Interbull Industry Meeting
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Identification and Management of Deleterious Mutations in Fleckvieh

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Deleterious mutations in Fleckvieh

(recessive inheritance, causal mutation known)

- Identified *via* *phenotype-driven* screens
  - Arachnomelia (Buitkamp et al., 2011)
  - Thrombopathia (Jansen et al., 2013)
  - Bovine male subfertility (Pausch et al., 2014)
  - Zinc deficiency-like syndrome (Jung et al., 2014)
  - Dwarfism (2013)

- Identified *via* *genotype-driven* screens
  - Fleckvieh Haplotype 2 (Pausch et al., 2015)
  - Fleckvieh Haplotype 4 (Pausch et al., 2015)
  - Braunvieh Haplotype 2 (2014)

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Recessive phenotypes in Fleckvieh

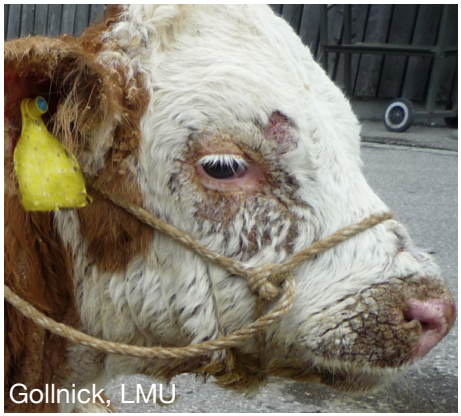
Buitkamp, LfL

Arachnomelia

Causal gene: *MOCS1*

Causal mutation: fs457X

Buitkamp et al., BMC Genetics 2011, 12:11


Gollnick, LMU

Zinc deficiency-like syndrome

Causal gene: *PLD4*

Causal mutation: p.Trp215X

Jung et al., *BMC Genomics 2014, 15:623*


Pfitzner, LMU

Thrombopathia

Causal gene: *RASGRP2*

Causal mutation: p.Leu234Pro

Boudreaux et al., Vet Pathol. 2007; 44(6):932-5

Jansen et al., BMC Genomics 2013,14:446


Schwarzenbacher,ZuchtData

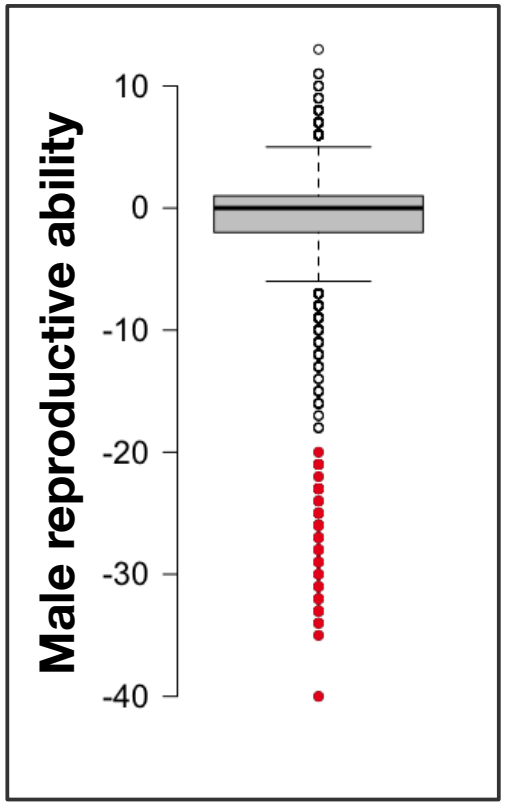
Dwarfism

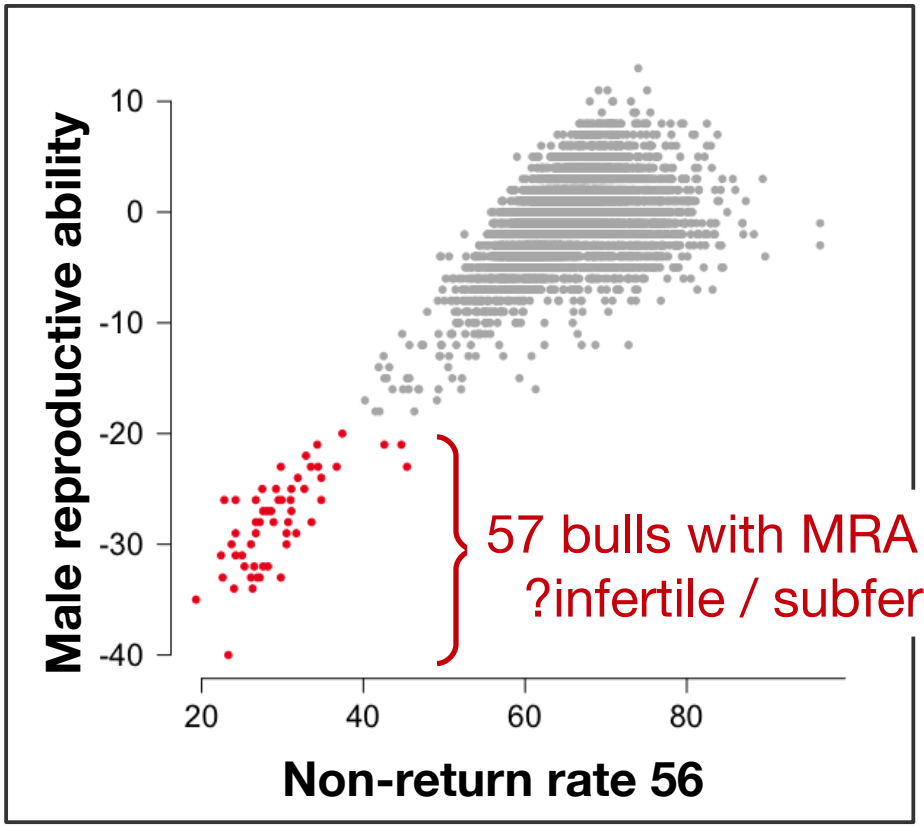
Causal gene: *XXXX*

Causal mutation: fsX

Bovine male subfertility

- Male reproductive ability (*a.k.a. sire conception rate)*
  - proxy for insemination success per bull
  - highly correlated with the non-return rate



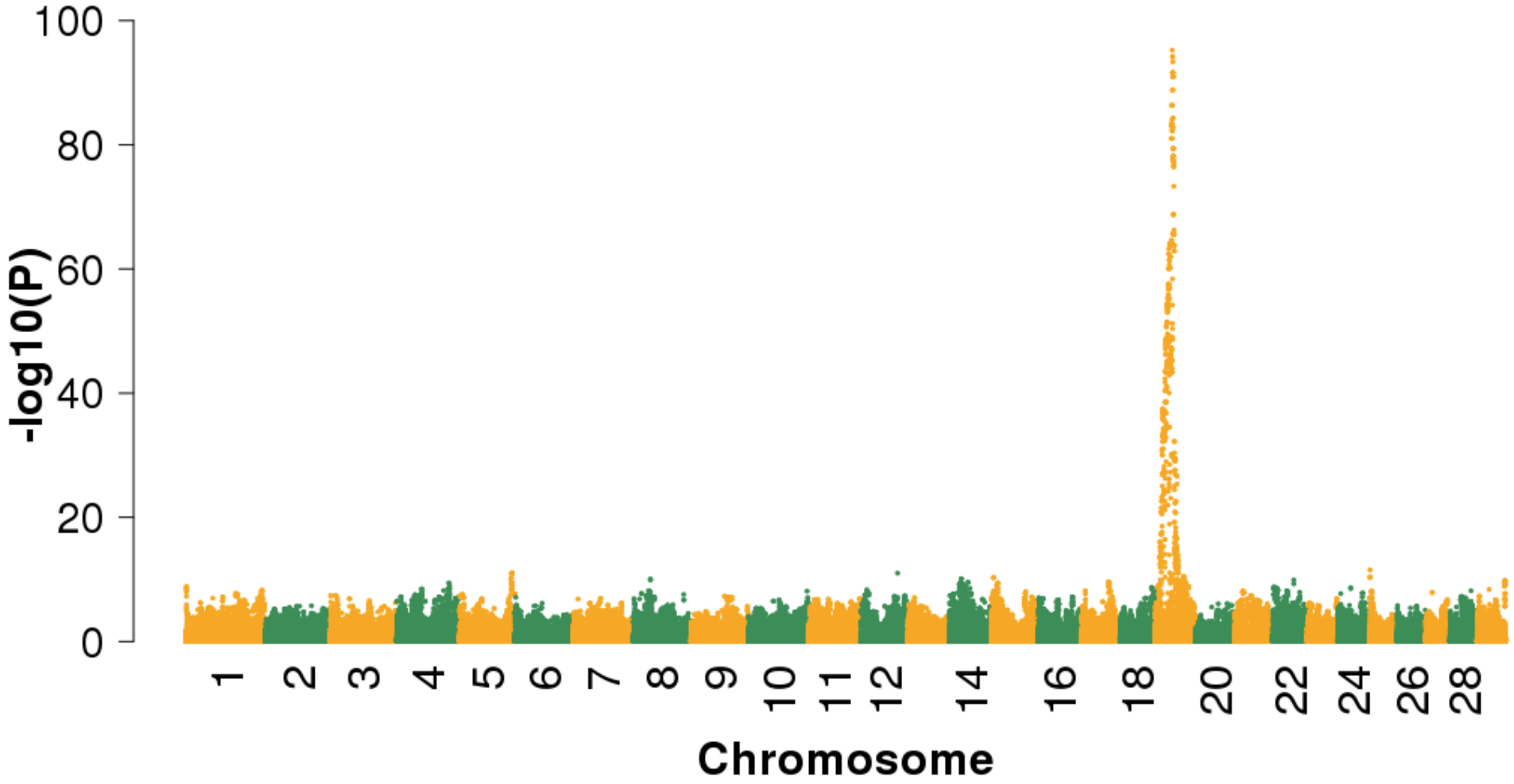


Insemination success with semen of subfertile bulls

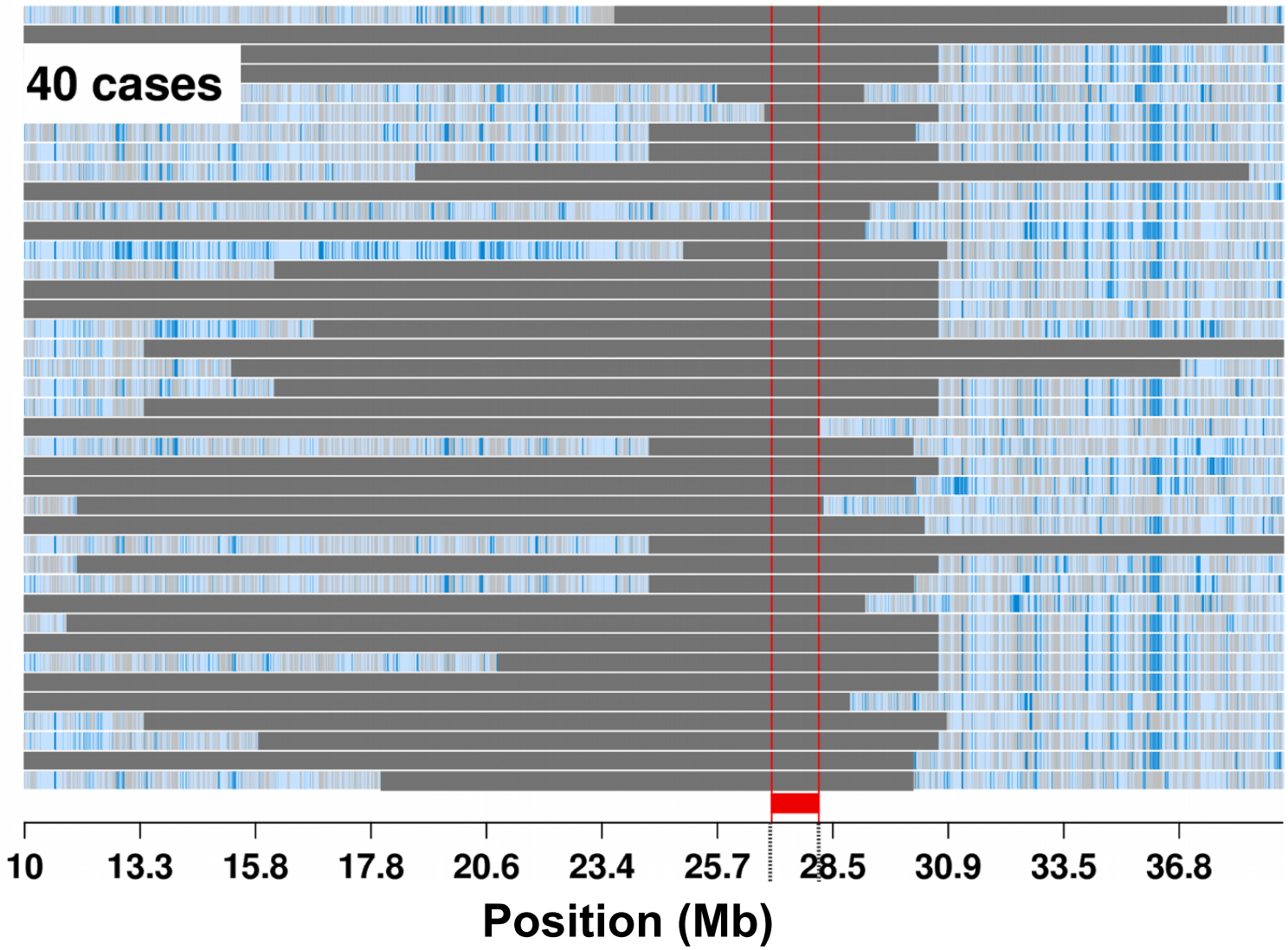
Name	Birth year	Number of inseminations	Successful inseminations	Male reproductive ability
subfertile_1	2003	1344	15 (1.12%)	-32
subfertile_2	2009	986	23 (2.33%)	-27
subfertile_3	2009	671	9 (1.34%)	-25
subfertile_4	2008	699	4 (0.57%)	-30
subfertile_5	2009	849	14 (1.65%)	-28
subfertile_6	2008	777	5 (0.64%)	-32

GWAS for MRA

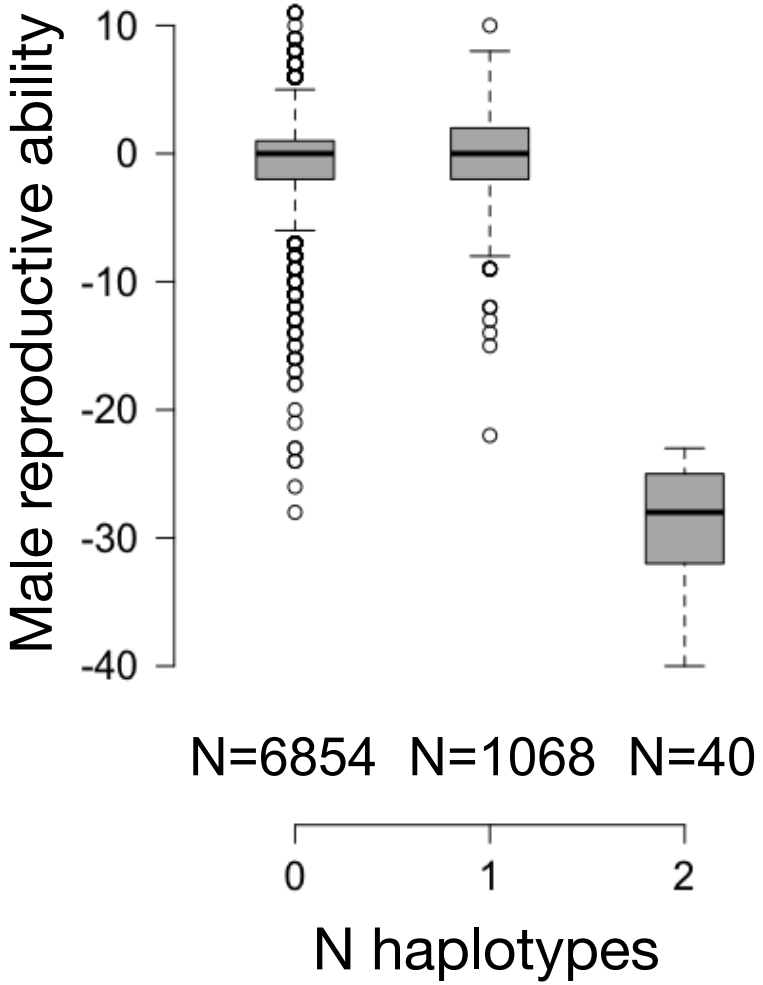
(57 cases vs. 8179 controls, 650K SNP)



Homozygosity mapping on bovine chromosome 19



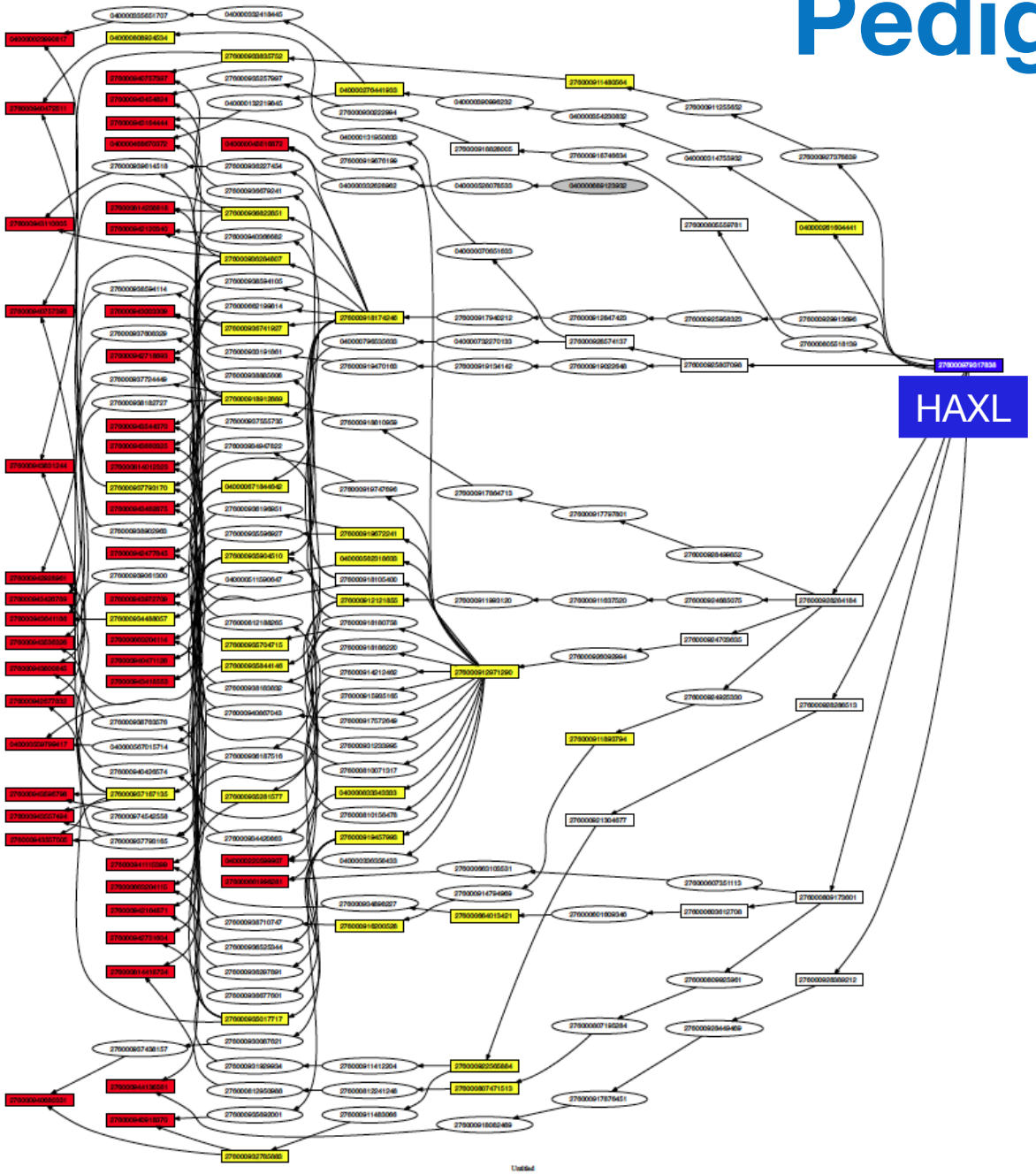
Haplotype analysis



- 40 subfertile bulls are homozygous for the haplotype
- None of the bulls with normal fertility is homozygous
- Frequency of the haplotype is 7.2%
- MRA of heterozygous bulls is normal

  => recessive inheritance

Pedigree analysis



HAXL is the founder of the mutation

- birth year 1966
- most important ancestor of the FV population
- present in >98% of all pedigrees

Ejaculate parameters of subfertile bulls are normal

(=idiopathic subfertility)

Ejaculate parameters in fresh semen:

	N	Ø number of ejaculates	Ejaculate volume (ml)	Sperm count (Mio/µl)	Spermatozoa with progressive motility (%)
wt/wt	177	52	5.14±1.08	1.53±0.22	74.73±5.21
wt/mt	21	52	5.15±1.03	1.47±0.23	75.15±4.92
mt/mt	5	63	5.56±1.75	1.42±0.29	70.82±7.06

Ejaculate parameters after thawing:

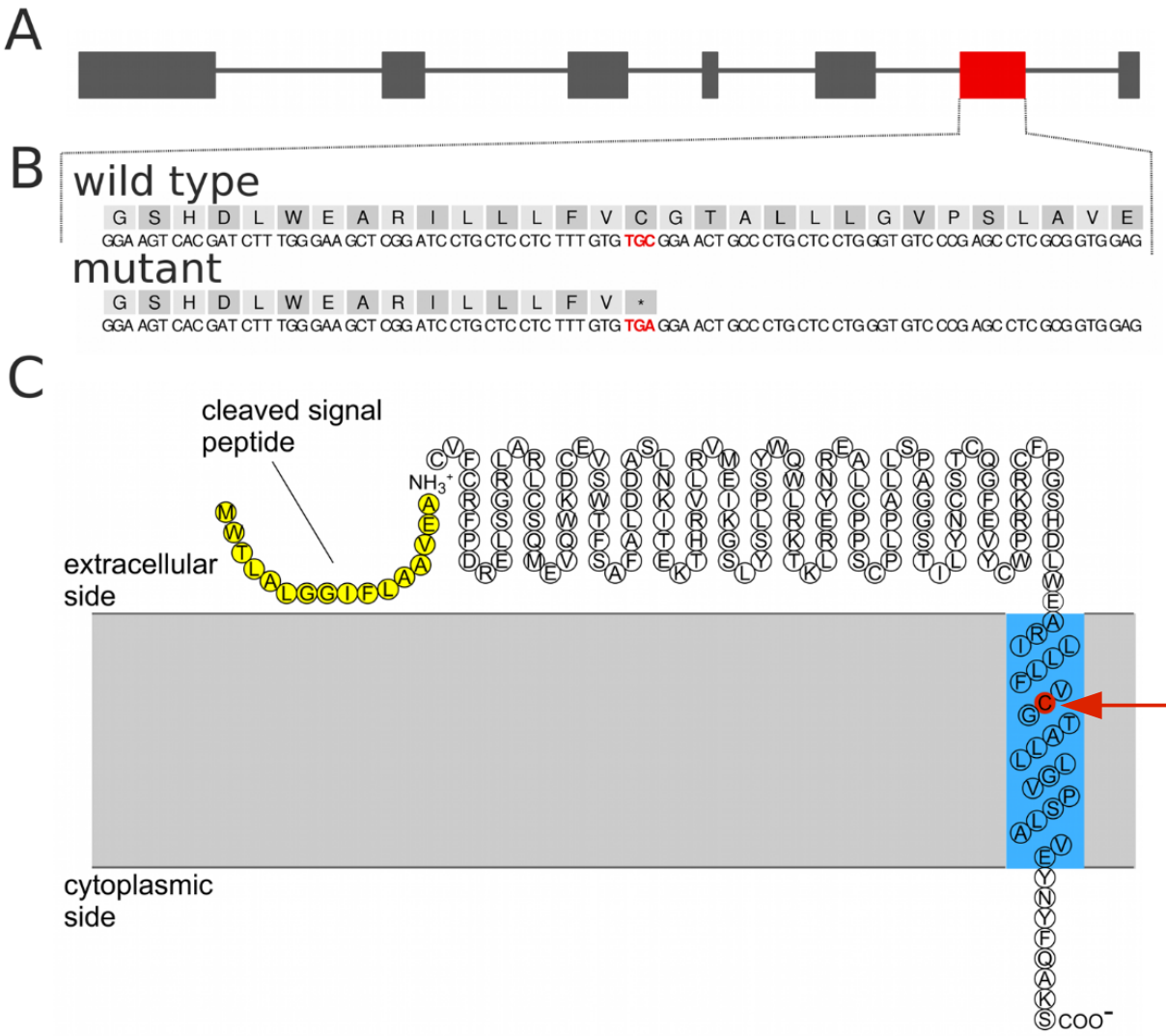
	N	Motile spermatozoa (%)	Vital spermatozoa (%)
wt/wt	10	53±9	56±11
wt/mt	10	61±13	65±11
mt/mt	10	48±13	51±12

Hunting down the causal mutation

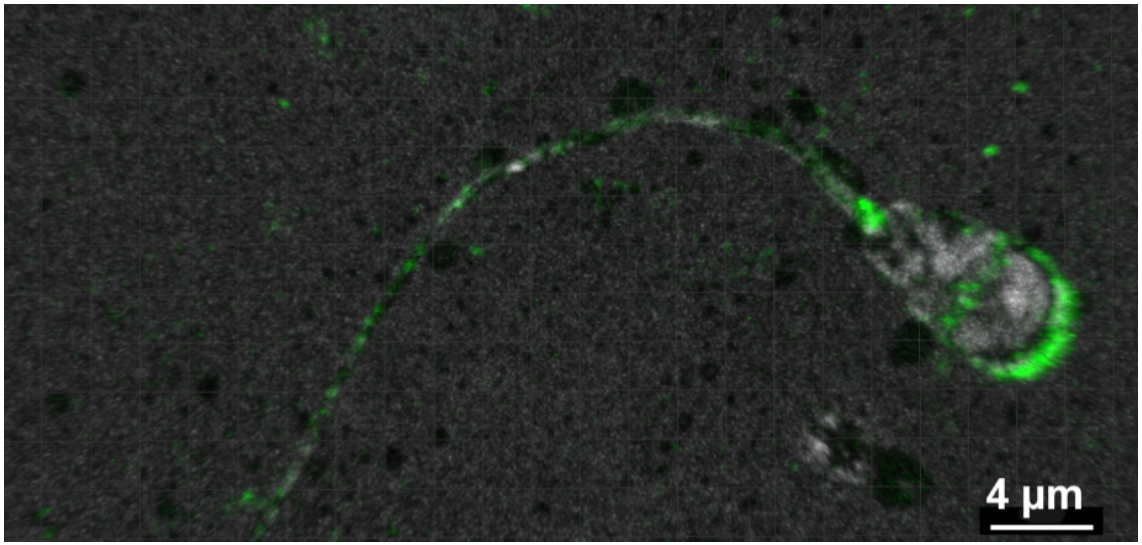
- Sequence data of 43 Fleckvieh animals
  - 6 of them are carriers of the subfertility-associated haplotype

- Tasks:
  - Identify variants that are heterozygous in 6 haplotype carriers and homozygous for the reference allele in all non-carriers
  - Predict the consequence of compatible variants (= *functional annotation*)
  - Identify putatively deleterious mutations

A nonsense mutation in *TMEM95* is perfectly associated with MRA

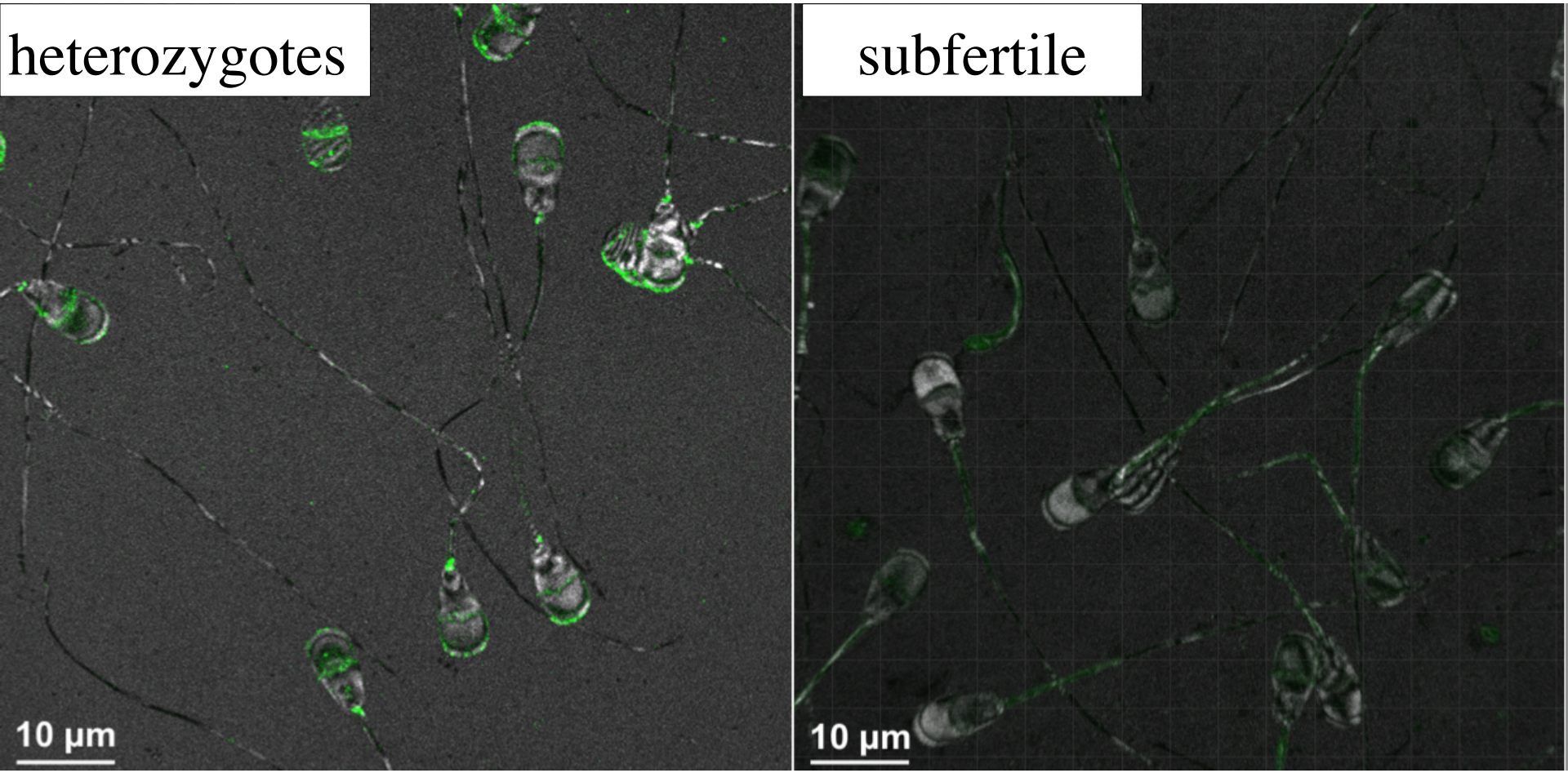


TMEM95 is located at the plasma membrane of spermatozoa of fertile bulls



Origin: Sabine Kölle, Urologische Klinik und Poliklinik, LMU München

TMEM95 is missing in spermatozoa of subfertile bulls



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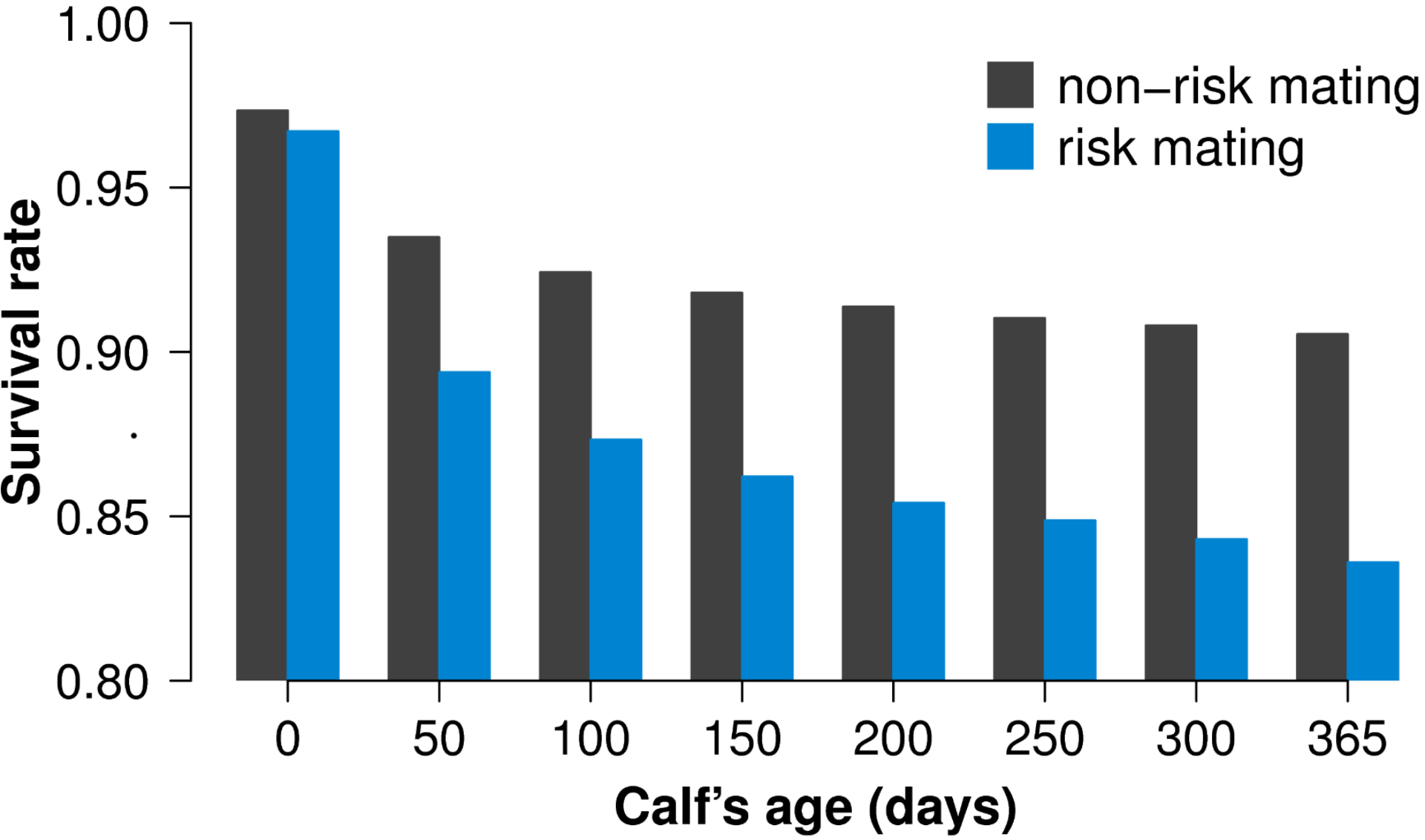
Identification of deleterious alleles *via* homozygosity depletion

- 25,000 animals genotyped (50K)
- Sliding window-based approach
- Identification of FH1-FH4
  - associated with embryonic losses and juvenile mortality

- **Fleckvieh Haplotype 2 (FH2)**
  - Chromosom 1: 96,000,000 bp - 98,000,000 bp
    - $freq_{HT}$ = 4%
    - **36 homozygotes expected but only 2 observed**
    - P=1.16 x 10^{-13}

First year survival rate



Hunting down the causal FH2-mutation

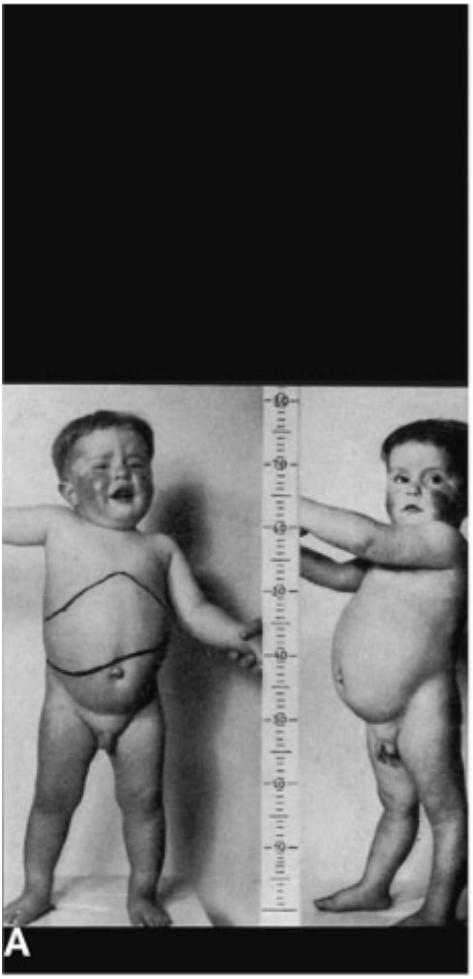
- Eight FH2-heterozygous animals have been sequenced
  - these carry a 4bp deletion in *SLC2A2* introducing premature translation termination

- Recessive mutations in *SLC2A2* cause Fanconi-Bickel syndrome in humans
  - impaired insulin/glucose metabolism

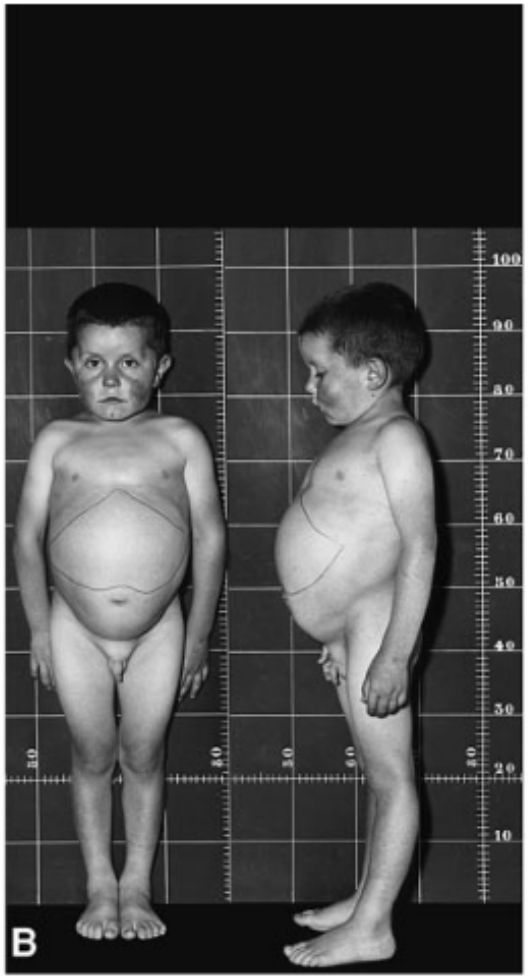
    => severe growth retardation

    => liver damage

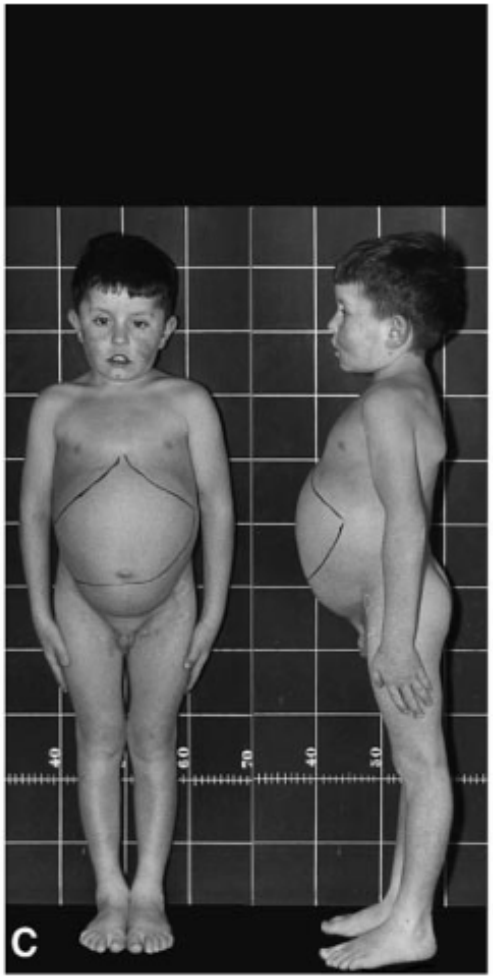
Fanconi-Bickel syndrome



4 years



10 years



12 years

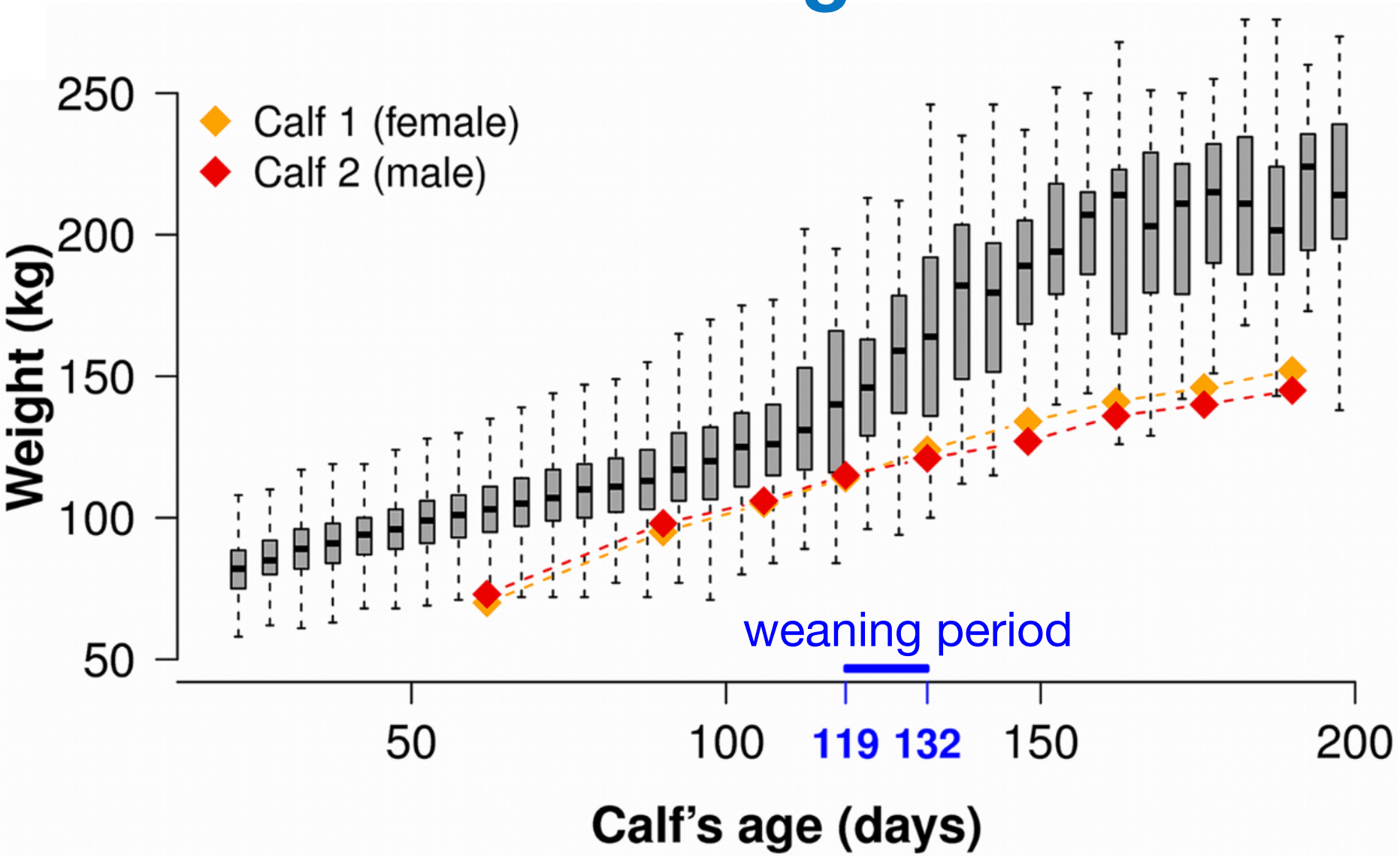
52 years

Santer et al., (1998), Eur J Pediatr 157: 783-797

Fanconi-Bickel syndrome in cattle?

courtesy of H. Schwarzenbacher, ZuchtData

Growth retardation starts after weaning



Management of deleterious alleles in Fleckvieh

- Establishment of a task force '***Genetic characteristics***'
  - Members from
    - Breeding organisations
    - Artificial insemination companies
    - Genetic evaluation team
    - University
  - Tasks:
    - Evaluation of scientific results
    - Which genetic characteristics should be considered in breeding programs and how?
    - Dissemination of information

=> RECOMMENDATIONS

Results of the task force

- *Transparency*
  - dissemination of **full information** to all parties involved
- *Declaration of carrier bulls*
  - direct **gene tests** for all artificial insemination bulls as soon as they are available
  - genetic characteristics are listed along with the breeding value information
- *Classification of genetic disorders depending on*
  - the phenotype of homozygotes
  - the allele frequency of deleterious alleles
  - pleiotropic effects / penetrance

Recommendations

- ***„Do not select carriers“***
  - *e.g.* for Arachnomelia and for the zinc deficiency-like syndrome

    RATIONALE: low frequency, animal welfare
- ***„Select only superior carriers“***
  - *e.g.* for Fleckvieh Haplotype 4 (embryonically lethal)

    RATIONALE: no immediate phenotype manifest
- ***„Do not select homozygous bulls“***
  - *e.g.* for bovine male subfertility

    RATIONALE: heterozygotes are fertile, no effect in females

Declaration in databases

RINDERZUCHT AUSTRIA

ZuchtData - Zuchtwertdatenbank

HENDEL

DE 09 40822798 HB: 10/169975 FH2, ZDL, TP

geb.: 28.03.2006
Züchter:
Station: EUROgenetik Neustadt/Aisch

ABSTAMMUNG		Stammbaum
HERICH AT 671.844.642 ZW: 111 / 108 / +399 -0.32 -0.02	**HERON** AT 444.171.941	**HORWEIN**
	BORIS AT 796.535.633	**GS STRICH**
LINA DE 09 36323018 ZW: 94 / 103 / +108 +0.06 -0.05 2/1 - 12189-4.18-3.48-934 HL: 1. - 12189-4.18-3.48-934	**HOCHREP** DE 09 15193492	**HODACH**
	LOROSA DE 09 32878743 1/1 - 7078-4.27-3.74-567	**POSTNER**

ZUCHTWERTE (AT/DE, 02.12.2014) **NK** Historie *GZW -2, MW -2, FW +1, FIT +0* **gGZW 101** (90)

MILCH -7 -0.04 -3 +0.00 +0 **MW 99** (93)

100-Tg.:	91	2425 - 4,08 - 3,22 - 177	Stall: 7389	Tö int.: 96 PM
1.Lakt.:	79	6511 - 4,18 - 3,49 - 500	7425	96 8
2.Lakt.:	62	7409 - 4,18 - 3,52 - 570	7580	74 8
3.Lakt.:	38	7707 - 4,16 - 3,49 - 589	7504	56 8

Unresolved questions...

- Should haplotypes be considered for breeding decisions without knowing the causal mutation?
- How to comply with export regulations?
- What is the optimal strategy to manage dozens of genetic characteristics?
- etc.

Conclusions

- Eight deleterious alleles are (currently) considered in the Fleckvieh breeding population
- Sequence data revealed many more putatively deleterious alleles
- Knowing a population's genetic load is a prereqisite for sustainable breeding decisions
- Animals carrying deleterious alleles need to be identified

Acknowledgements

- Dr. Hermann Schwarzenbacher, ZuchtData, Vienna
- Chair of Animal Breeding, TU München
- German-Austrian working group '*genetic characteristics*'
- ASR, AGÖF, FBF, DHV

Thank you for your attention!