Gene Therapy In and Around the Germline

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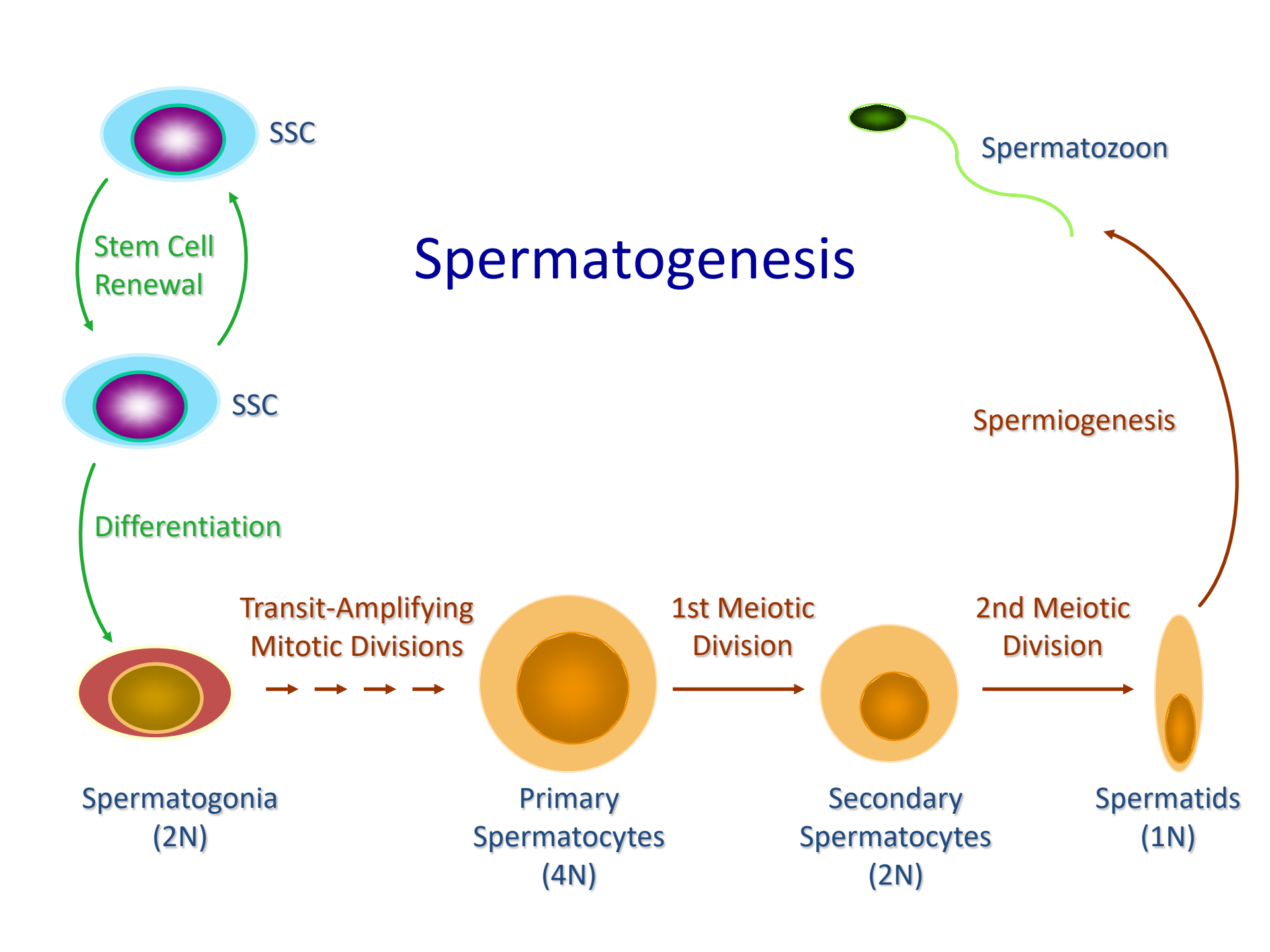
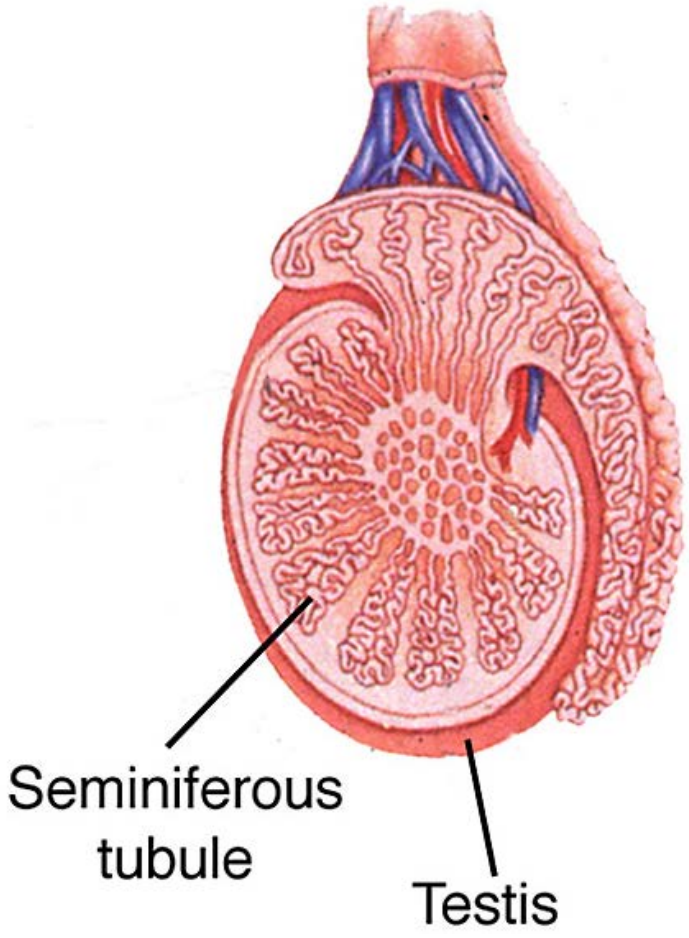
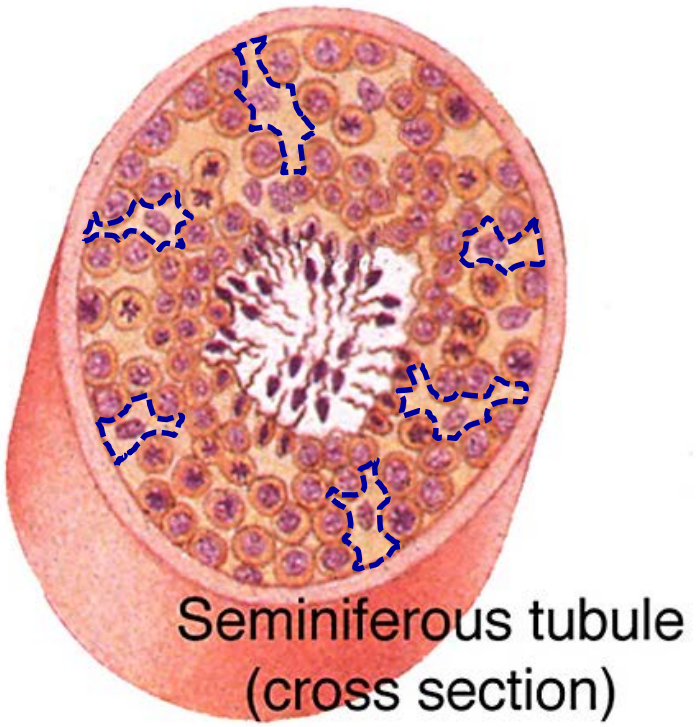
International Summit on Human Gene Editing:
A Global Discussion

December 1-3, 2015

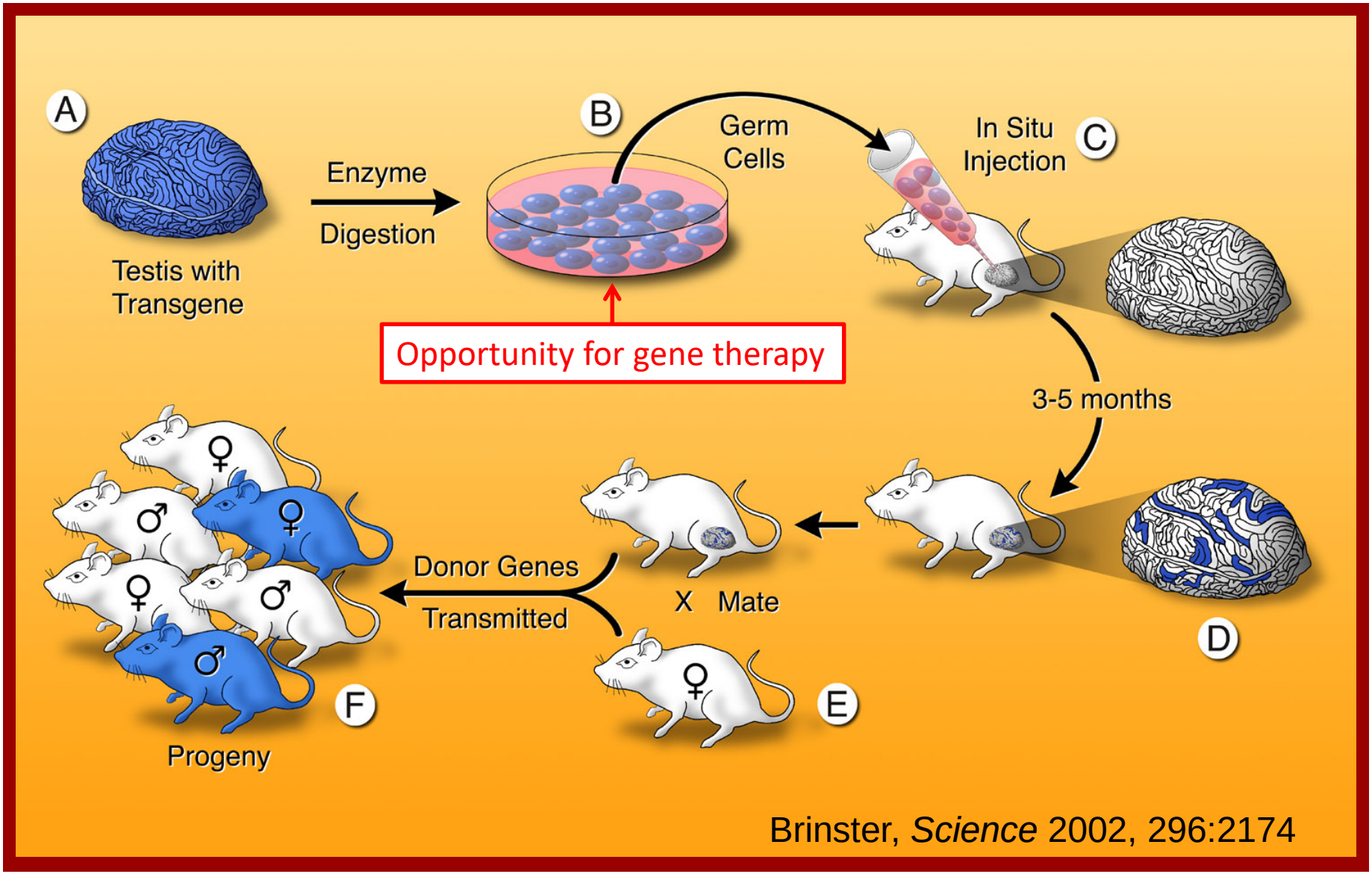

Spermatogenesis
SSC
Stem Cell Renewal
SSC
Differentiation
Spermatogonia (2N)
Transit-Amplifying Mitotic Divisions
Primary Spermatocytes (4N)
1st Meiotic Division
Secondary Spermatocytes (2N)
2nd Meiotic Division
Spermatids (1N)
Spermiogenesis
Spermatozoon

Anatomy of the Testis





Spermatogonial Stem Cell Transplantation



Brinster, *Science* 2002, 296:2174

Ex vivo Gene therapy and Transplantation of Spermatogonial Stem Cells (rat)

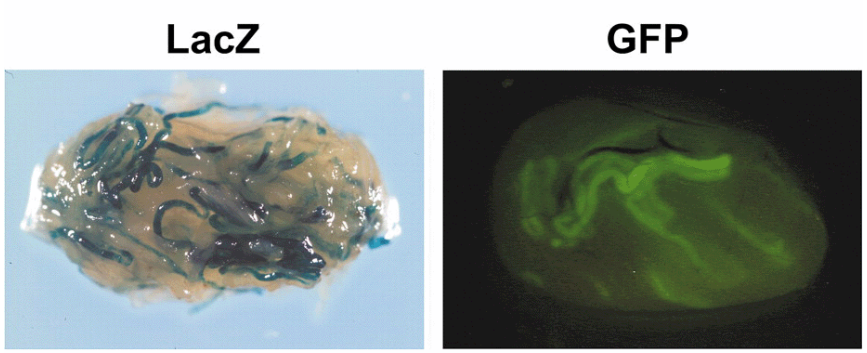
LacZ+ donor testis cells

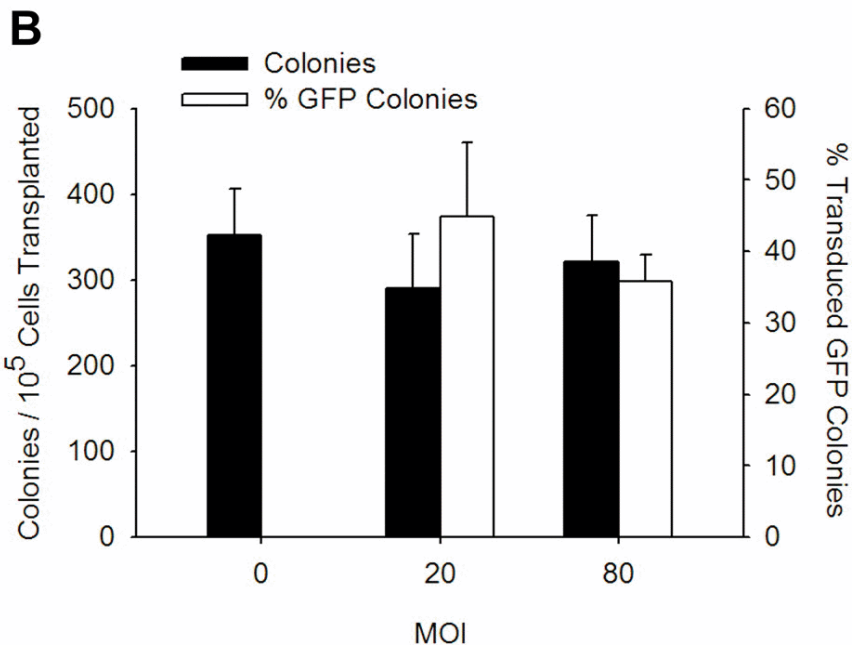
↓

Lentiviral-EF1a-EGFP
Transduction (O/N)

↓

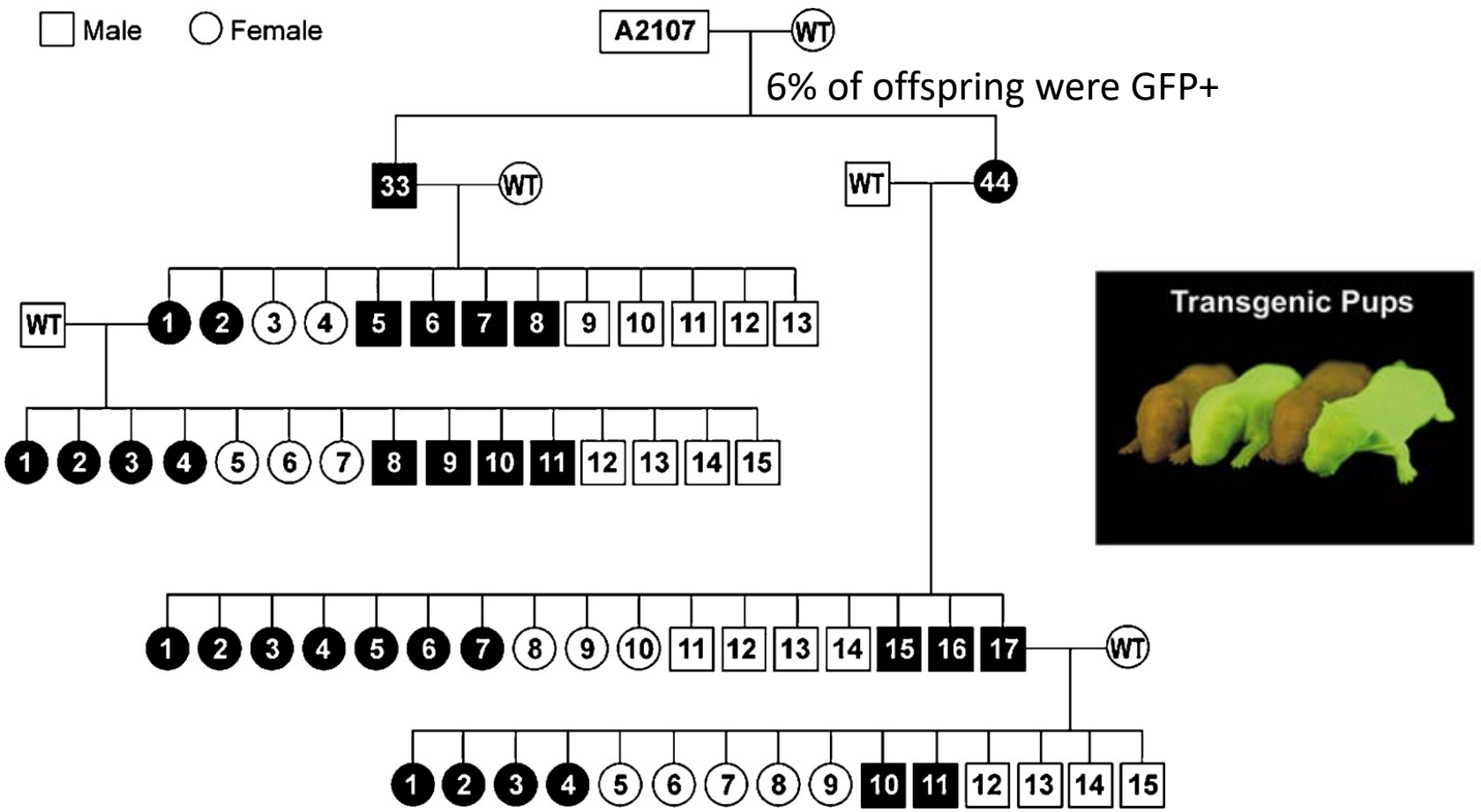
Transplant into the testes
of infertile recipient mice





Ryu et al., *J. Androl.* 2007

Ex vivo Gene therapy and Transplantation of Spermatogonial Stem Cells



Ryu et al., *J. Androl.* 2007

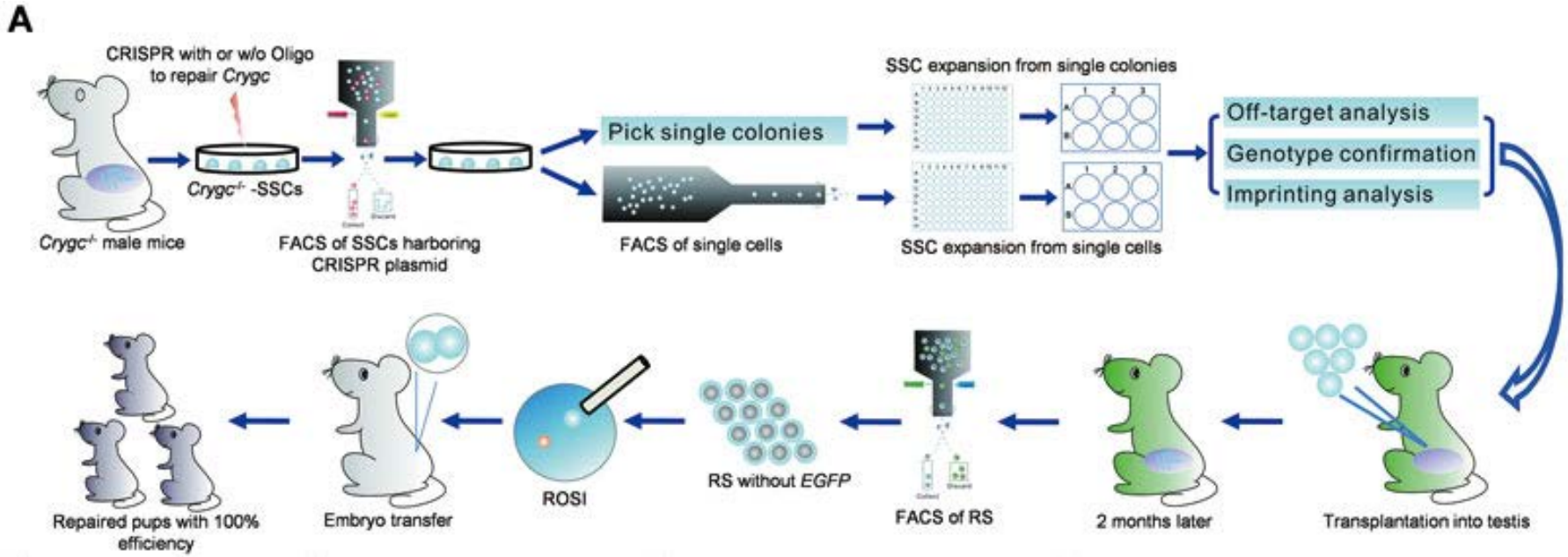
Progress since 2007

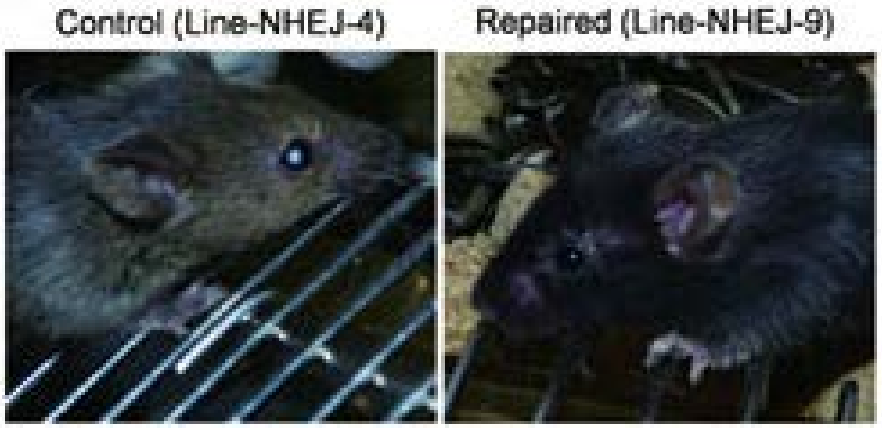
- Long-term culture of rodent SSCs is routine
- Advances in gene editing technologies
  - ZFN, TALENs, CRISPR/Cas9
- Precision gene therapy

Wu et al., *Cell Res* 2015; Chapman et al., *Cell Rep* 2015

Correction of a genetic disease by CRISPR-Cas9-mediated gene editing in mouse spermatogonial stem cells

Wu et al., *Cell Res* 2015



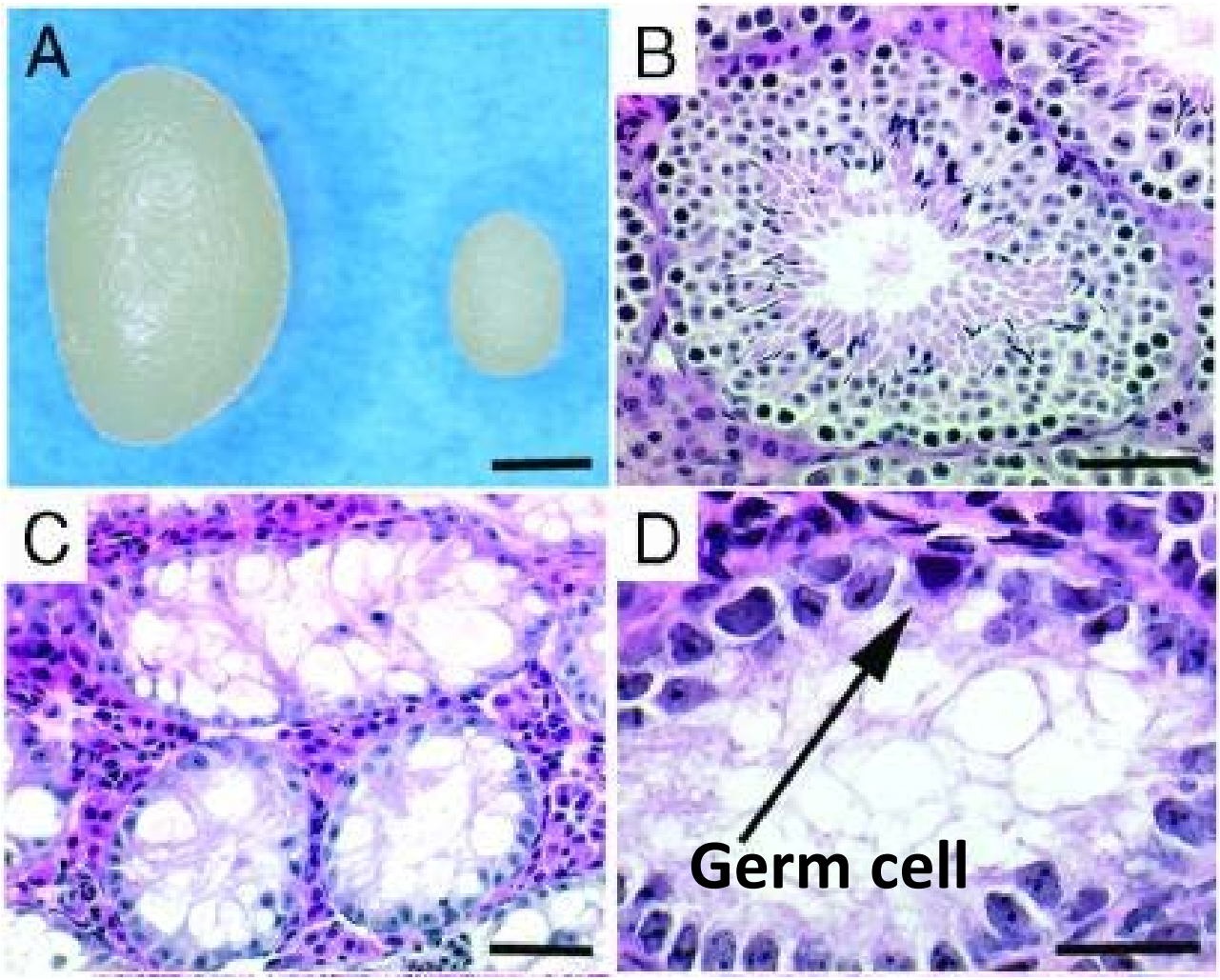


Male Infertility

- 12% of men in the United States are subfertile or infertile
- Half of male infertility is idiopathic
- Definitions:
  - Oligospermia - <15 million sperm/ml of ejaculate
  - Azoospermia – no sperm in the ejaculate
    - Obstructive – plumbing problem
    - Nonobstructive (NOA) – spermatogenesis problem
      - NOA-maturation arrest (350,000 between 20-49)

Nonobstructive Azoospermia-Maturation Arrest

(Steel mice – Sertoli cell defect)



Kanatsu-Shinohara et al., *PNAS* 2002

Adenovirus-mediated gene delivery and *in vitro* microinsemination produce offspring from infertile male mice

Mito Kanatsu-Shinohara*, Atsuo Ogura†, Masaya Ikegawa‡, Kimiko Inoue†, Narumi Ogonuki†, Kei Tashiro‡, Shinya Toyokuni§, Tasuku Honjo*, and Takashi Shinohara*¶

PNAS

Restoration of spermatogenesis by lentiviral gene transfer: Offspring from infertile mice

Masahito Ikawa*, Vinay Tergaonkar*, Atsuo Ogura†, Narumi Ogonuki†, Kimiko Inoue†, and Inder M. Verma*‡

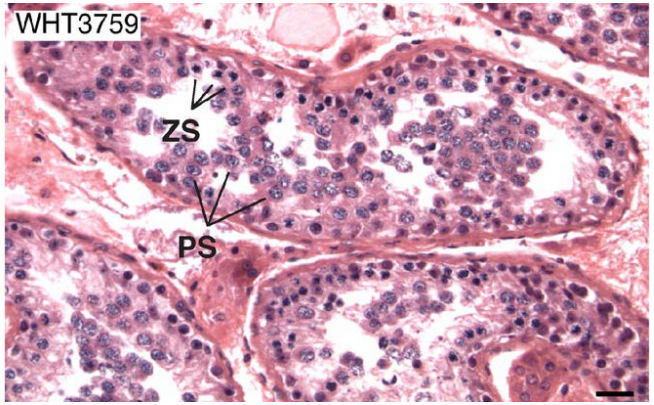
PNAS

- Spermatogenesis restored
- Mice by testicular biopsy and ICSI
- No germline transmission (35 pups)

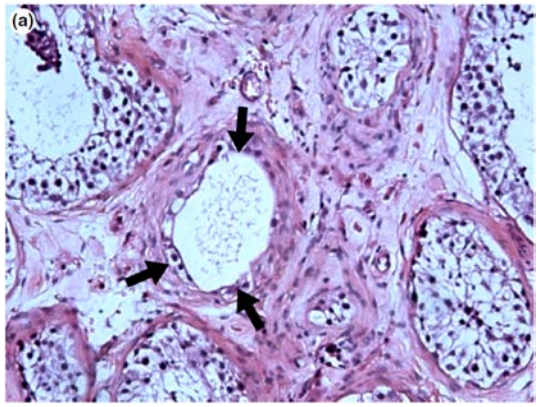
Also by electroporation: Yomogida et al., *Biol Reprod* 2002

Azoospermia-Maturation Arrest

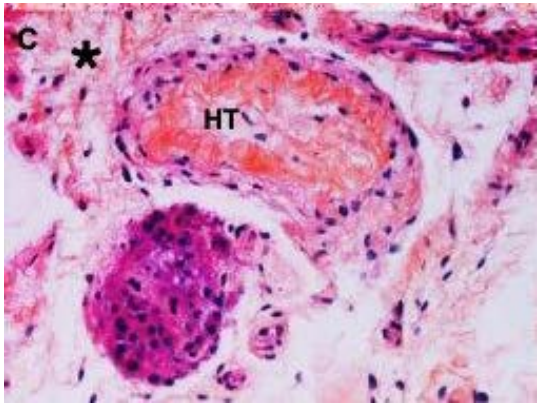
(human)



TEX11
(Germ)



AR
(Somatic)

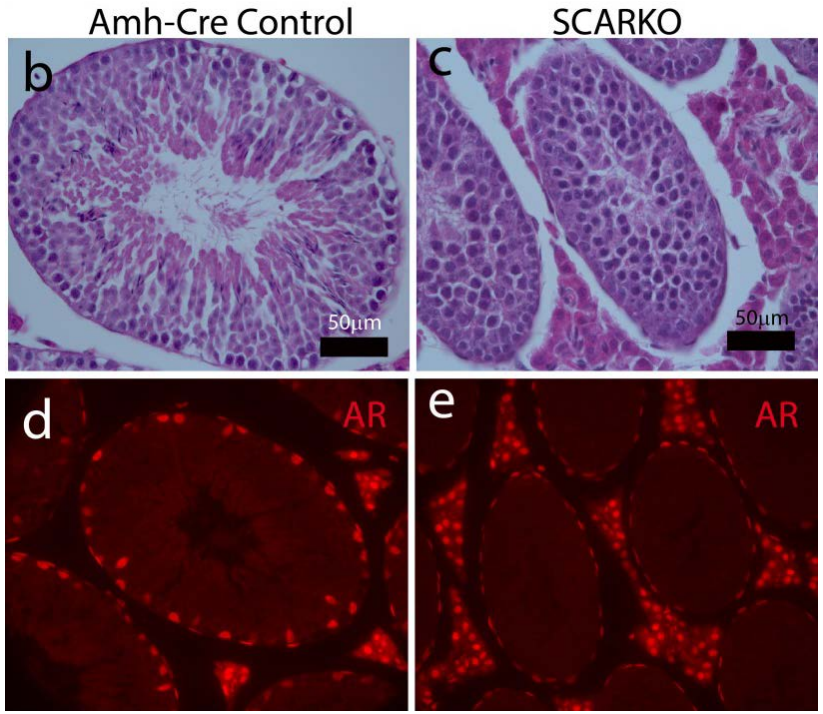


NR5A1
(Somatic)

Yatsenko et al., *J Clin Invest* 2015; Yang et al., *EMBO* 2015; Mirfakhraie et al., *J Androl* 2011; Goglia et al., *Fert Steril* 2011; Massin et al., *Clin Endocrinol* 2012; Chen et al., *As J Androl* 2015

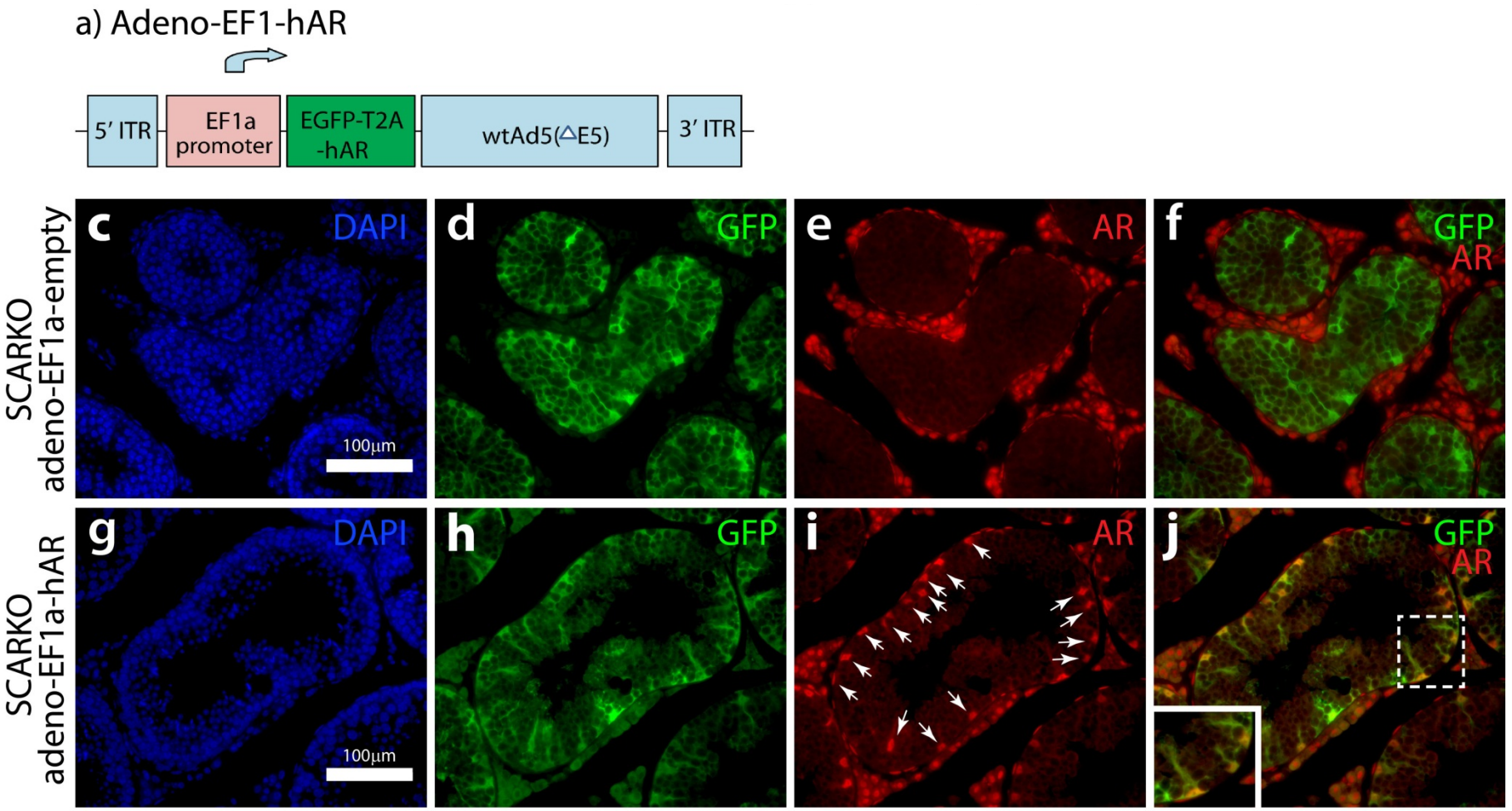
SCARKO Mice

Sertoli Cell Androgen Receptor Knockout



SCARKO mice from: Chakraborty et al., *Mol Endocrinol* 2014

Sertoli Gene Therapy in SCARKO Mice



Tests of Fertility, germline transmission and genomic integration are ongoing

Yatsenko, Walker, Braun and Orwig, Unpublished

Summary/Conclusion

Sertoli cell gene therapy

- *In Vivo* Sertoli gene therapy for male infertility is technically feasible today
  - Whole genome/exome sequencing to identify causative genes
  - It may be possible to correct somatic defects without germline modification
  - Need to map integrations and quantify risk of germline transmission
  - Studies in higher primates are needed

Summary/Conclusion

Germline gene therapy

- Germline gene therapy is technically feasible today
  - *Ex vivo* gene therapy and transplantation of SSCs
- Challenges
  - Stable, long-term human SSCs culture systems still under development – nothing independently replicated yet
  - Precision gene therapy requires culture system to select and expand accurate clones
  - Is clonal selection desirable?
    - Paternal age effect (e.g., FGFR2: Apert; LaminA: Progeria)