performed at a very young age. During this operation, a biopsy of the testis is normally taken in order to evaluate their infertility potential and the presence of malignant cells. This study evaluated the morphology and functional capacity of cryopreserved testes biopsies and their possible use in fertility preservation. **METHODS:** Biopsies from 11 testes (eight boys) were obtained. Each biopsy was subdivided into six pieces and two pieces were frozen in each of two different cryoprotectants. One fresh and two cryopreserved pieces were cultured for 2 weeks. All pieces were prepared for histology. Used culture media were analysed for testosterone and inhibin B concentrations. **RESULTS:** The morphology of the fresh and frozen–thawed samples was similar, with well-preserved seminiferous tubules and interstitial cells. A similar picture appeared after 2 weeks of culture, but a few of the cultured biopsies contained small necrotic areas. The presence of spermatogonia was verified by c-kit-positive immunostaining. Production of testosterone and inhibin B (ng/mm³ testis tissue) in the frozen–thawed pieces was on average similar to that of the fresh samples. **CONCLUSIONS:** Intact testicular tissue from young boys with non-descended testes tolerates cryopreservation with surviving spermatogonia and without significant loss of the ability to produce testis-specific hormones *in vitro*. It may be an option to freeze part of the testis biopsy, which is routinely removed during the operation for cryptorchidism, for fertility preservation in adult life.

Further basic research – Waiting for funding

Research by others:

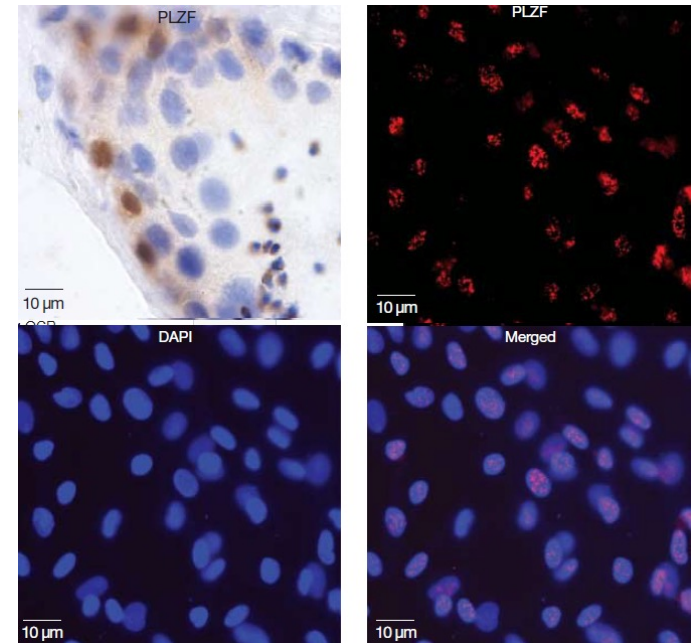
Long-term survival of human spermatogonial stem cells in mouse testes.

Nagano M, Patrizio P, Brinster RL.
Fertil Steril. 2002;78:1225-1233.



Propagation of human spermatogonial stem cells in vitro.

Sadri-Ardekani H, Mizrak SC, van Daalen SK, Korver CM, Roepers-Gajadien HL, Koruji M, Hovingh S, de Reijke TM, de la Rosette JJ, van der Veen F, de Rooij DG, Repping S, van Pelt AM.
JAMA. 2009;302:2127-34



[Experimental testicular tissue banking to generate spermatogenesis in the future: A multidisciplinary team approach.](#)

Sadri-Ardekani H, McLean TW, Kogan S, Sirintrapun J, Crowell K, Yousif MQ, Hodges SJ, Petty J, Pranikoff T, Sieren L, Zeller K, Atala A. *Methods*. 2016 Apr 15;99:120-7.

Material: Parents of 19 prepubertal boys aged 8 months–11 years (median: 5 years old).

Results: Acceptance rate: 79% (15/19)



Material: Parents of 41 prepubertal boys aged 4–45 (median: 13) months old.
(14: 2-stage procedure & 27: 1-stage procedure)

Results: Acceptance rate: 90% (37/41)

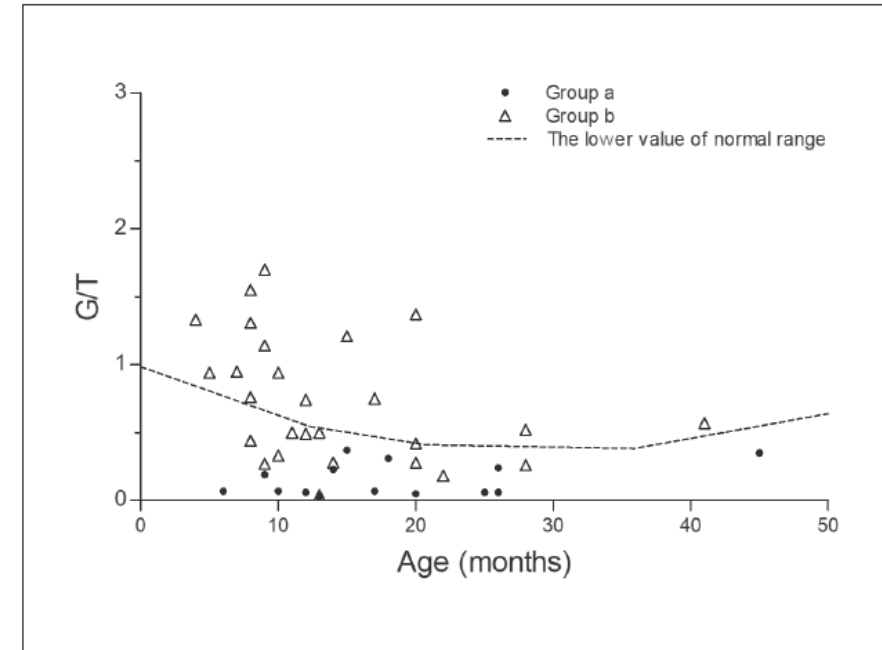


Fig. 2. The number of germ cells per cross-sectional tubule (G/T) of 41 boys with cryptorchidism in relation to age. The dotted line represents the lower value of normal range of G/T [Cortes, 1990; Cortes et al., 1995].

(Group a; n=14: 2-stage procedure & Group b; n=27: 1-stage procedure)

Propagation of Spermatogonial Stem Cell-Like Cells From Infant Boys

Published
2019

Lihua Dong^{1*}, Stine Gry Kristensen¹, Simone Hildorf², Murat Gul^{1,3}, Erik Clasen-Linde⁴,
Jens Fedder^{5,6}, Eva R. Hoffmann⁷, Dina Cortes^{8,9}, Jorgen Thorup^{2,9} and
Claus Yding Andersen^{1,9}

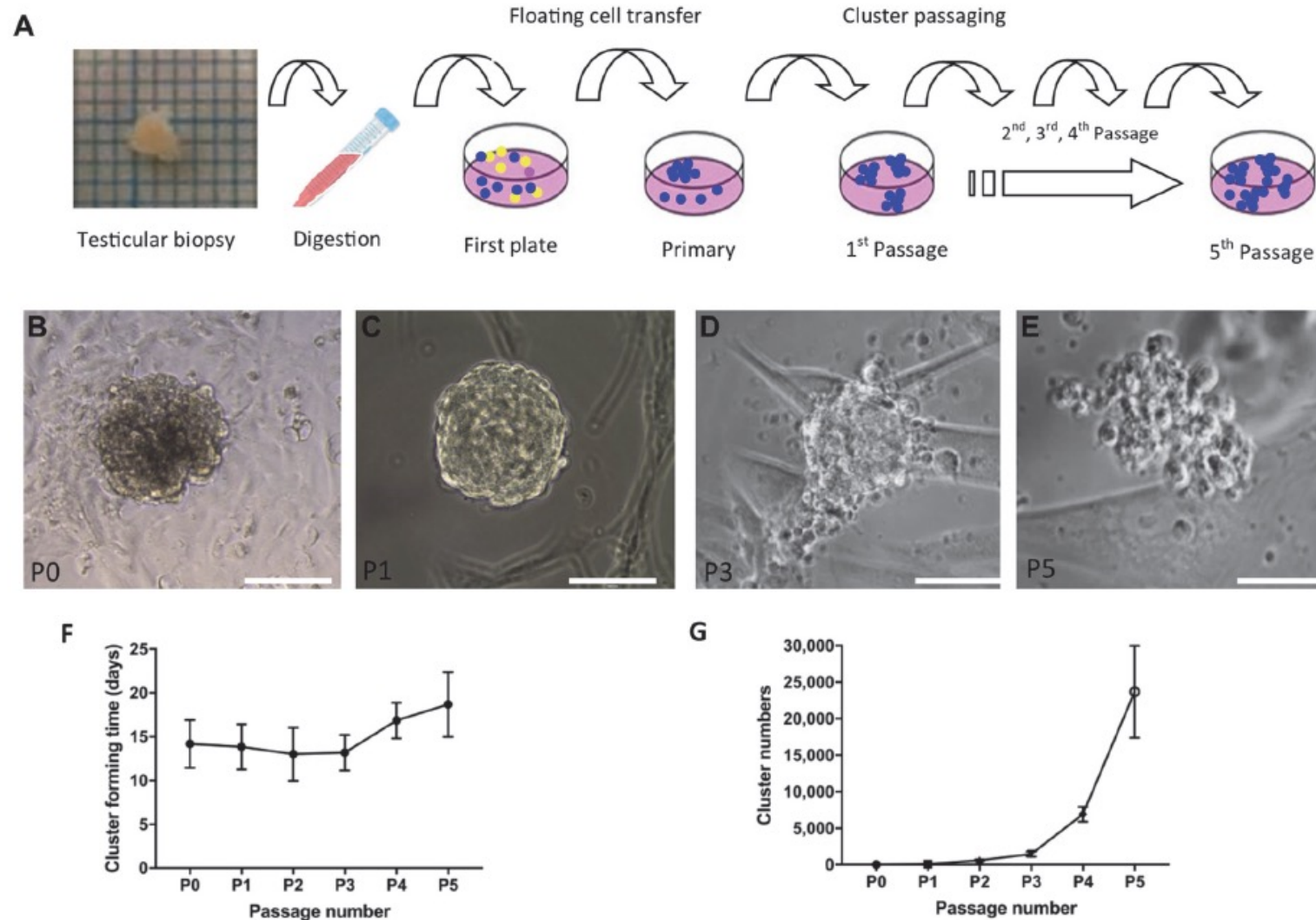
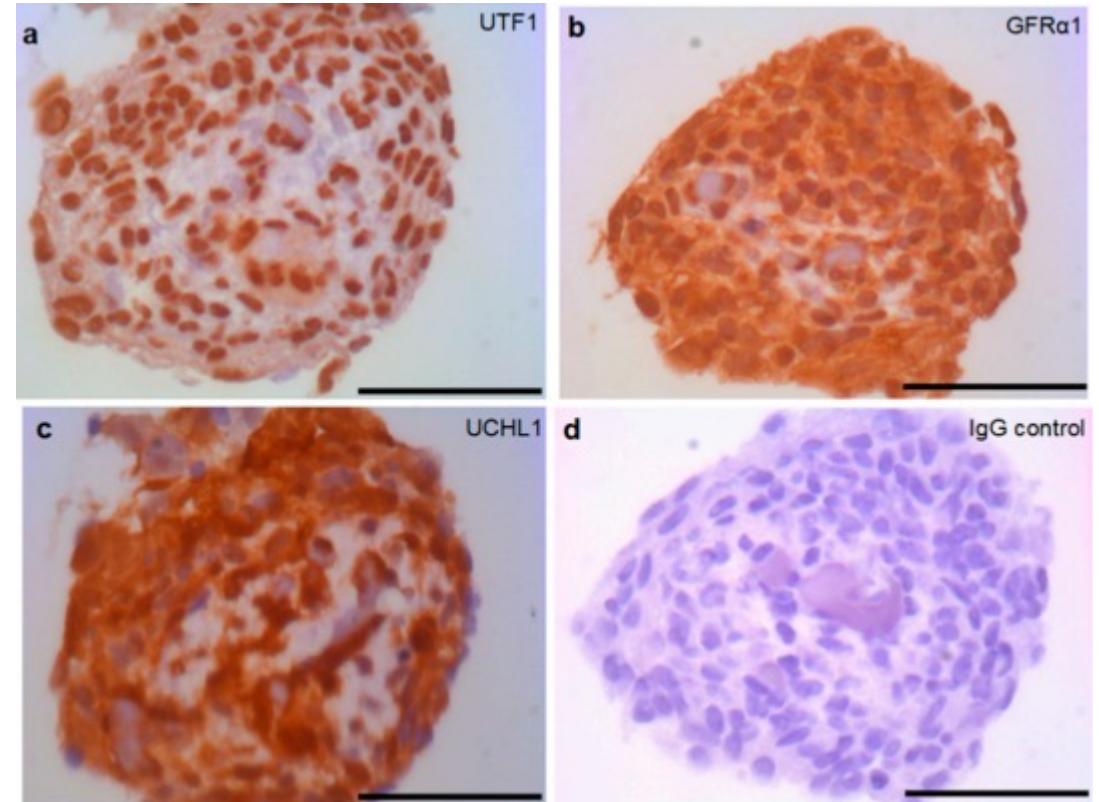
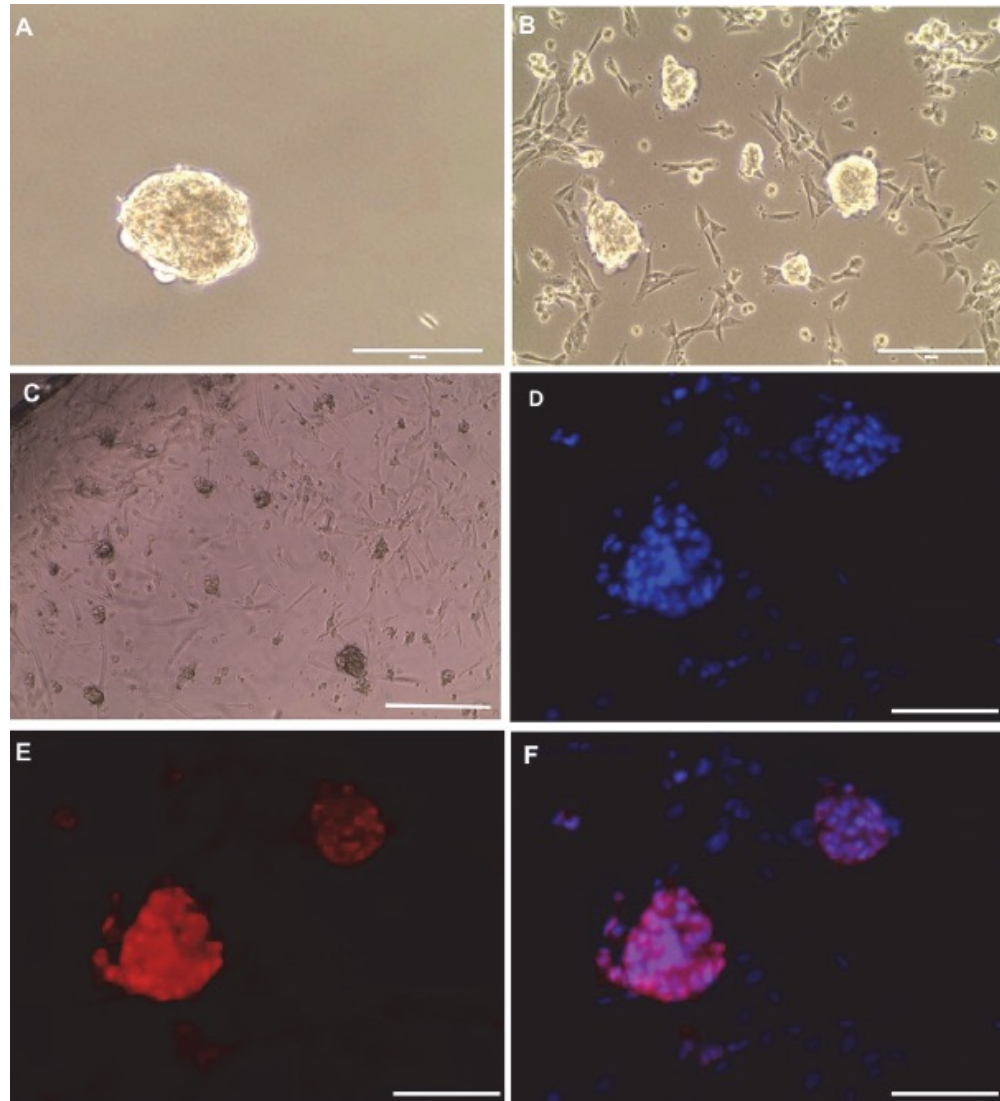


FIGURE 3 | Derivation and propagation of human spermatogonial stem cell like cell clusters (SSCLCs) from infant boys. **(A)**, Methodology of deriving SSCLCs from testicular biopsies. One square is 1 mm². **(B)**, SSCLCs forming from primary cell culture. **(C)**, First passage. **(D)**, Third passage. **(E)**, Fifth passage. Scale bar 100 μm. **(F)**, Cluster forming time after passaging (mean and standard deviations). **(G)**, Estimated SSCLC numbers (mean and standard deviations).

Propagation of Spermatogonial Stem Cell-Like Cells From Infant Boys

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Autologous Grafting of Cryopreserved Prepubertal Rhesus Testis Produces Sperm and Offspring

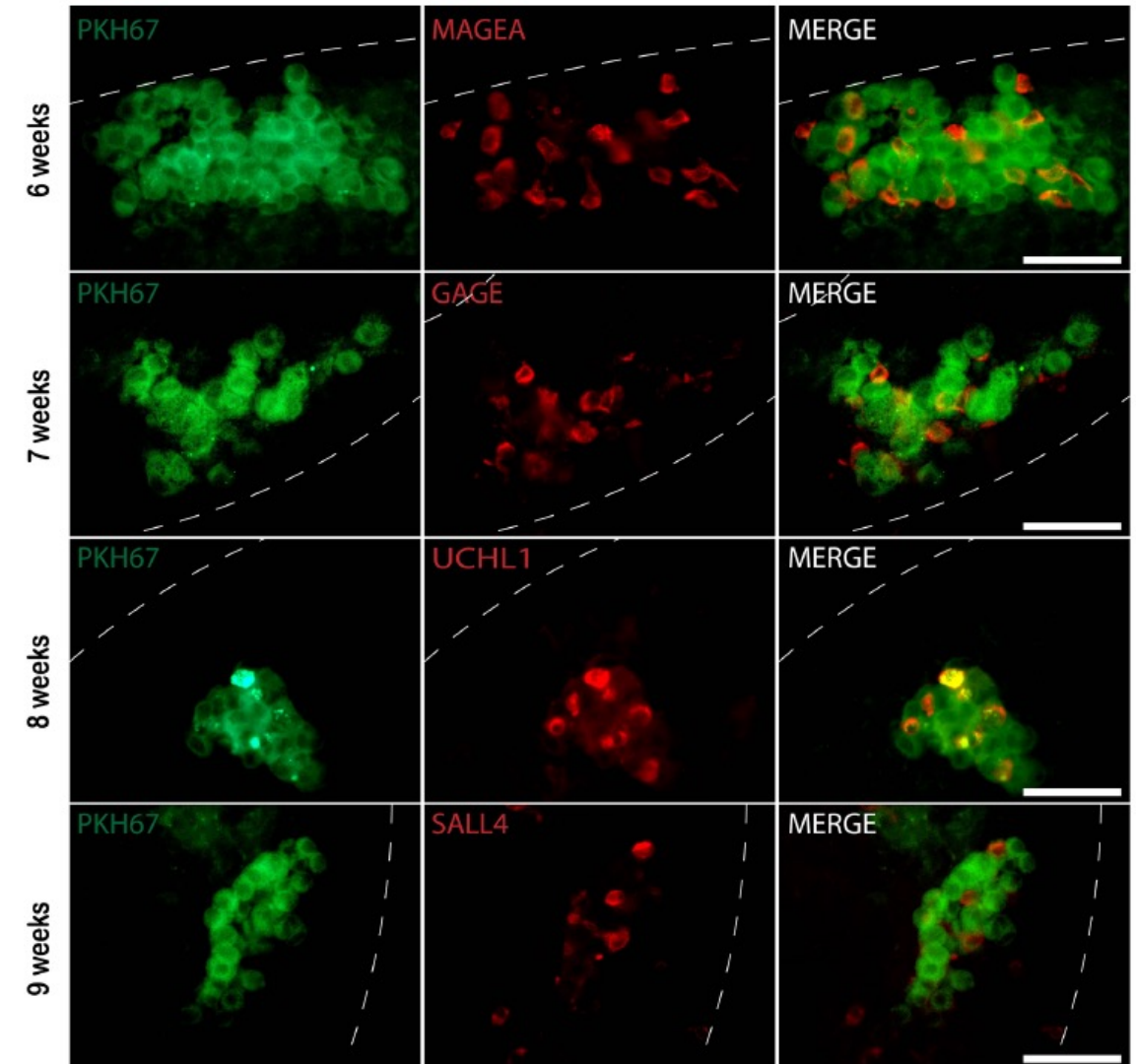
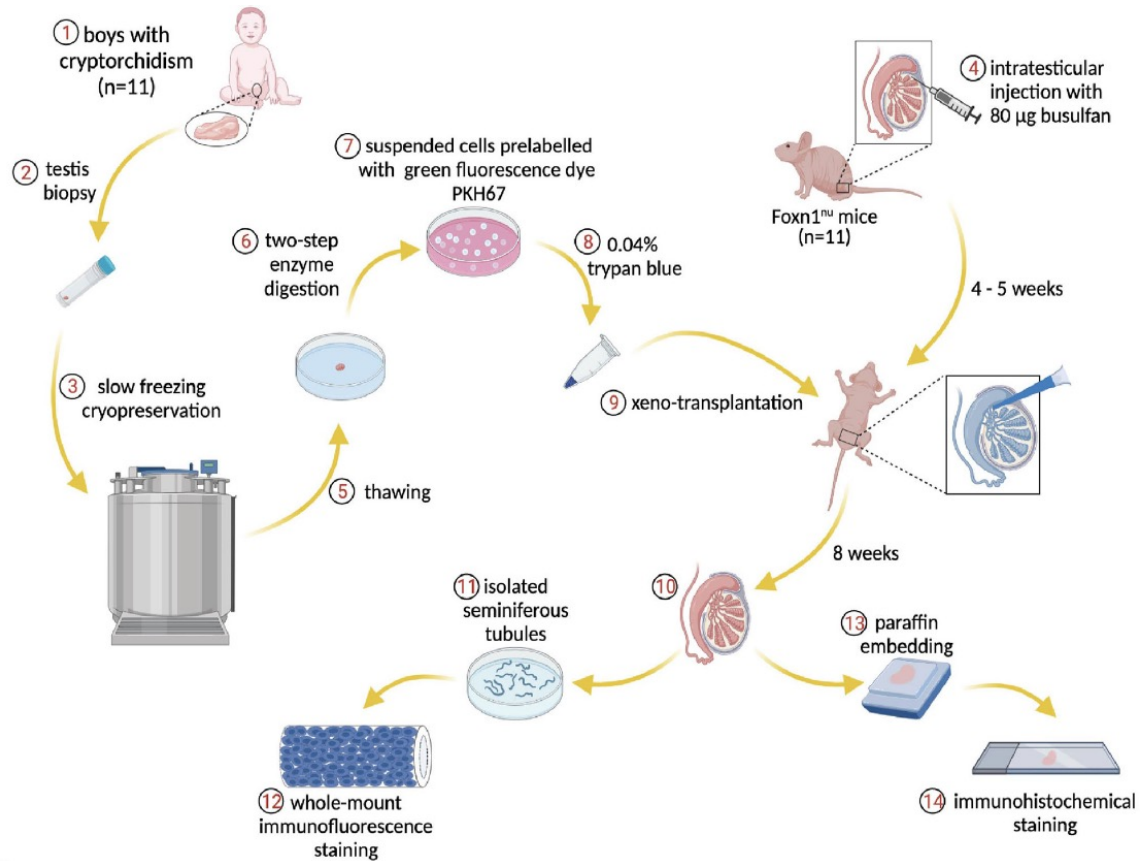
Adetunji P. Fayomi^{1,2,3}, Karen Peters³, Meena Sukhwani³, Hanna Valli-Pulaski^{2,3}, Gunapala Shetty⁴, Marvin L. Meistrich⁴, Lisa Houser⁵, Nicola Robertson⁵, Victoria Roberts⁵, Cathy Ramsey⁵, Carol Hanna⁵, Jon D. Hennebold⁵, Ina Dobrinski⁶, and Kyle E. Orwig^{1,2,3,*}



Characterization and Survival of Human Infant Testicular Cells After Direct Xenotransplantation

Published
2022

Danyang Wang^{1,2*}, Simone Hildorf^{2,3}, Elissavet Ntemou¹, Lihua Dong¹,
Susanne Elisabeth Pors¹, Linn Salto Mamsen¹, Jens Fedder^{4,5}, Eva R. Hoffmann⁶,
Erik Clasen-Linde⁷, Dina Cortes^{2,8}, Jorgen Thorup^{2,3} and Claus Yding Andersen^{1,2}

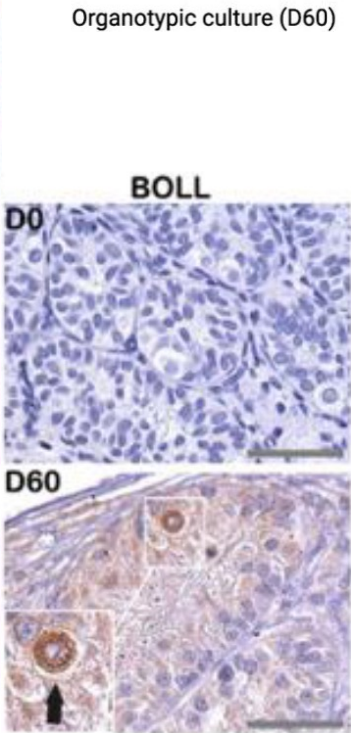
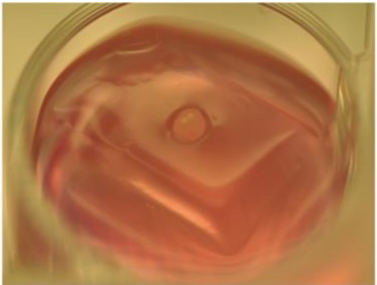
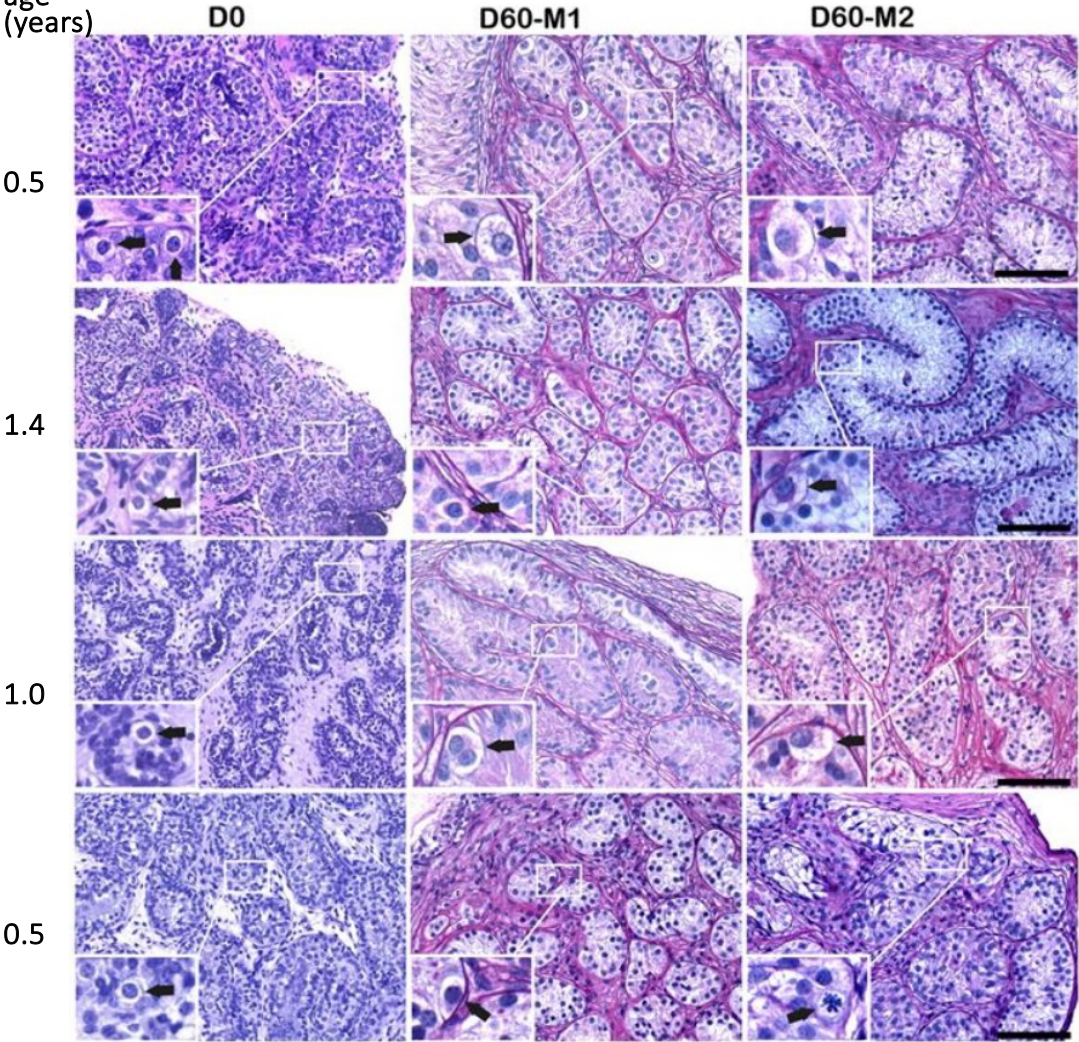


Organotypic Culture of Testicular Tissue from Infant Boys with Cryptorchidism

Danyang Wang^{1,2,*}, Simone Hildorf^{2,3}, Elissavet Ntemou¹, Linn Salto Mamsen¹, Lihua Dong¹, Susanne Elisabeth Pors¹, Jens Fedder^{4,5}, Erik Clasen-Linde⁶, Dina Cortes^{2,7}, Jørgen Thorup^{2,3} and Claus Yding Andersen^{1,2}

Published
2022

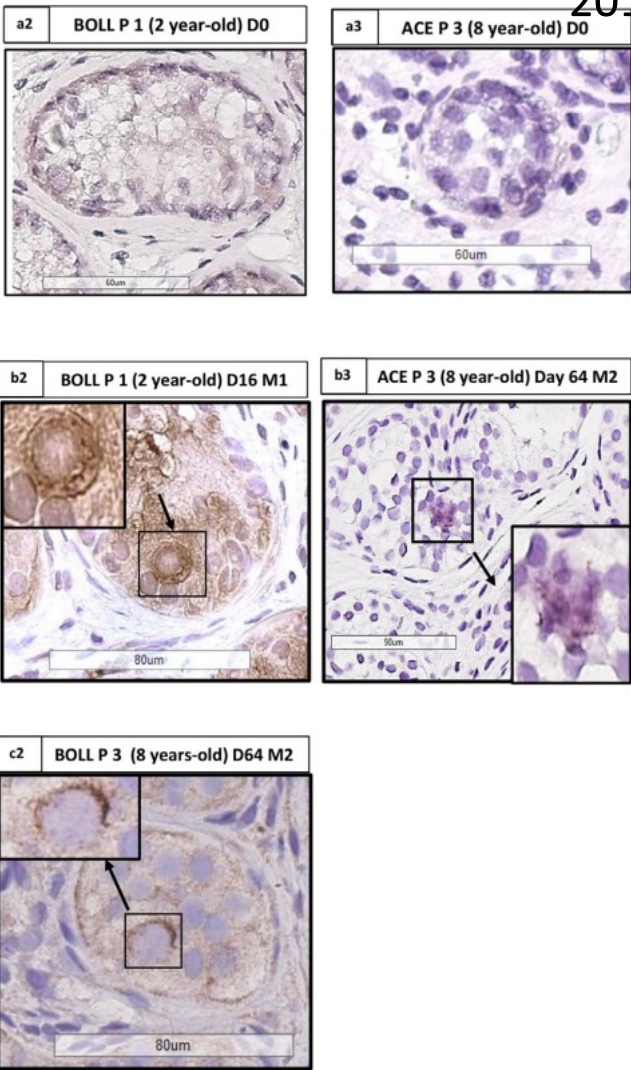
Patient
age
(years)



Haploid Germ Cells Generated in Organotypic Culture of Testicular Tissue From Prepubertal Boys

Francesca de Michele^{1,2*}, Jonathan Poels¹, Maxime Vermeulen², Jérôme Ambroise³, Damien Gruson⁴, Yves Guiot⁵ and Christine Wyns^{1,2*}

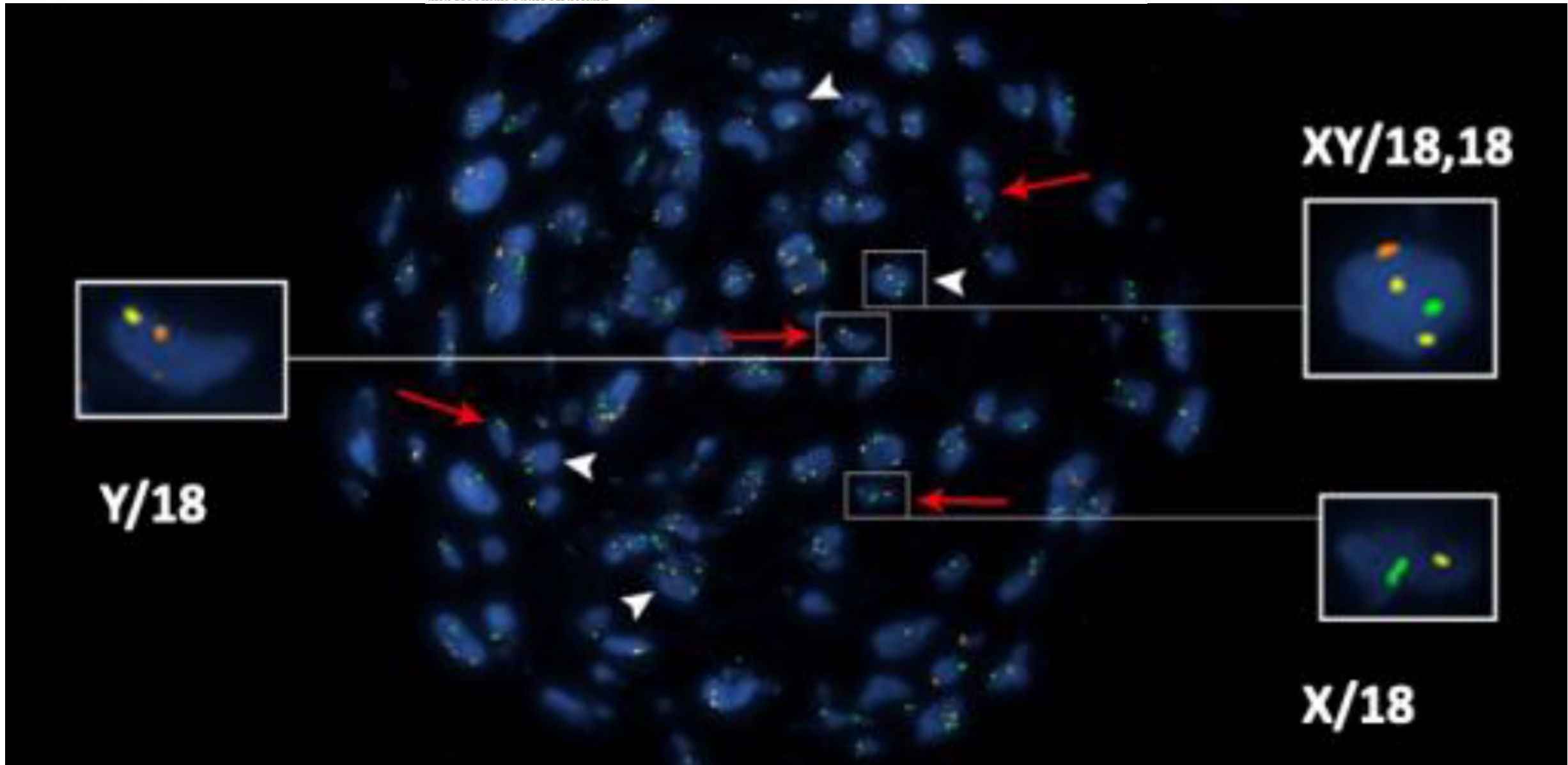
Published
2018



In Vitro Generation of Haploid Germ Cells from Human XY and XXY Immature Testes in a 3D Organoid System

Published
2024

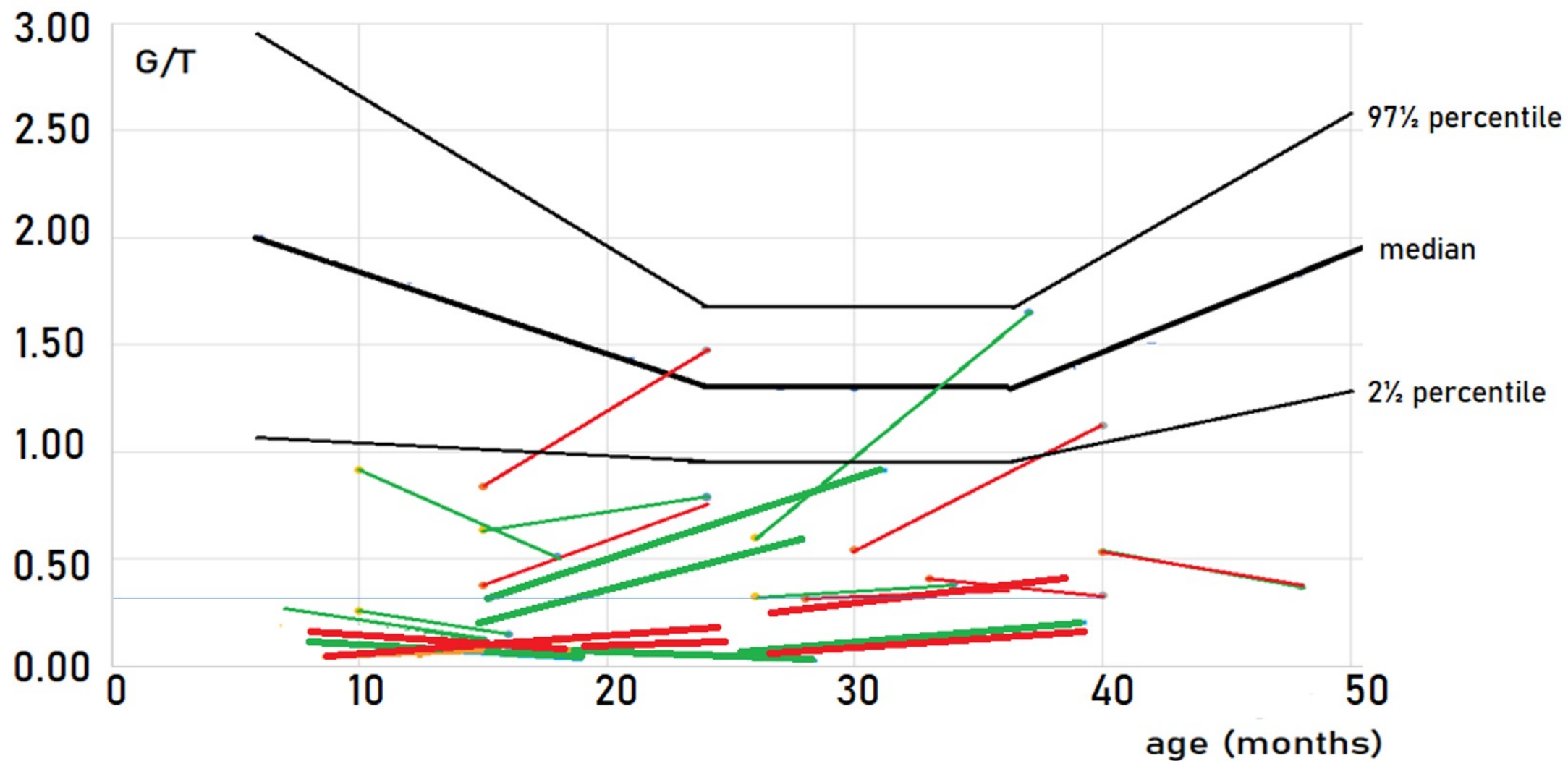
Guillermo Galdon ^{1,2,*} , Nima Pourhabibi Zarandi ^{1,3} , Nicholas A. Deebel ^{1,4} , Sue Zhang ^{1,5}, Olivia Cornett ¹, Dmitry Lyalin ^{6,7}, Mark J. Pettenati ⁶, YanHe Lue ⁸ , Christina Wang ⁸, Ronald Swerdloff ⁸, Thomas D. Shupe ¹, Colin Bishop ¹, Kimberly Stogner ^{1,6}, Stanley J. Kogan ¹, Stuart Howards ^{1,4}, Anthony Atala ^{1,4} , and Hooman Sadri-Ardekani ^{1,4,6,*} 



Results

green = GnRH (Kryptocur[®]) treated

red = Surgery only



Published
2021

The impact of early and successful orchidopexy on hormonal follow-up for 208 boys with bilateral non-syndromic cryptorchidism

Simone Hildorf^{1,2} · Dina Cortes^{2,3} · Erik Clasen-Linde⁴ · Magdalena Fossum^{1,2} · Jorgen Thorup^{1,2}

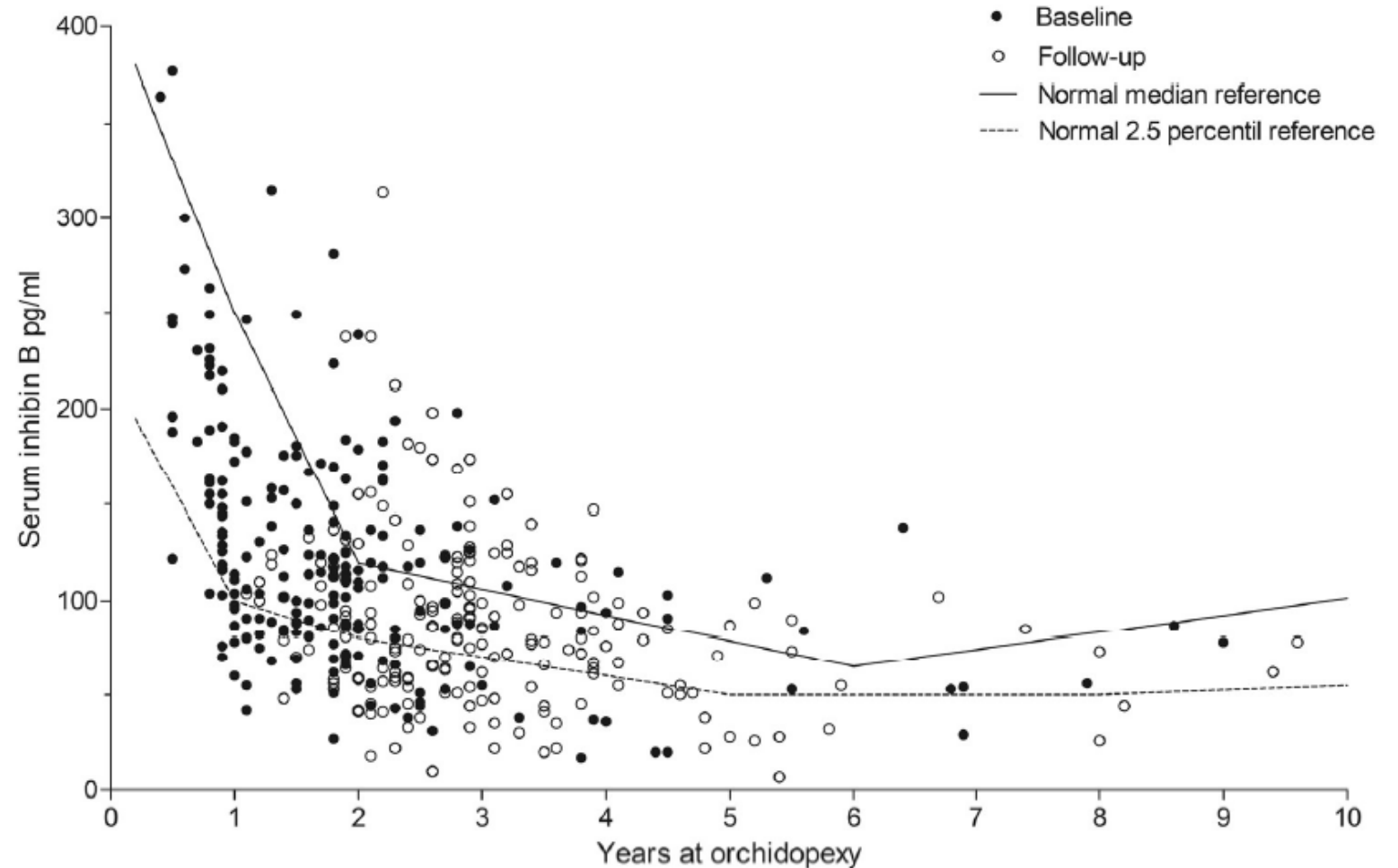
208 boys with bilateral cryptorchidism.

Age at orchidopexy:

Median: 1 year and 8 months

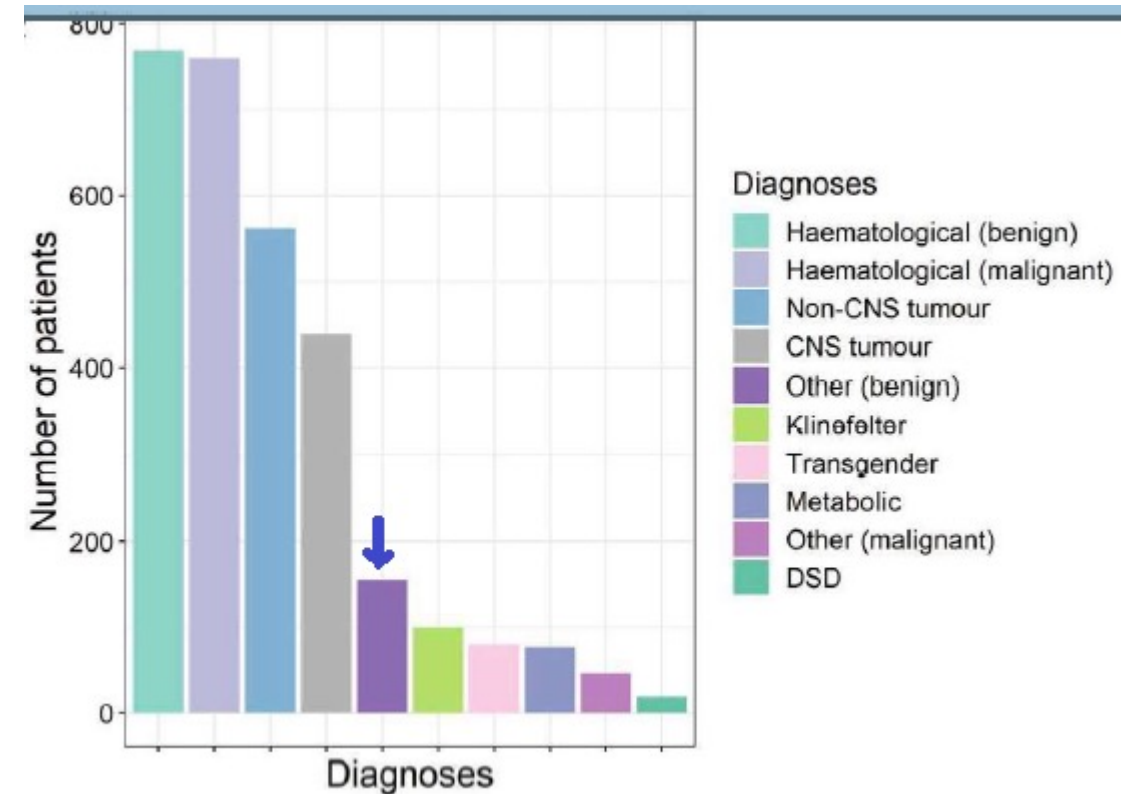
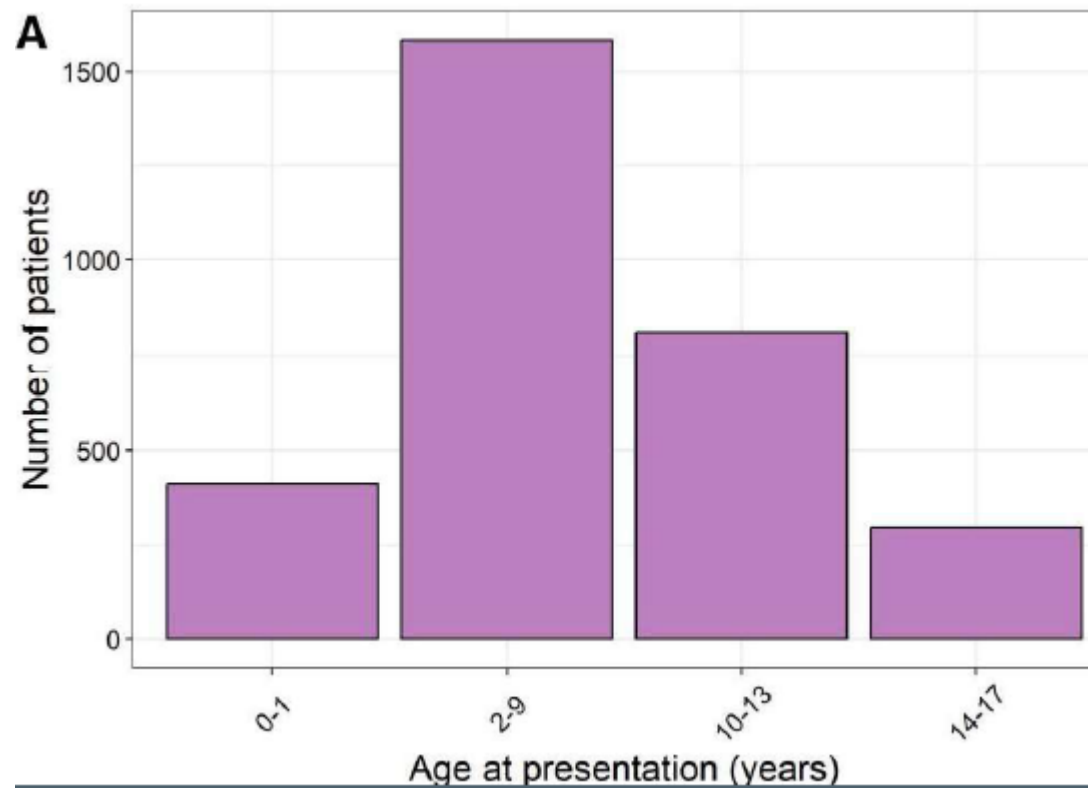
Range: 4 months – 9½ years

At surgery the serum level of inhibin B correlated positively to G/T (Spearman $r = 0.3072$, $p < 0.0001$)



Collected data from the ORCHID-NET consortium 2022

Edinburgh,Scotland; Münster,Germany; Copenhagen,Denmark; Paris,France; Brussels,Belgium; Bizkaia,Spain; Rouen,France; Philadelphia,USA; Melbourne,Australia; Stockholm,Sweden; Helsinki,Finland; Oxford,UK; Amsterdam,Netherlands; Pittsburgh,USA; Winston-Salem,USA; Utrecht,Netherlands; Tel-Aviv,Israel



Conclusion

- Cryopreservation of testicular biopsies may be indicated for selected boys with cryptorchidism although no later beneficial use can be guaranteed
- Indication for discussion:
- Bilateral cases with a germ cell number per tubular cross section below 0.3
- Bilateral and unilateral cases with serum inhibin-B level less than 2½ percentile of normal.
- Other groups ?? Parental request ?? Low EGR4 gene expression ??

Thank you !

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Simone E Hildorf	Danyang Wang
Hans Peter Larsen	Kolja Kvist
Tina E Olsen	Erik Clasen-Linde
Magdalena Fossum	Anne Grete Byskov
Mahboobeh Amoushahi	Jens Fedder
Stine G Kristensen	Christian FS Jensen
Murat Gül	Eva R Hoffmann

Slides for questions

Low-dose every-second-day LHRH treatment following bilateral orchidopexy in children with bilateral cryptorchidism may improve their fertility outcome.

Bogaert G, Vanhoyland M, Hadziselimovic F.
J Pediatr Urol. 2023 Feb;19(1):128.e1-128.e7.

Sperm analysis group	Low doze LH-RH Age at surgery (years)	Sperm concentration (million/ml)	No hormonal treatment Age at surgery (years)	Sperm concentration (million/ml)
Normal	4.8	57.6		
Normal	9.5	31.4	9.3	40.1
Normal	1.6	14.2		
Mild Oligospermia	4.1	10.2		
Mild Oligospermia	2.1	8.5		
Mild Oligospermia	2.0	8.0		
Mild Oligospermia	11.3	5.2		
Oligospermia	2.2	1.8	11.4	0.5
Oligospermia	10.4	1.2	7.3	0.4
Azoospermia	10.8	<0.3	9.2	<0.3
Azoospermia	5.3	<0.3	11.2	0

Association between male genital anomalies and adult male reproductive disorders: a population-based data linkage study spanning more than 40 years

Francisco J Schnever, Elizabeth Milne, Sarra E Jamieson, Gavin Pereira, Michele Hansen, Andrew Barker, Andrew J A Holland, Carol Bower, Natasha Nassar

Summary

Background The male genital anomalies hypospadias and undescended testes have been linked to adult male reproductive disorders, testicular cancer, and decreased fertility. Few population-based studies have evaluated their effects on adult fertility outcomes and, in the case of undescended testes, the importance of early corrective surgery (orchidopexy).

Methods We did a population-based cohort study of all liveborn boys in Western Australia in 1970–99, and followed them up until 2016 via data linkage to registries for hospital admissions, congenital anomalies, cancer, and assisted reproductive technologies (ART). Study factors were hypospadias or undescended testes, and study outcomes were testicular cancer, paternity, and use of ART for male infertility. Cox regression was used to calculate hazard ratios (HRs) for the associations between genital anomalies and testicular cancer or paternity, and log-binomial regression was used to calculate relative risks (RRs) for the associations between genital anomalies and use of ART.

Findings The cohort comprised 350 835 boys, of whom 2484 (0.7%) had been diagnosed with hypospadias and 7499 (2.1%) with undescended testes. There were 505 (0.1%) cases of testicular cancer, 109 471 (31.2%) men had fathered children, and 2682 (0.8%) had undergone fertility treatment with ART. Undescended testes was associated with a more than two times increase in risk of testicular cancer (HR 2.43, 95% CI 1.65–3.58) and hypospadias with an almost 40% increase (1.37, 0.51–3.67), although this increase was not significant. Both hypospadias and undescended testes were associated with a 21% reduction in paternity (adjusted HR 0.79 [95% CI 0.71–0.89] for hypospadias and 0.79 [0.74–0.85] for undescended testes). Undescended testes was associated with a two times increase in use of ART (adjusted RR 2.26, 95% CI 1.58–3.25). For every 6 months' delay in orchidopexy, there was a 6% increase in risk of testicular cancer (HR 1.06, 95% CI 1.03–1.08), a 5% increase in risk of future use of ART (1.05, 1.03–1.08), and a 1% reduction in paternity (RR 0.99, 95% CI 0.98–0.99).

Interpretation Undescended testes is associated with an increased risk of testicular cancer and male infertility, and decreased paternity. We provide new evidence to support current guidelines for orchidopexy before age 18 months to decrease the risk of future testicular cancer and infertility.