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OMICS Group is an amalgamation of Open Access publications and worldwide international science conferences and events. Established in the year 2007 with the sole aim of making the information on Sciences and technology 'Open Access', OMICS Group publishes 500 online open access scholarly journals in all aspects of Science, Engineering, Management and Technology journals. OMICS Group has been instrumental in taking the knowledge on Science & technology to the doorsteps of ordinary men and women. Research Scholars, Students, Libraries, Educational Institutions, Research centers and the industry are main stakeholders that benefitted greatly from this knowledge dissemination. OMICS Group also organizes 500 International conferences annually across the globe, where knowledge transfer takes place through debates, round table discussions, poster presentations, workshops, symposia and exhibitions.

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OMICS International is a pioneer and leading science event organizer, which publishes around 500 open access journals and conducts over 500 Medical, Clinical, Engineering, Life Sciences, Pharma scientific conferences all over the globe annually with the support of more than 1000 scientific associations and 30,000 editorial board members and 3.5 million followers to its credit.

OMICS Group has organized 500 conferences, workshops and national symposiums across the major cities including San Francisco, Las Vegas, San Antonio, Omaha, Orlando, Raleigh, Santa Clara, Chicago, Philadelphia, Baltimore, United Kingdom, Valencia, Dubai, Beijing, Hyderabad, Bengaluru and Mumbai.

.

A comparative transcriptome provides candidate genes for determination the cause of males infertility

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Infertility

- is defined as lack of conception following of frequent unprotected sexual intercourse
during 1 year for age < 35
or 6 months for age > 35

- approximately affects 15% of reproductive couples, although this prevalence intensifies with increasing age

- men and women are equally affected

Causes for male infertility

- ETOH
- drugs
- tobacco
- health problems
- radiation and chemotheraphy
- enviromental factors
  - pesticides or lead
  - high temperature

Most of male infertility cases are due to :

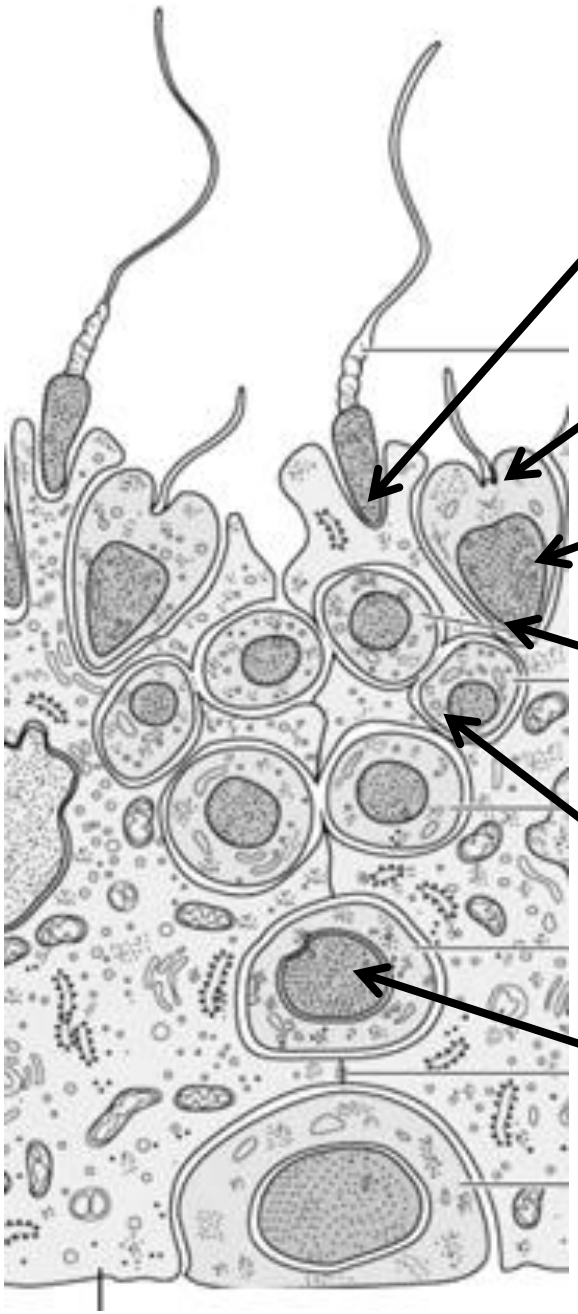
- low sperm counts
- poor sperm quality
- or both

The remaining cases of male infertility can be caused:

- anatomical problems
- hormonal imbalances
- genetic defects

Idiopathic 30-40% of cases

European Association of Urology 2015



(Croxford et al.,2011)

Acrosin prepropeptide (*Acr*)

proteolysis of the zona pellucida during fertilization

(Adham *et al*., 1997; Honda *et al*., 2002)

Testis express protein 22 (*Tex22*)

biogenesis of the acrosome and midpiece of the sperm tail

(Neesen *et al*., 2002)

Transition protein 2 (*Tnp2*)

replacement of histones and chromatine condensation

(Reinhart *et al*., 1991)

cAMP responsive element binding protein 3-like 4 (*Creb3l4*)

transcription factor active in respond to stress conditions

(Adham *et al*., 2005)

Testicular haploid expressed gene (*Theg*)

upregulted by some factors from Sertoli cells

(Mannan *et al*., 2000)

Histone cluster 1 (*Hist1h1t*)

replacement of histones H1.1 and H1.2

(Meistrich *et al*., 1985; Drabent *et al*., 1993/1996)

Determination of infertility causes in mutant mouse line with the deletion of six germ cell-specific genes

All six genes are expressed exclusively in male germ cells

All single knockout males were fertile

fertile 1 - KO

Moreover:

fertile	2 - KO	Acr/Tnp2
fertile	3 - KO	Acr/Tnp2/Hist1h1t
fertile	4 - KO	Acr/Tnp2/Hist1h1t/Theg
fertile	5 - KO	Acr/Tnp2/Hist1h1t/Theg/Creb3l4
fertile/infertile	6 - KO	Acr/Tnp2/Hist1h1t/Theg/Creb3l4/Tep22

fertile 4 - KO Acr/Tnp2/Hist1h1t/Tep22

Determination of infertility causes in mutant mouse line with the deletion of six germ cell-specific genes

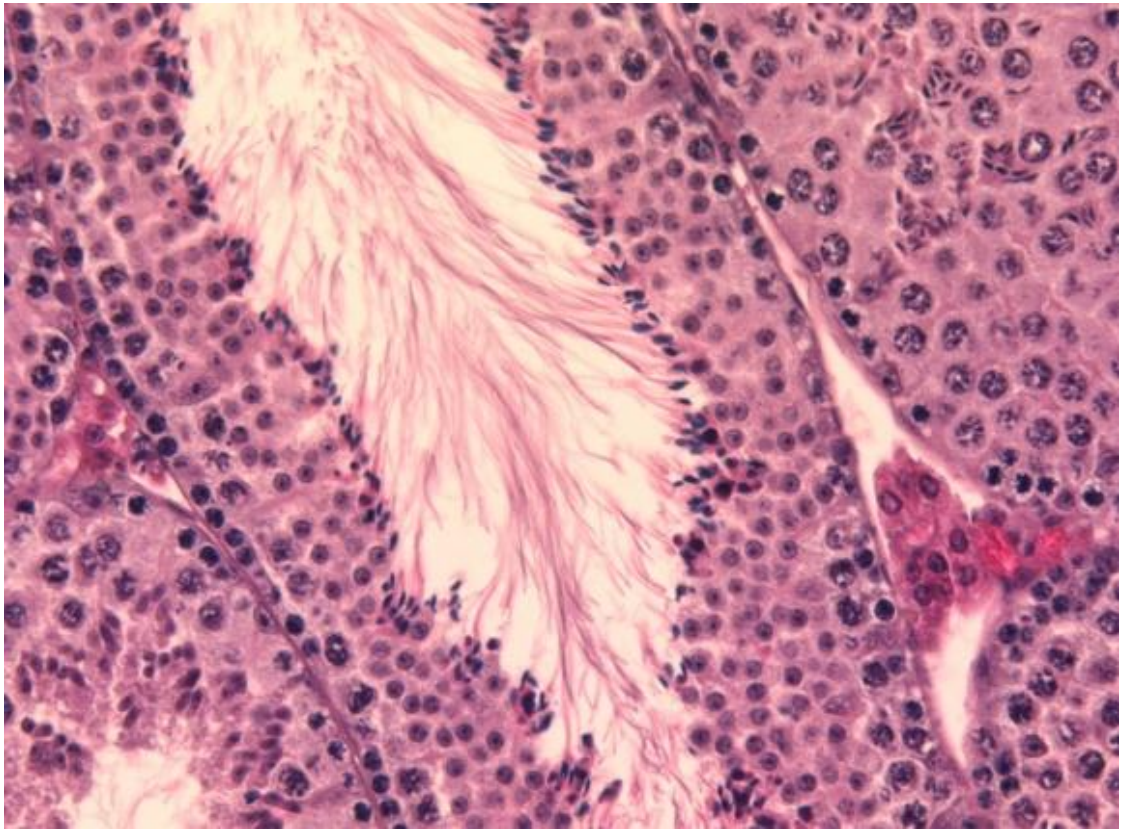
- the analysis of the phenotype of 6xKO males

- determination of infertility causes

- identification and analysis of new gene which might be important for fertility

The phenotype of 6 times mouse mutants

About 20% are infertile



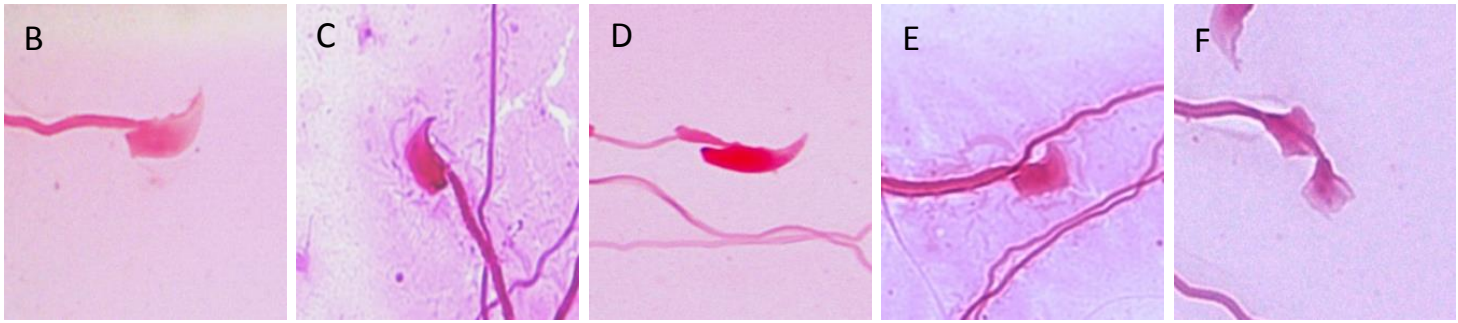
Histology of testis (hematoxyline – eosine staining) from adult 6xKO infertile male - **all stages of spermatogenesis** are present

The phenotype of 6 times mouse mutants

Serum testosterone level

The total sperm number of 6xKO infertile are similar to wild type control

6xKO males have reduced sperm speed, acrosome reaction and incresed number of sperm with abnormal head



Transcriptome assay

clusters of co-regulated genes

clusters of genes involved in biological processes

clusters of genes expressed in the same organelle

- 2700 of novel transcripts were identified in purified mouse germ cells

- cytochrome c oxidase regulate apoptosis in germ cells

- NF-kappaB (nuclear factor kappaBetta), SP1 (trans-acting transcription factor1), AP-1 (activator protein 1), EGR (nerve growth factor) were identified as transcription regulators in spermatogenesis

Gene Chip Mouse
Gene 1.0 ST Array
(Affymetrix)

Comparison of the transcriptome of 5- and 6-times knockout

From 20986 tested genes:

55 genes are down- or up-regulated

41 genes have known expression in testis

↓

4 are testis specific

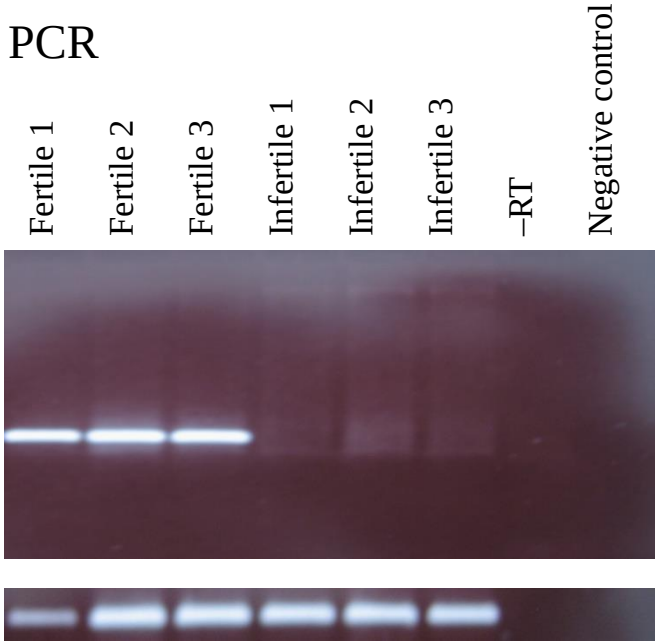
4 are unknown genes

4933400A11Rik

10 have known expression in other organs than testis, but for most of them the expression in testis was never tested

4933400A11Rik gene

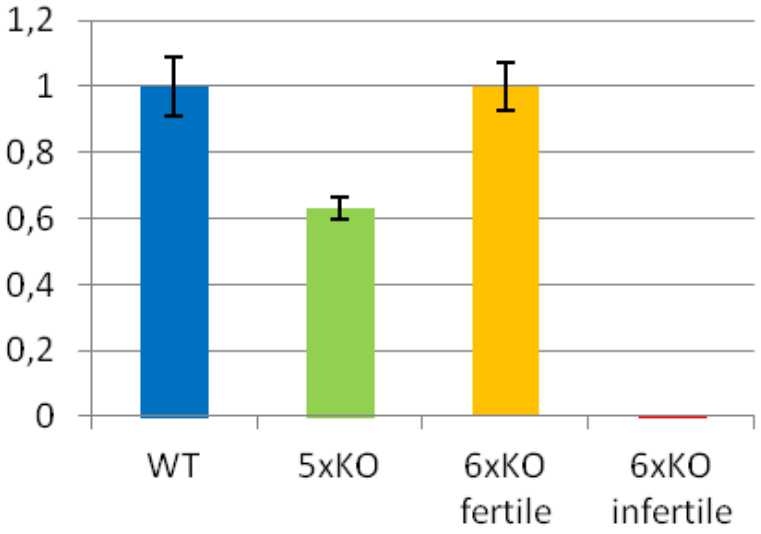
PCR



165 bp
4933400A11Rik

222 bp Hprt

qPCR

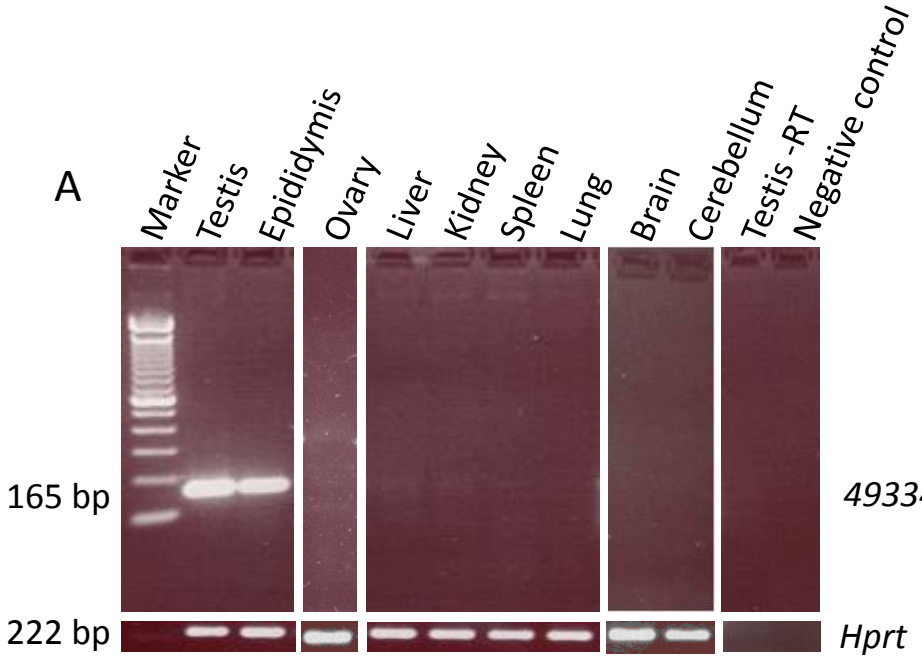


4933400A11Rik is expressed in testis of fertile 6xKO males, but is not expressed in infertile mouse

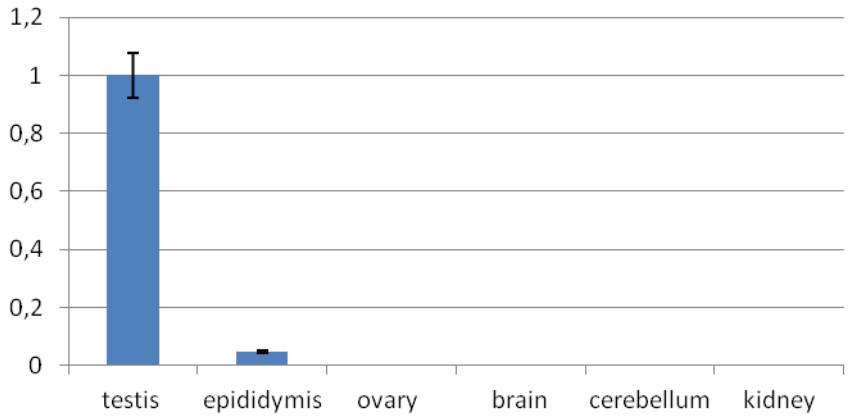
4933400A11Rik

PCR

A

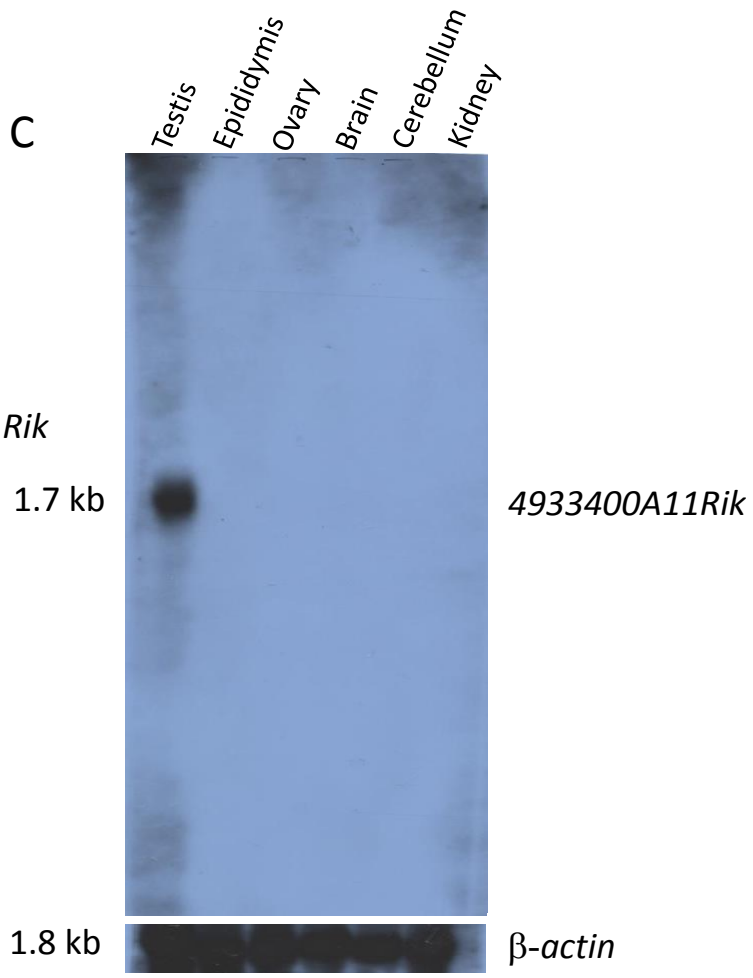


qPCR

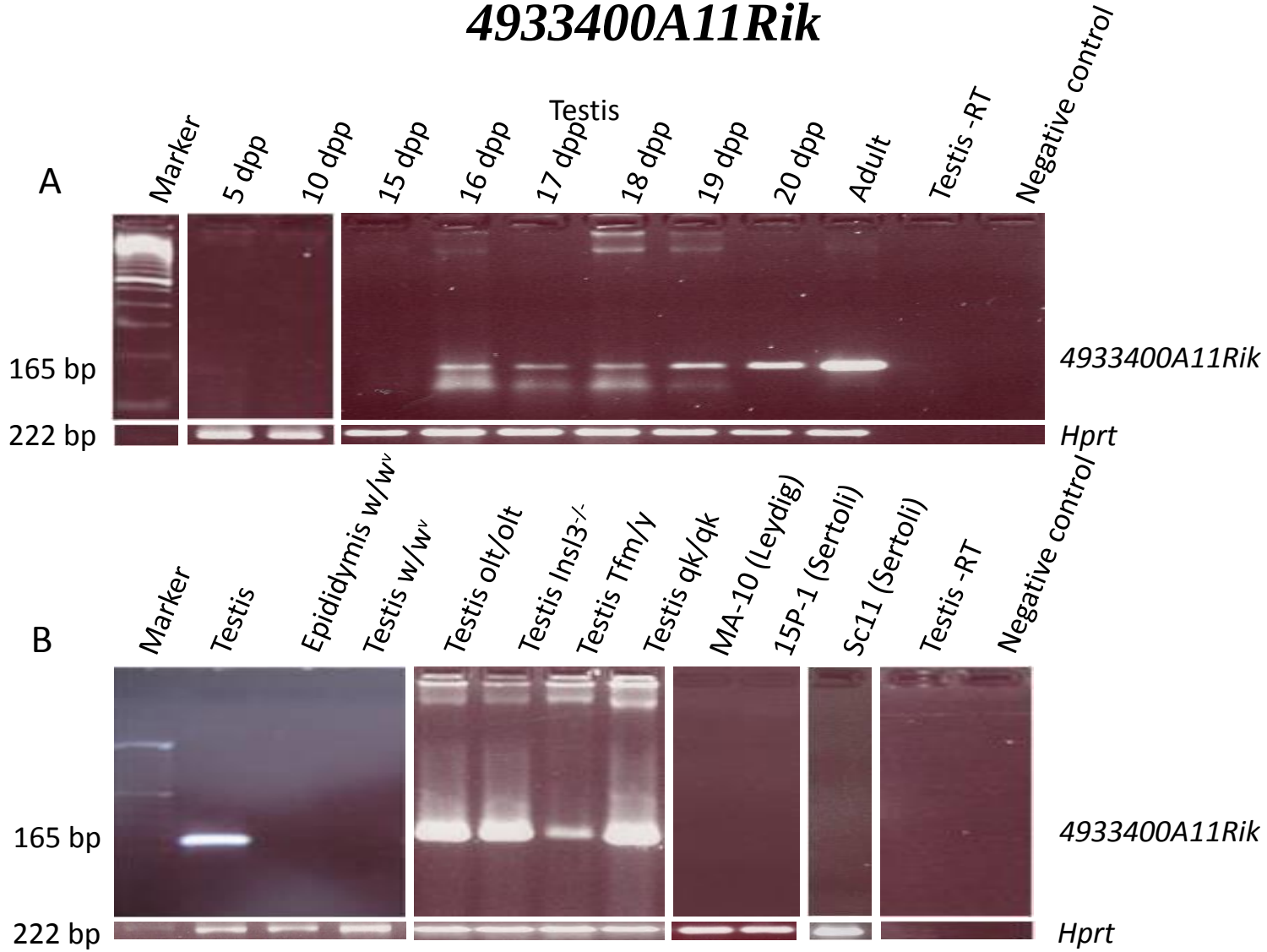


Northern blot

C

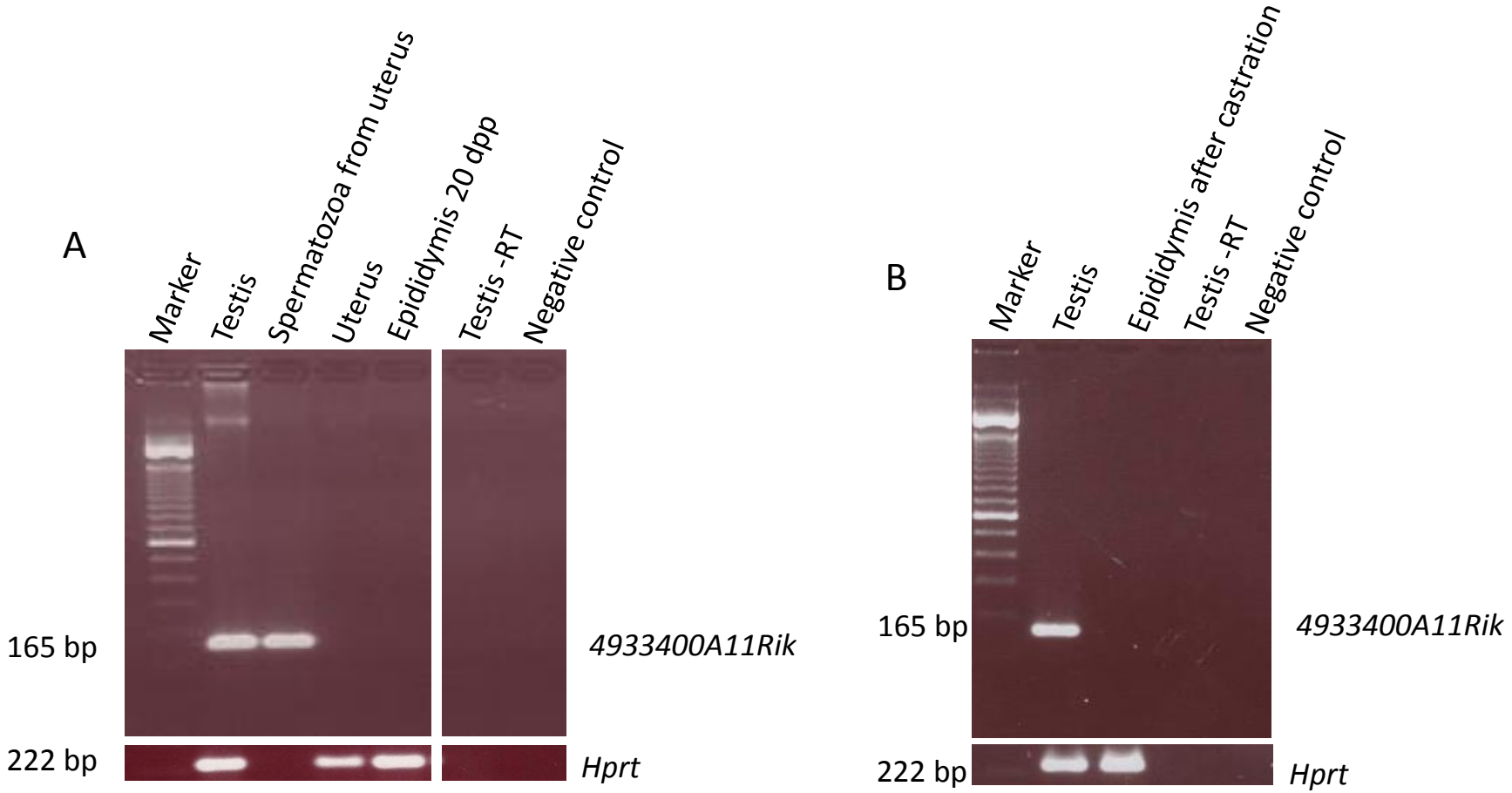


4933400A11Rik



4933400A11Rik is expressed in germ cells

4933400A11Rik



4933400A11Rik RNA seems to be stored in spermatozoa

Examples from literature:

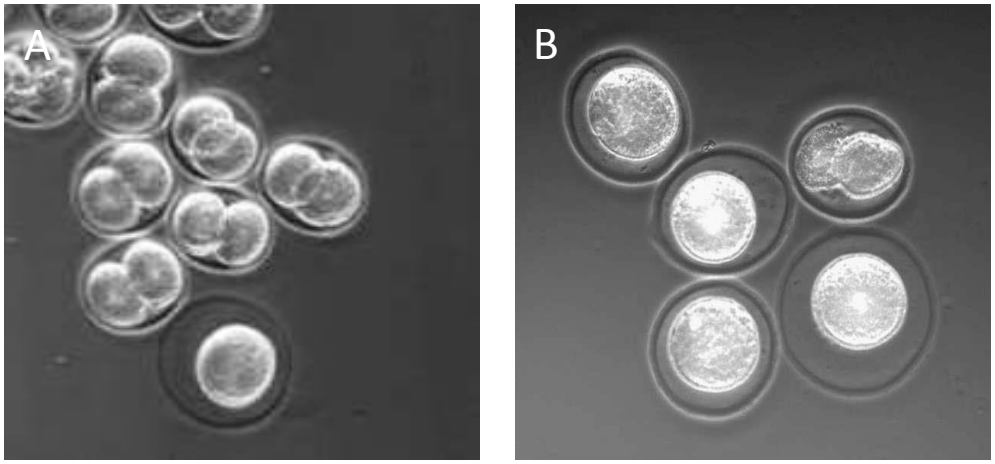
- mRNAs might play certain roles in fertilization and embryonic development (Ostermeier *et al*.)

- The organization of DNA, pronuclear formation, oocyte activation and the establishment of imprinting in early embryos (Ostermeier *et al*.; Boerke *et al*.,

- 15 iRNA specifically inhibit genes which are active exclusively during early embryonic development (Boerke *et al*.)

Analysis of fertilization and developing of fertilized oocytes

2-cell stage



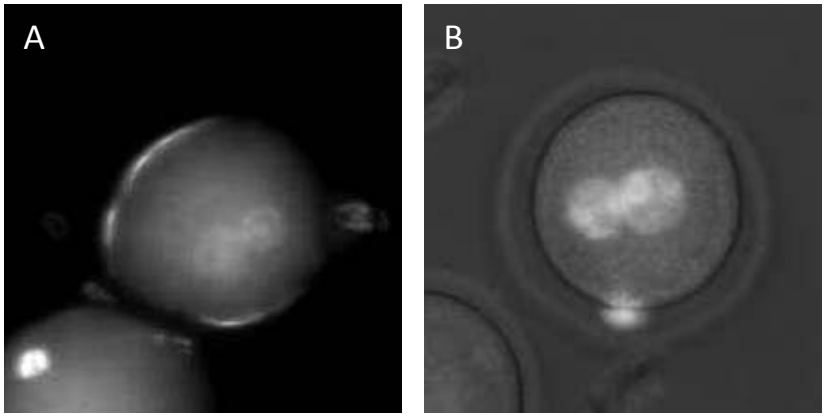
fertile 6xKO
35 %

infertile 6xKO
0 %

Infertile 6xKO didn’t develop to 2-cell stage

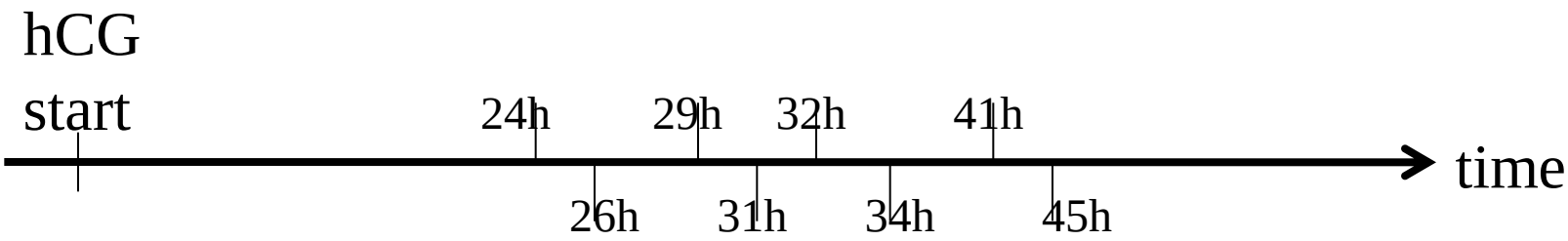
Analysis of fertilization and developing of fertilized oocytes

2 pronuclei stage



WT
21 %

fertile 6xKO
3 %



Process of developing is every time different and it depends on time of fertilization, which takes place during 48 h after breeding

In Vitro fertilization

To answer if it is problem during fertilization we have done IVF with complete oocyte and with oocyte without zona pellucida

	IVF - oocyte with zona pellucida						
	1-cell stage	%	2-cell stage	%	degenerated	%	total
WT	14	17.3	41	50.6	26	32.1	81
6xKO fertile	102	65.0	14	8.9	41	26.1	157

In fertile 6xKO group percent of 2 cell stage is reduced in comparison to wild type

IVF without zona pellucida

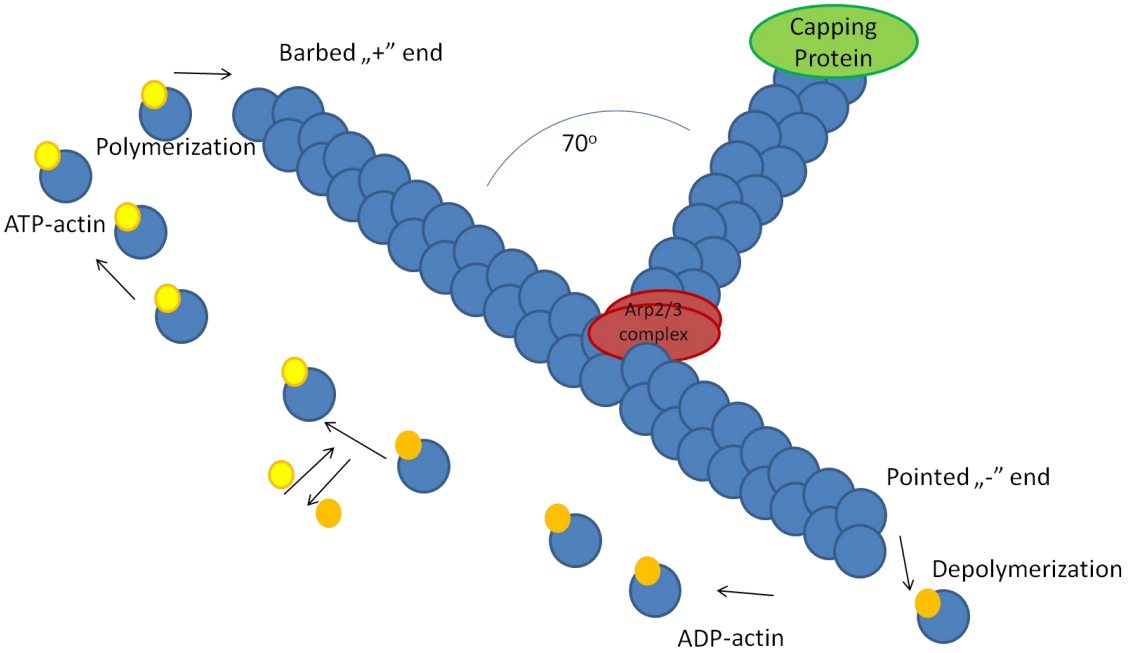
	IVF zona pellucida free						
	2 pronuclei	%	1 cell stage	%	degradeted	%	total
WT	60	66,7	10	11,1	20	22,2	90
6xKO fertile	27	56,3	18	37,5	3	6,3	48
6xKO infertile	15	65,2	4	17,4	4	17,4	23

According to all IVF experiments and the phenotype it seems probable that sperm of 6xKO infertile males are not able to penetrate ZP intact oocytes

4933400A11Rik

- *4933400A11Rik* encodes a protein similar to capping protein alpha subunit family

- members of this protein family regulate growth of the actin filament by capping the barbed end of growing actin filaments



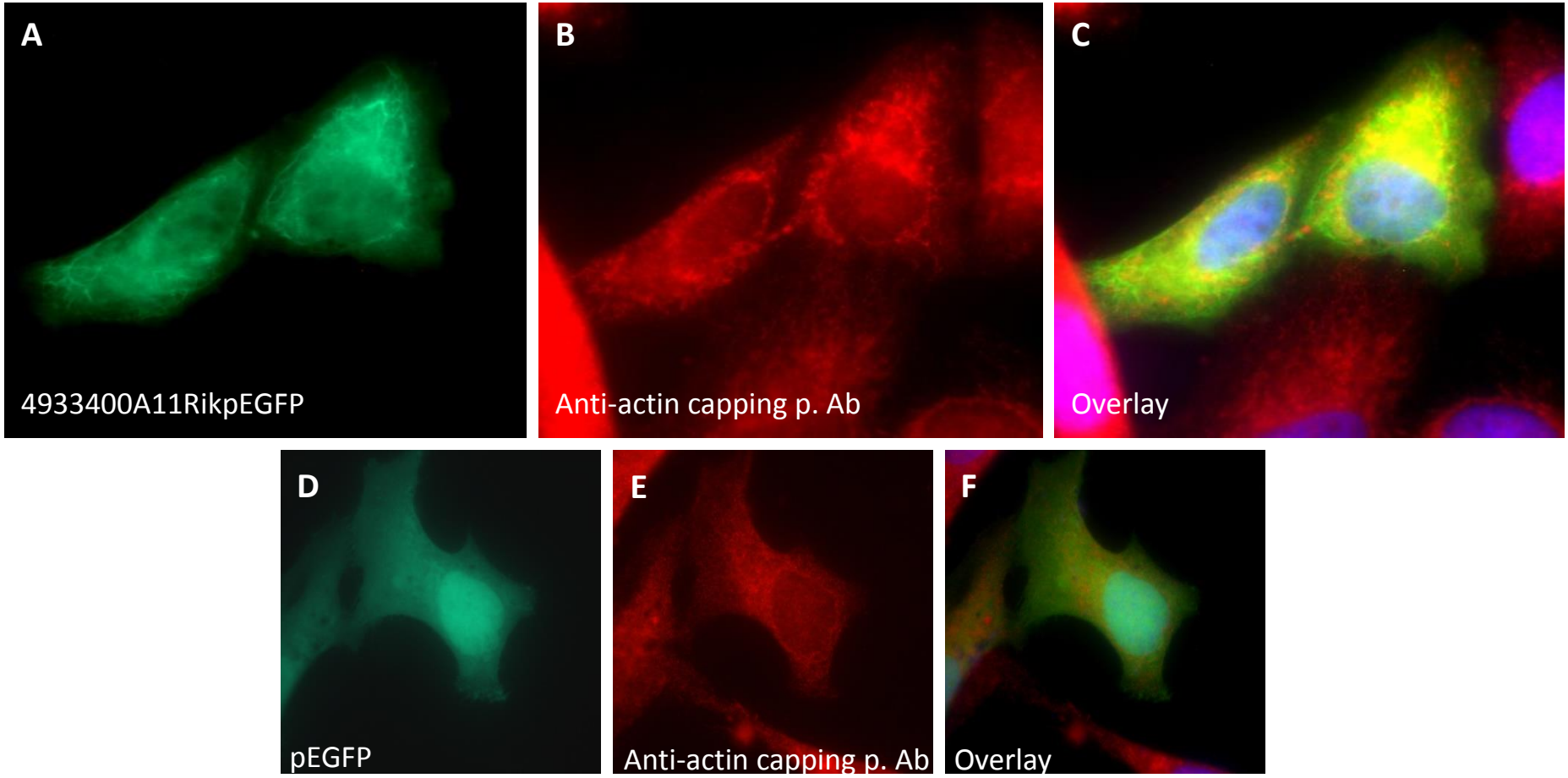
(Schafer, 2004; Nicholson-Dykstra *et al*., 2005; Lowery and van Vactor, 2009; Kim *et al*., 2010; modified).

Actin:

- changes in cell shape
- cell divisions
- depolymerised to allow the activation of the outer acrosomal membrane
- preventing the sperm DNA from incorporation into oocytes cytoplasm
- testicular sperm maturation and can block sperm motility

Co-localization of 4933400A11RikpEGFP with actin capping protein subunit alpha-1

HeLa cells



4933400A11Rik protein is new capping protein

Conclusions
What might be reason of infertility?

We suggest that disregulation of germ cells actin cytoskeleton reorganisation may be the underlying cause of male infertility in 6xKO mice

- gene *4933400A11Rik* is not expressed in infertile 6xKO males

- increase number of abnormal head

- reduced of acrosome reacted sperm

- reduced speed of sperm

- problems with fertilisation

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Thank you for your attention

Conclusions
Why only about 20 % of 6xKO males are infertile?

It might be effect of the mixed genetic background
CD-1 × C57Bl/6J × 129/Sv

0.1 % of base pair is different between mouse line = 2000 genes

Different effect of genetic background on gene expression

ob-/- and *db* -/- :	B6	→ obesity and transient diabetes
	C57BLKS	→ obesity and overt diabetes

(Coleman, 1973/1978)

Tnp2-/-:	129Sv	→ males are infertile
	C57BL/6Jx129/Sv	→ males are fertile

(Adham *et al*., 2001)

Future perspective

Characterization of the gene encoding 4933400A11RIK:

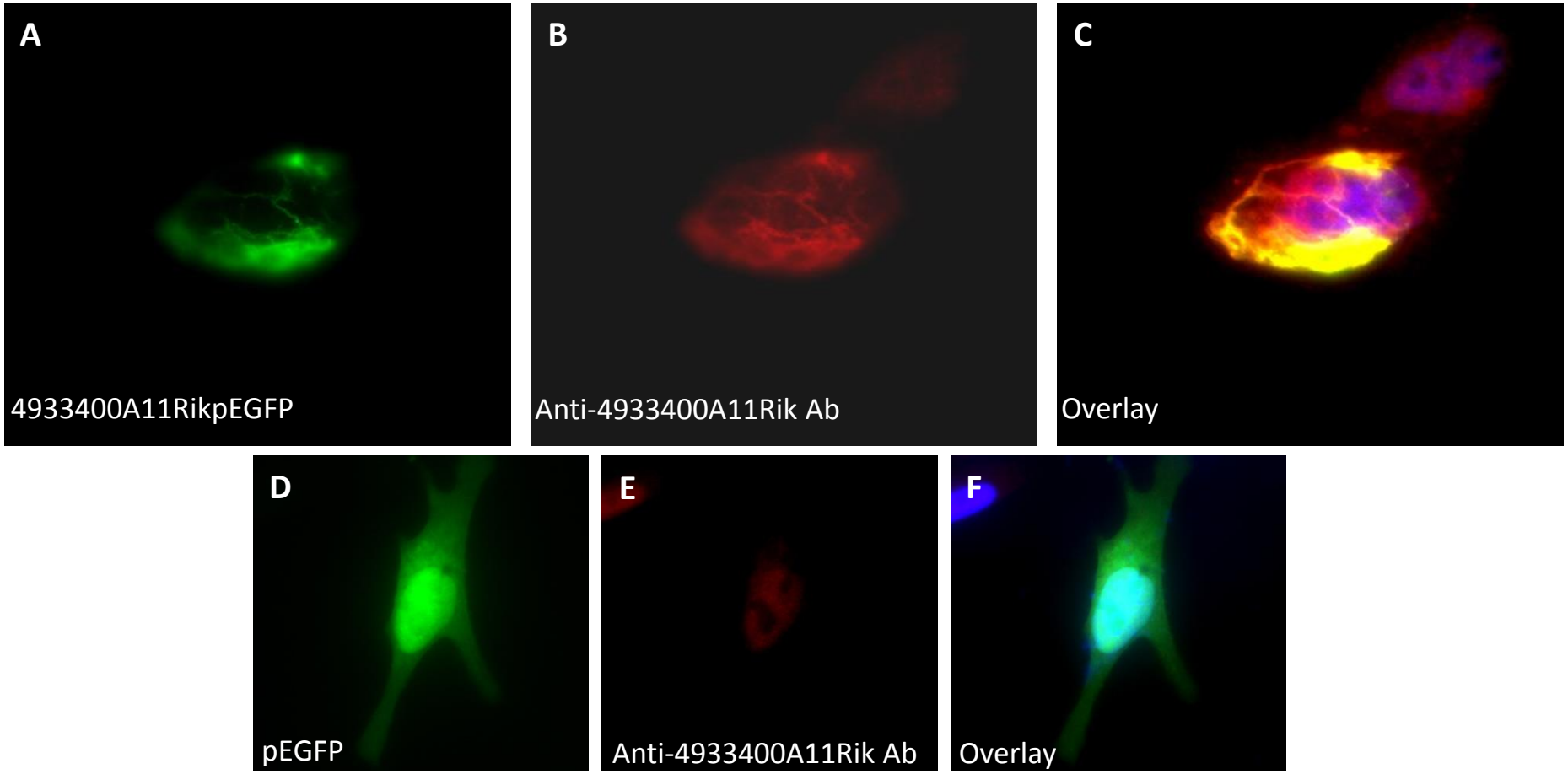
- determination the role for spermatogenesis

- generation of conditional knockout mice

- rescue experiment will give answer to question wheter this mRNA is necessary for the early embryonic development

Co-localization of 4933400A11RikpEGFP with anti-4933400A11Rik antibody

HeLa cells

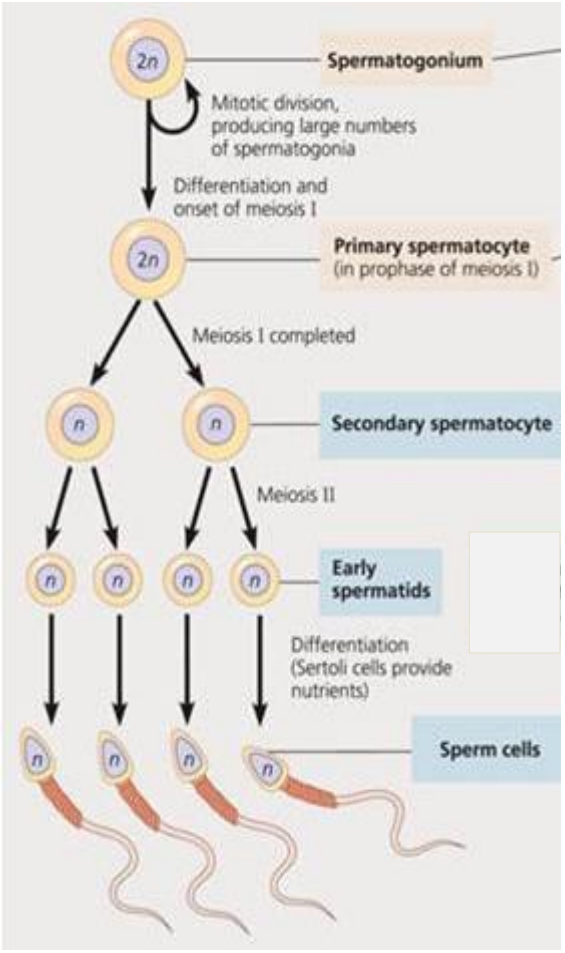


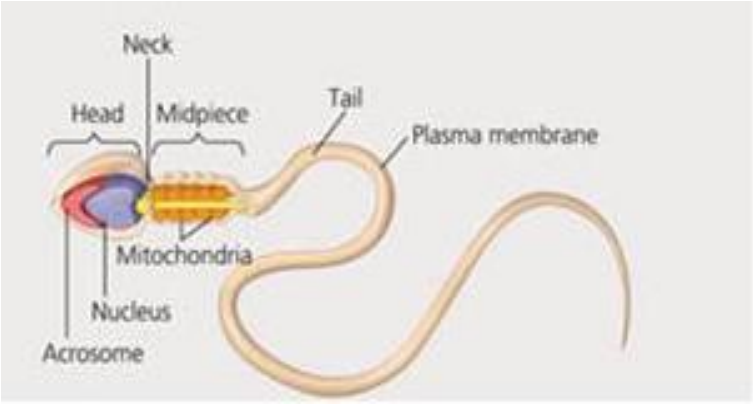
4933400A11Rik protein is localised in cytoplasm

Histone cluster 1 (*Hist1h1t*)
replaces in male germ cells somatic linker histones H1.1 and H1.2 during the meiotic prophase

Testicular haploid expressed gene (*Theg*)
in spermatids, upregulted by some factors from Sertoli cells

Testis express protein 22 (*Tex22*)
biogenesis of the acrosome and midpiece of the sperm tail

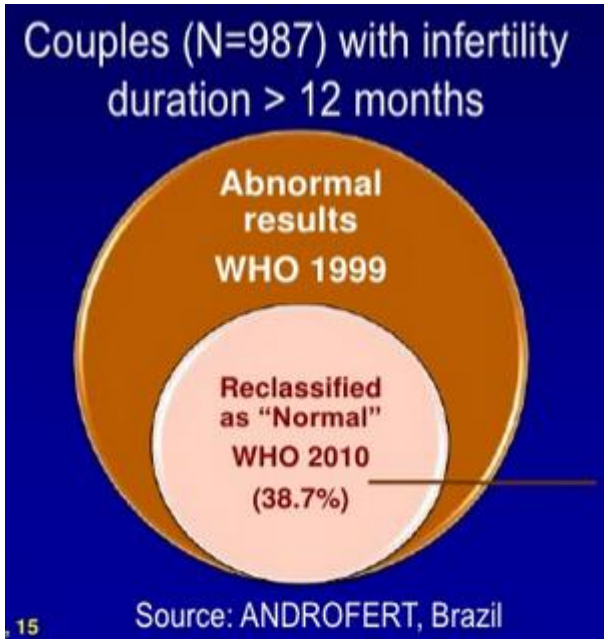




Transition protein 2 (*Tnp2*)
replacement of histones and chromatine condensation in elongated spermatids

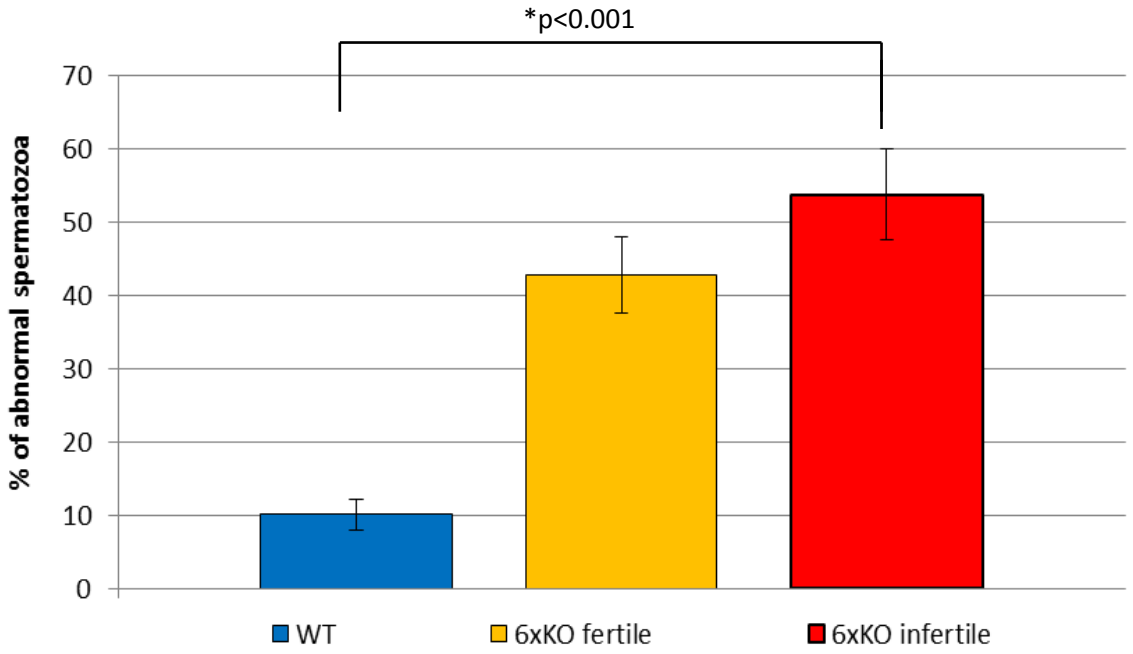
cAMP responsive element binding protein 3-like 4 (*Creb3l4*)
CREB/ATF family transcription factors, especially active in respond to a variety of stress conditions

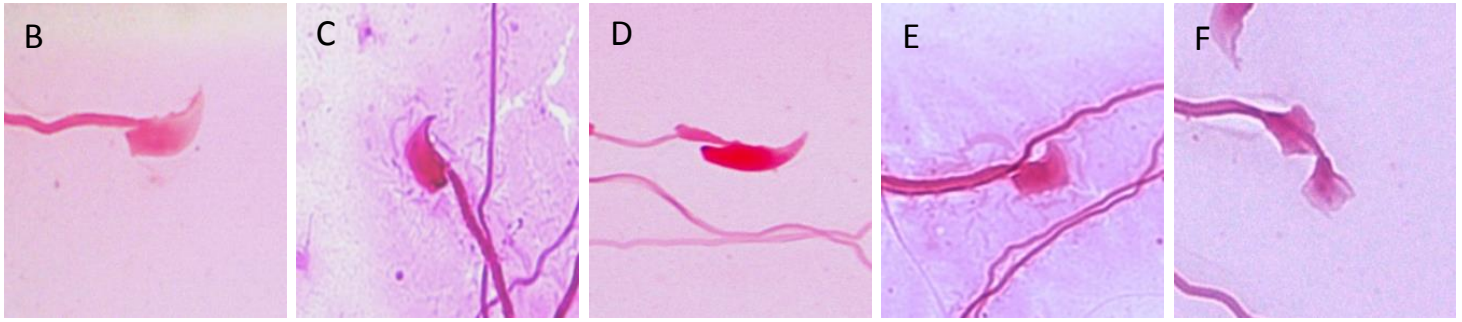
Acrosin prepropeptide (*Acr*)
proteolysis of the zona pellucida of the oocyte


Couples (N=987) with infertility duration > 12 months
Abnormal results
WHO 1999
Reclassified as "Normal"
WHO 2010
(38.7%)
Source: ANDROFERT, Brazil
15

The phenotype of 6 times mouse mutants

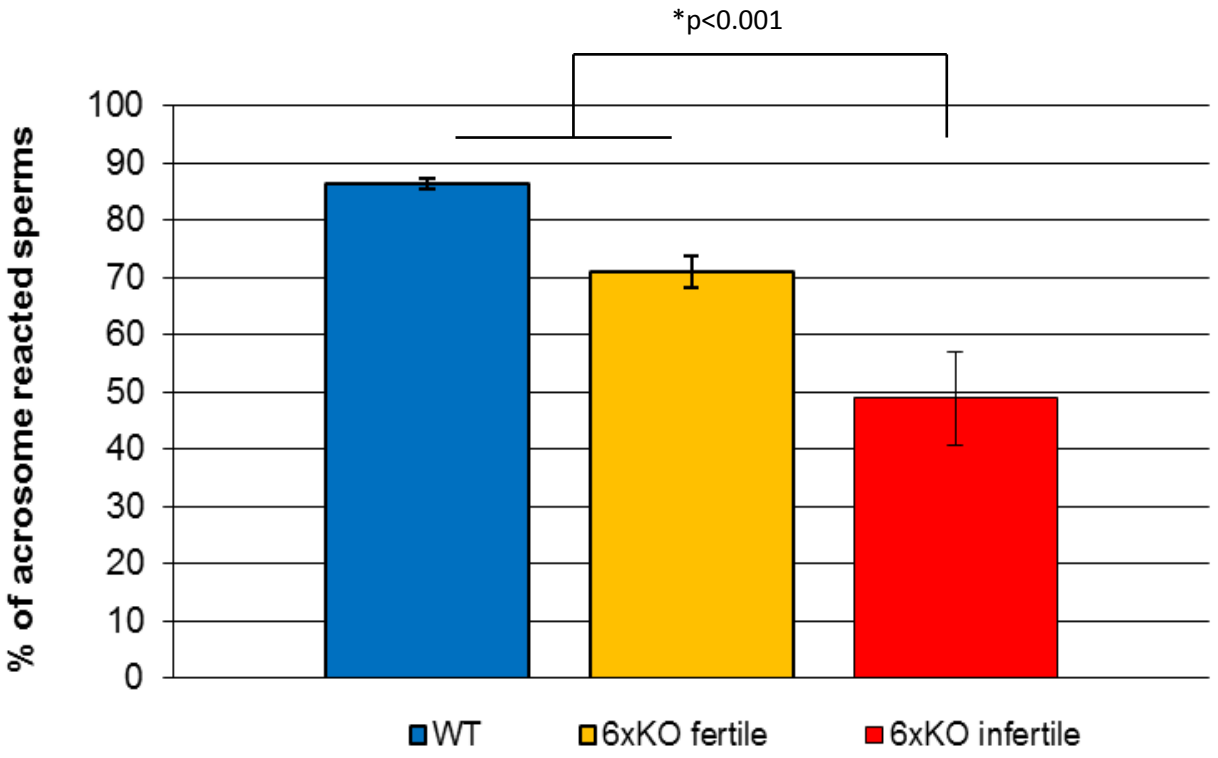
Abnormal head - in infertile 6xKO mice about 55% of spermatozoa have abnormal head





The phenotype of 6 times mouse mutants

Acrosome reaction was significantly reduced in 6xKO infertile



6xKO males have reduced sperm speed, acrosome reaction and incresed number of sperm with abnormal head

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