Protecting breeding populations through genomics

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4TH INTERNATIONAL WORKSHOP ON REGULATORY APPROACHES FOR AGRICULTURAL APPLICATIONS OF ANIMAL BIOTECHNOLOGIES

WISCONSIN
UNIVERSITY OF WISCONSIN–MADISON

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Conservation and adaptation perspective

It works by first understanding and identifying what genetic features make members of a species more adapted to an **environment** than others.

We then look for genes that are present in individuals that adapt or present increased performance over specific conditions.

Over time, the use of genomic selection as a breeding strategy can increase the average ability of these individuals in the breeding population to survive by promoting adaptation.

Eusebi et al. 2020, Hohenlohe et al. 2021

Allelic Variations in Genes are Responsible for Phenotypic Differences
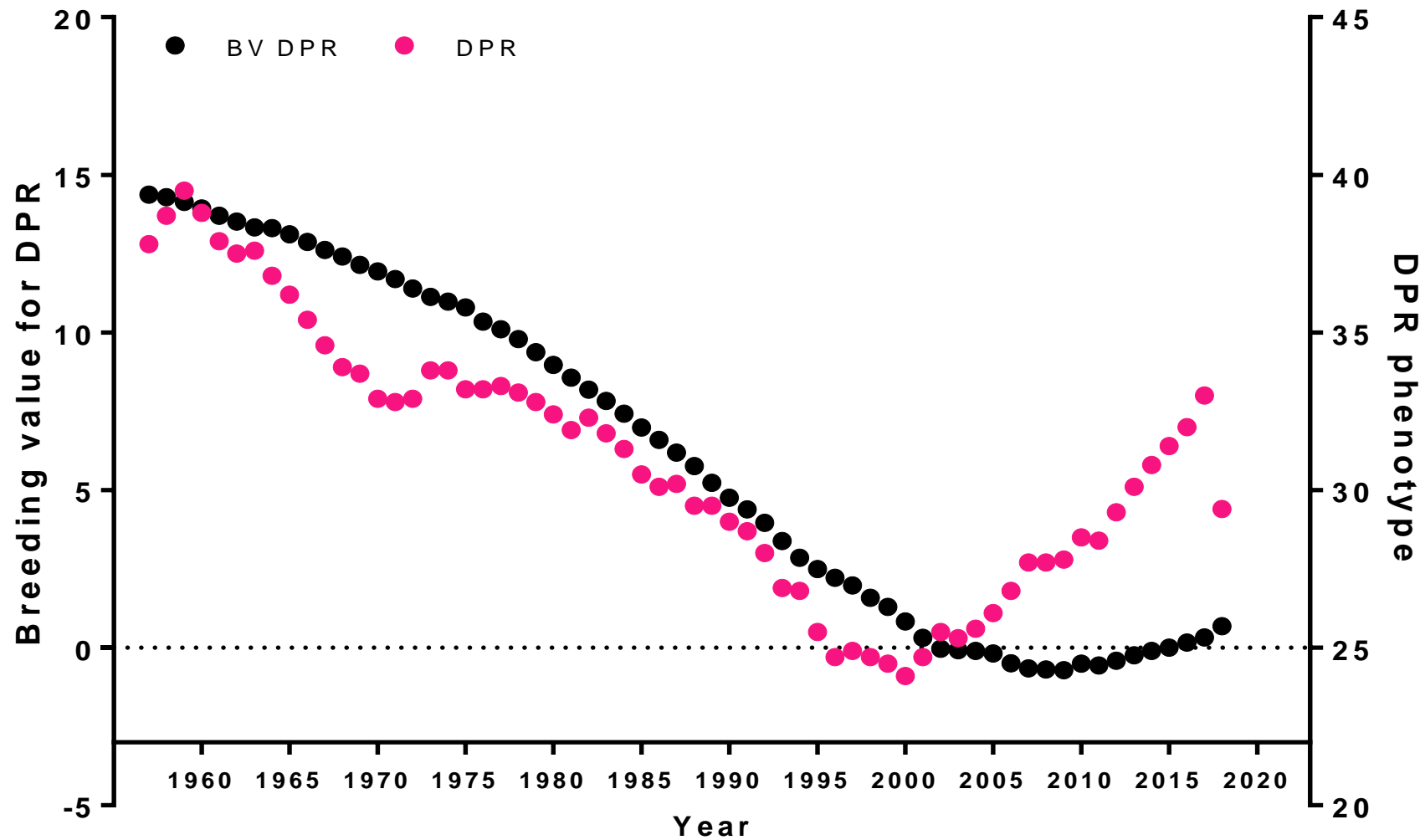

Single nucleotide polymorphism

- Select animals for reproductive function
- Insights into physiology
- Precision animal agriculture

Bovine Genome

- Length 2,715,853,792 bp
- 98,844,739 SNP
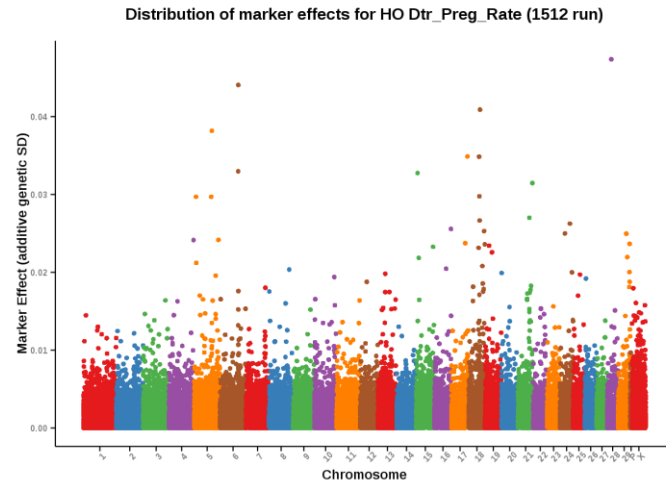- 18,942 structural variants (>50 bp)
- Assemblies: ARS-UCD 1.2

The Holstein Fertility Story

Heritability= 0.04

Higher the DPR value, less time for the female to get pregnant
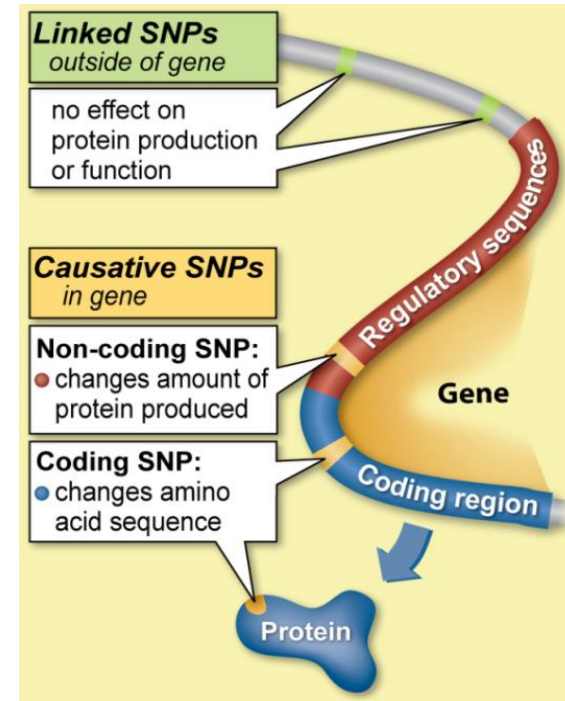
How can we identify genes involved in reproduction
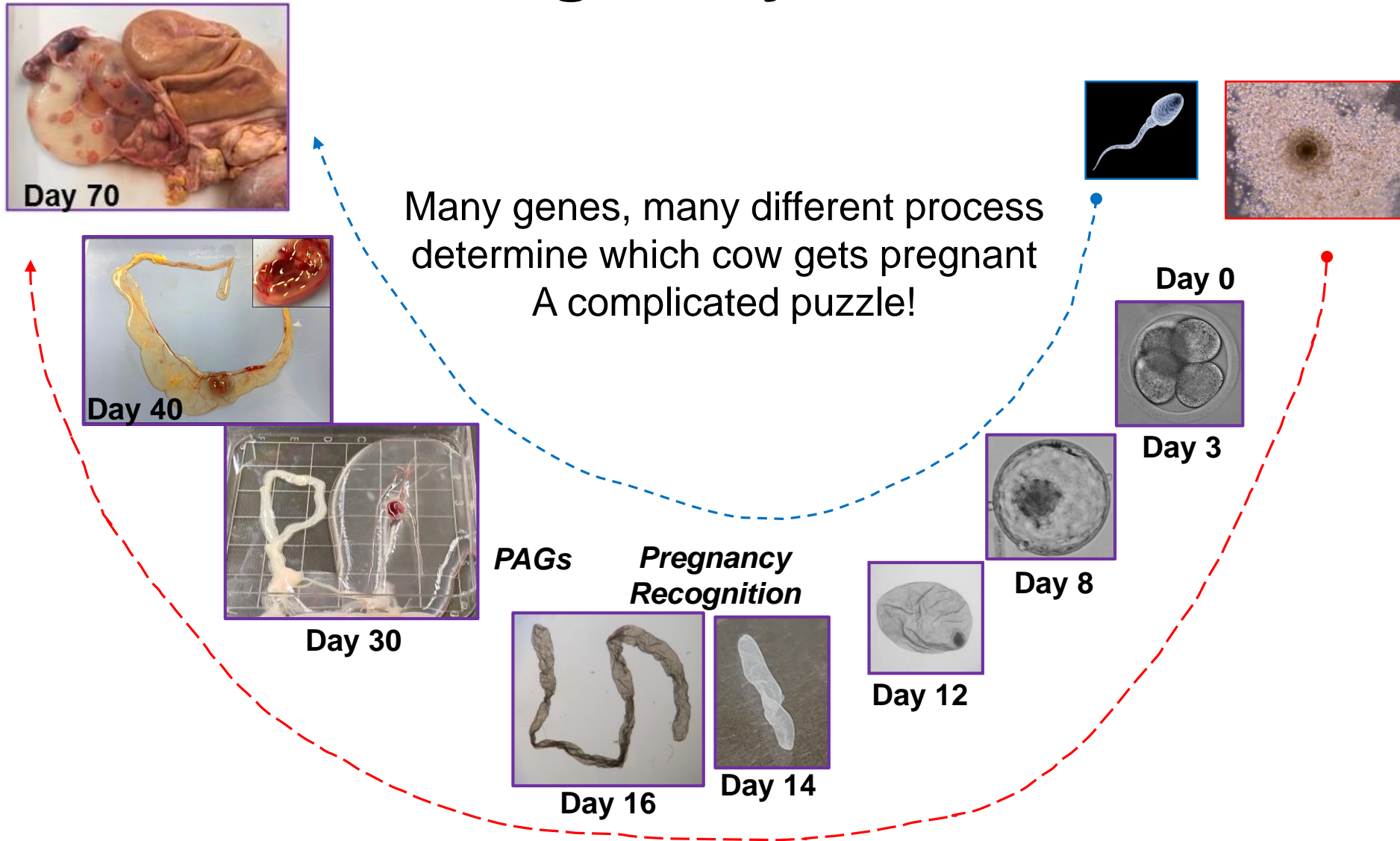

Genome-wide association studies

Whole genome sequencing

Candidate genes

Pregnancy in cattle

Many genes, many different process determine which cow gets pregnant
A complicated puzzle!

Day 70

Day 40

Day 30

PAGs

Pregnancy Recognition

Day 16

Day 14

Day 12

Day 8

Day 3

Day 0

Embryo
-survival to transfer (El-Sayed et al, 2006)
-oocyte vs. 8 cell embryo (Misirlioglu et al., 2006)
-blastocyst vs. morula (Mamo et al., 2006)
-upregulated after cryopreservation (Kuzmany et al., 2011)
-Superovulated vs. unstimulated (Gad et a., 2011)
-WOW vs. group culture (Hoelker et al., 2009)
-genes regulated by CSF2 (Loureiro et al., 2011)
-genes regulated by IGF1 (Bonilla et al., 2011)

Genes Associated with Reproductive Traits
-interval to insemination (Pimentel et al., 2011)
-56 d non-return rate (Pimentel et al., 2011)

Brain
- strong vs. weak estrus (Kommadath et al., 2011)

Oviduct
- diestrus vs estrus (Bauersachs et al., 2004)

Oocyte
-stages of maturation (Fair et al., 2007)

Liver
-differentially regulated during transition period (Graber et al., 2010)

Follicle
-oocyte maturation (Salhab et al., 2011)
-*in vivo* vs. *in vitro* (Tesfaye et al., 2009)
-regulated by LH surge (Assidi et al., 2010)
-dominant vs. subordinate (Fayad et al., 2004; Pfeffer et al., 2007: Zielak et al, 2007; Luo et al., 2011)
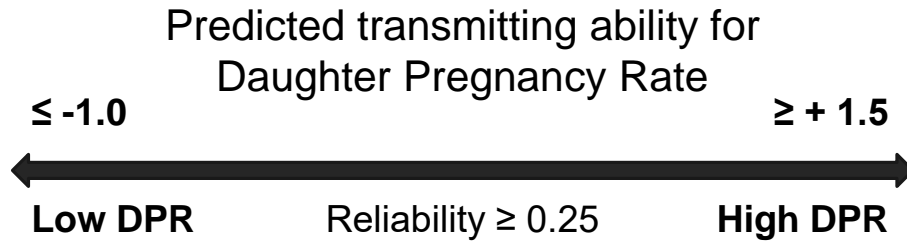
Mammary
-lactating vs. nonlactating (Mani et al., 2010)

Endometrium
-pregnant vs. non pregnant (Cerri et al., 2012)
-lactating vs. Non lactating (Cerri et al., 2012)
-embryo survival (Beltman et al., 2010)
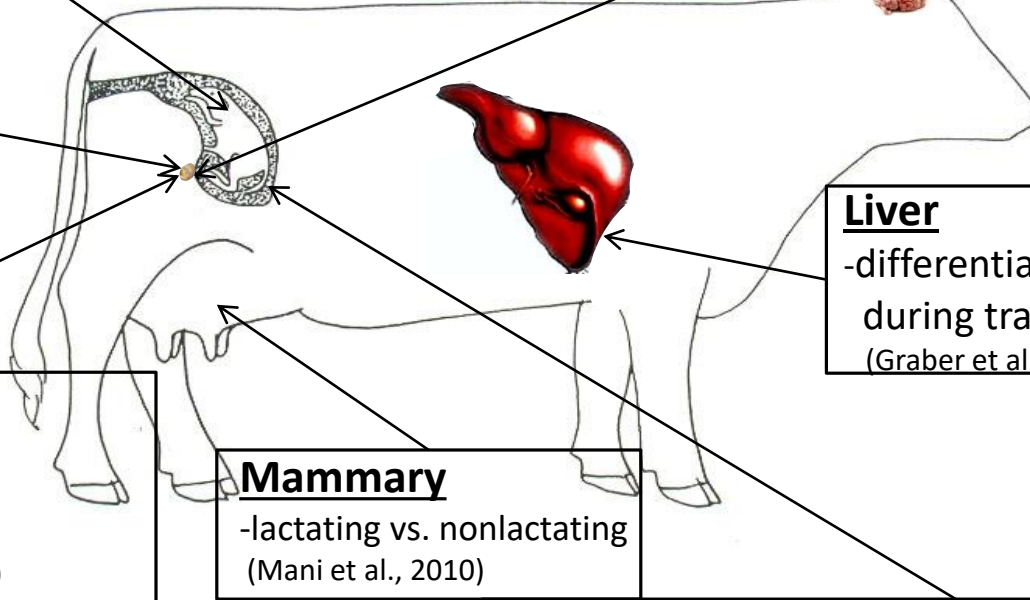
Holstein Population Characteristics

Predicted transmitting ability for
Daughter Pregnancy Rate

≤ -1.0   ≥ + 1.5

Low DPR   Reliability ≥ 0.25   **High DPR**

Location	# Dairies	High DPR	Low DPR	Total
Florida	6	677	137	814
California	5	394	1129	1523
Total	11	1071	1266	2337

**Genetic information
(PTA values)**

- Daughter pregnancy rate (DPR)
- Heifer conception rate (HCR)
- Cow conception rate (CCR)

**Phenotypic information
(Farm data)**

- Pregnancy rate at first service
- Services per conception
- Days open

77 SNP (1 SNP per gene)

Reliability Gain for DPR by using UF in Genetic Evaluations (AGIL, USDA)

Use of a more dense chip (300K Markers) improve reliability for daughter pregnancy rate by 0.4%

VanRaden et al. (2013)

SNP panel	(%)			
	Reliability, parent averages	Genomic reliability	Reliability gain	Diff. against USDA
USDA	30.44	60.79	30.35	-
USDA+UF	30.44	60.99	30.55	0.20
USDA (-100 kb) + UF	30.44	61.12	30.68	0.33
USDA (-200 Kb) + UF	30.44	61.17	30.73	0.38
UF	30.44	33.20	2.76	-

UF = 39 markers associated with DPR

Fertility is Different Between High and Low DPR Groups

Trait	N	LSMEANS (%)		P value
		High DPR	Low DPR	
Preg. rate first service (Lact1)	2213	53.1	28.6	<0.0001
Preg. rate first service (Lact2)	1969	43.9	23.0	<0.0001
Preg. rate first service (Lact3)	1321	41.0	25.0	<0.0001

Trait	N	LSMEANS		P value
		High DPR	Low DPR	
Services /conception (Lact1)	2213	1.93	3.26	<0.0001
Services /conception (Lact2)	1969	2.09	3.30	<0.0001
Services /conception (Lact3)	1321	2.20	3.20	<0.0001
Days open (Lact 1)	2213	98	163	<0.0001
Days open (Lact 2)	1969	112	167	<0.0001
Days open (Lact 3)	1321	110	158	<0.0001

COQ9 genotype affects phenotype for fertility without affecting production

Trait	Genotype			Additive effect	Additive P value
	AA	AG	GG		
Pregnancy rate (%)	36.0	34.0	33.0	1.00	<0.001
Services per conception (n)	2.6	2.7	2.8	-0.03	0.014
Days open (d)	138	145	146	-4.21	0.037
Milk yield (kg)	11,395	11,416	11,406	-5.87	0.860
Fat yield (kg)	937	936	938	-0.39	0.880
Protein yield (kg)	772	774	773	-0.32	0.880

Values are presented as least-squares means

Mitochondria DNA Copy Number in the Oocyte is Affected by *COQ9* Genotype

COX1 was used as reference to amplify mitochondrial DNA

Standard curve with 6, 10- fold dilution. Number of copies of transcript ranged from 1.2×10^6 to 1.2 copies

Ct of samples were compared to Ct on standard curve to determine the amount of transcript

Higher oocyte Mitochondria number is associated with increased quality and viability

P value <0.001

N: **AA** (11) **AG** (20) **GG** (12)

HSPA1L

HSPA1A/ HSPA1L
P<0.0001

Fold change

oocyte, 2 cell, 3-4 cell, 5-8 cell, 9-16 cell, morula, blast.

± 1 µM Melatonin

38.5 °C, 5% O_2, 5% CO_2	
40.0 °C, 5% O_2, 6% CO_2	38.5 °C, 5% O_2, 5% CO_2
38.5 °C, 21% O_2, 6% CO_2	38.5 °C, 5% O_2, 5% CO_2
40.0 °C, 21% O_2, 6% CO_2	38.5 °C, 5% O_2, 5% CO_2

0   24   72   168

Hours after insemination   ----assess development---
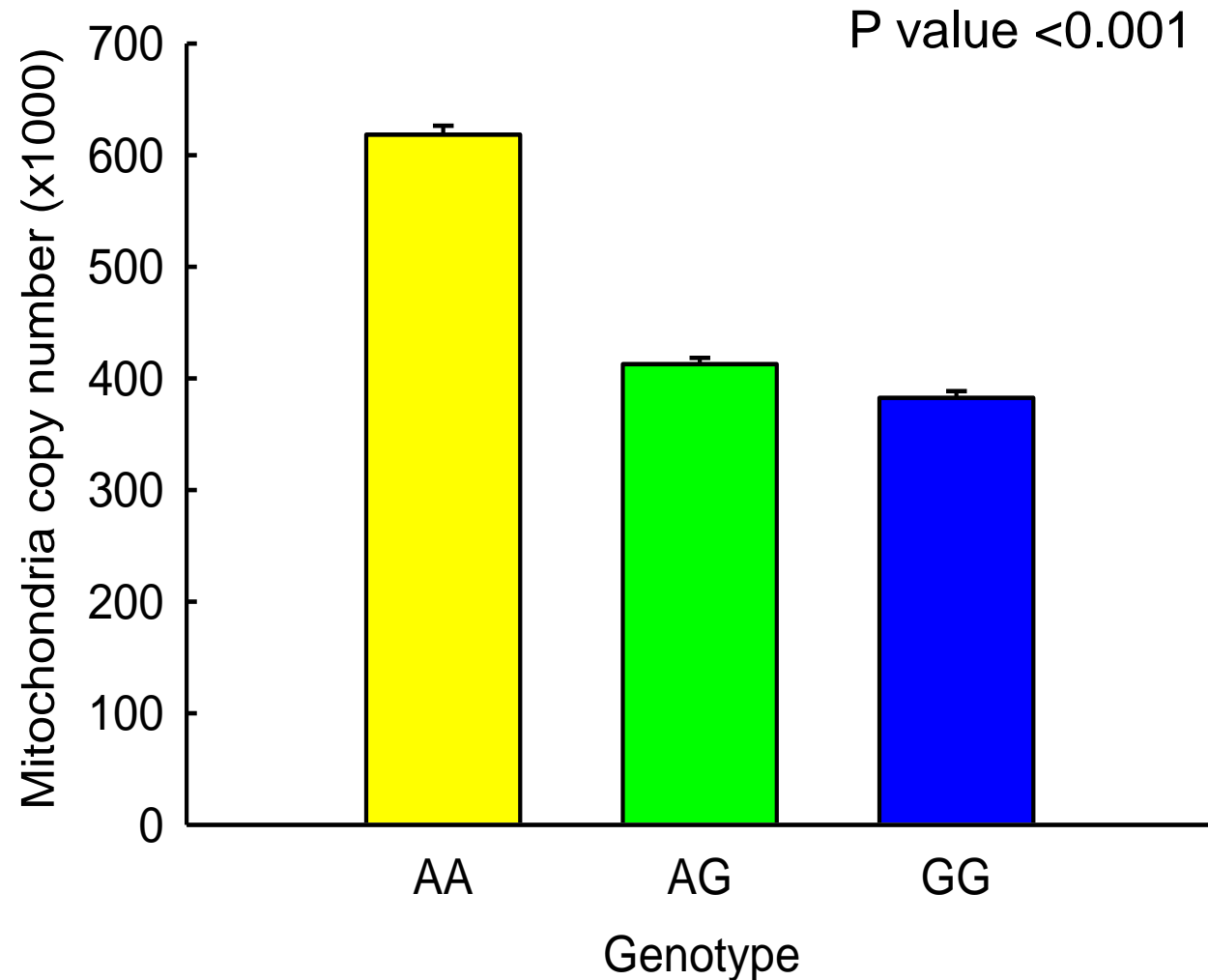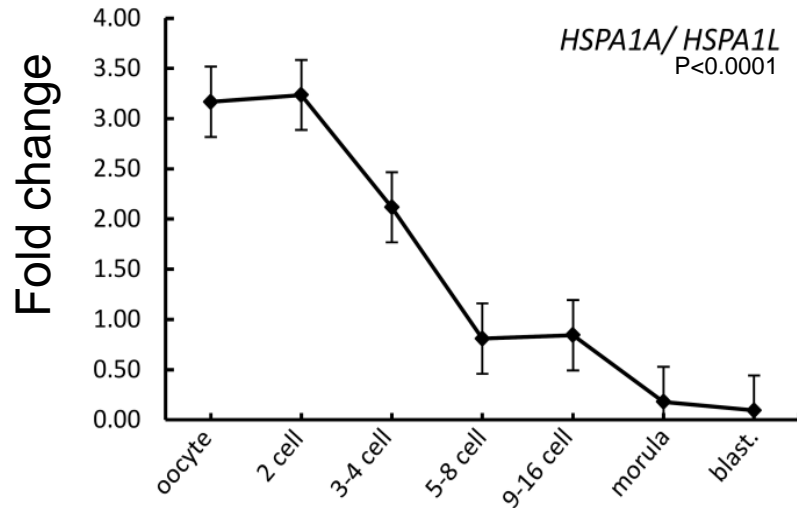
- Are the embryos inheriting the D allele for *HSPA1L* more likely to survive heat shock conditions than embryos carrying the wild type allele (C)?
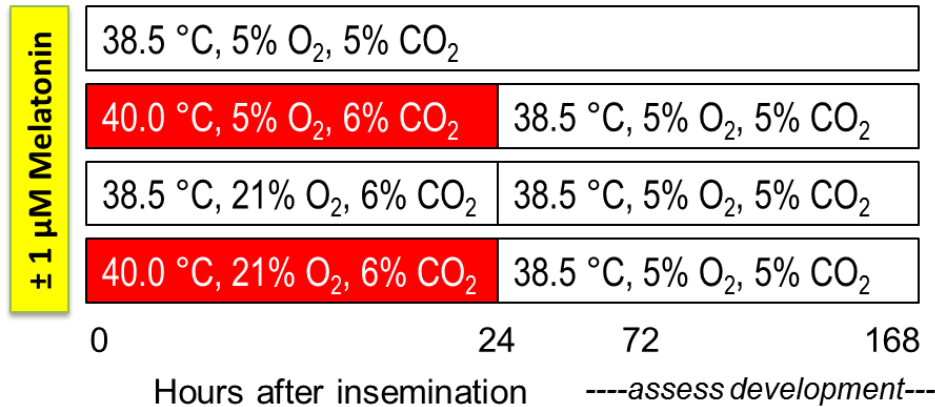
- 8 replicates
- 19 bulls
- 4359 embryos (510-578 per group)

Endpoints:
- Cleavage %
- Blastocyst %
- Blastocyst/cleaved %
- Genotype frequency for *HSPA1L*

Heat shock decreases development of the embryo to the blastocyst stage under low and high oxygen conditions
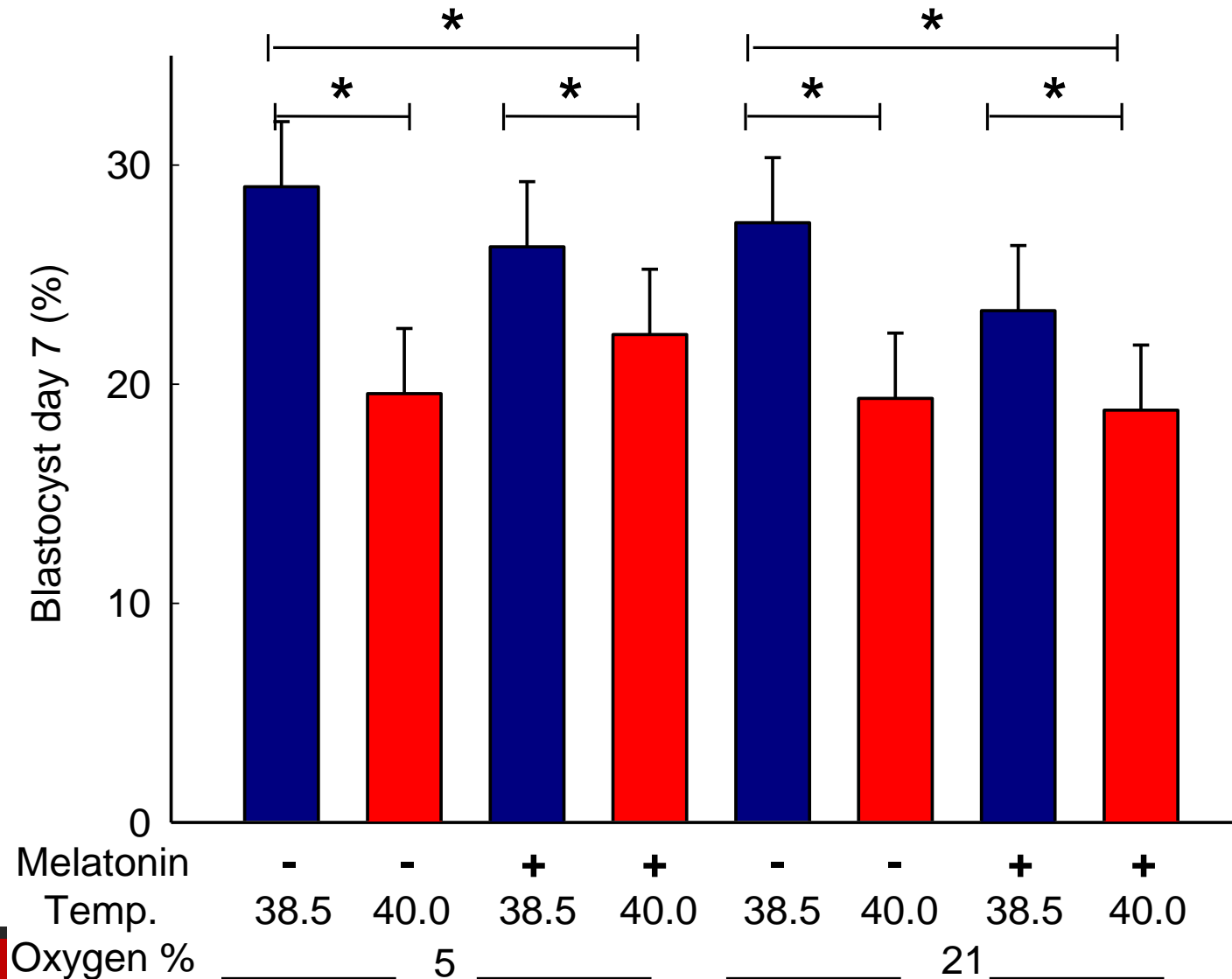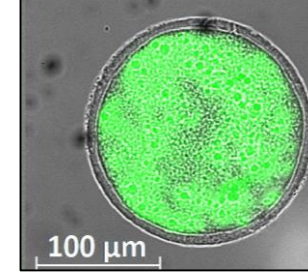
Mutation on *HSPA1L* improves survival of embryos exposed to heat stress
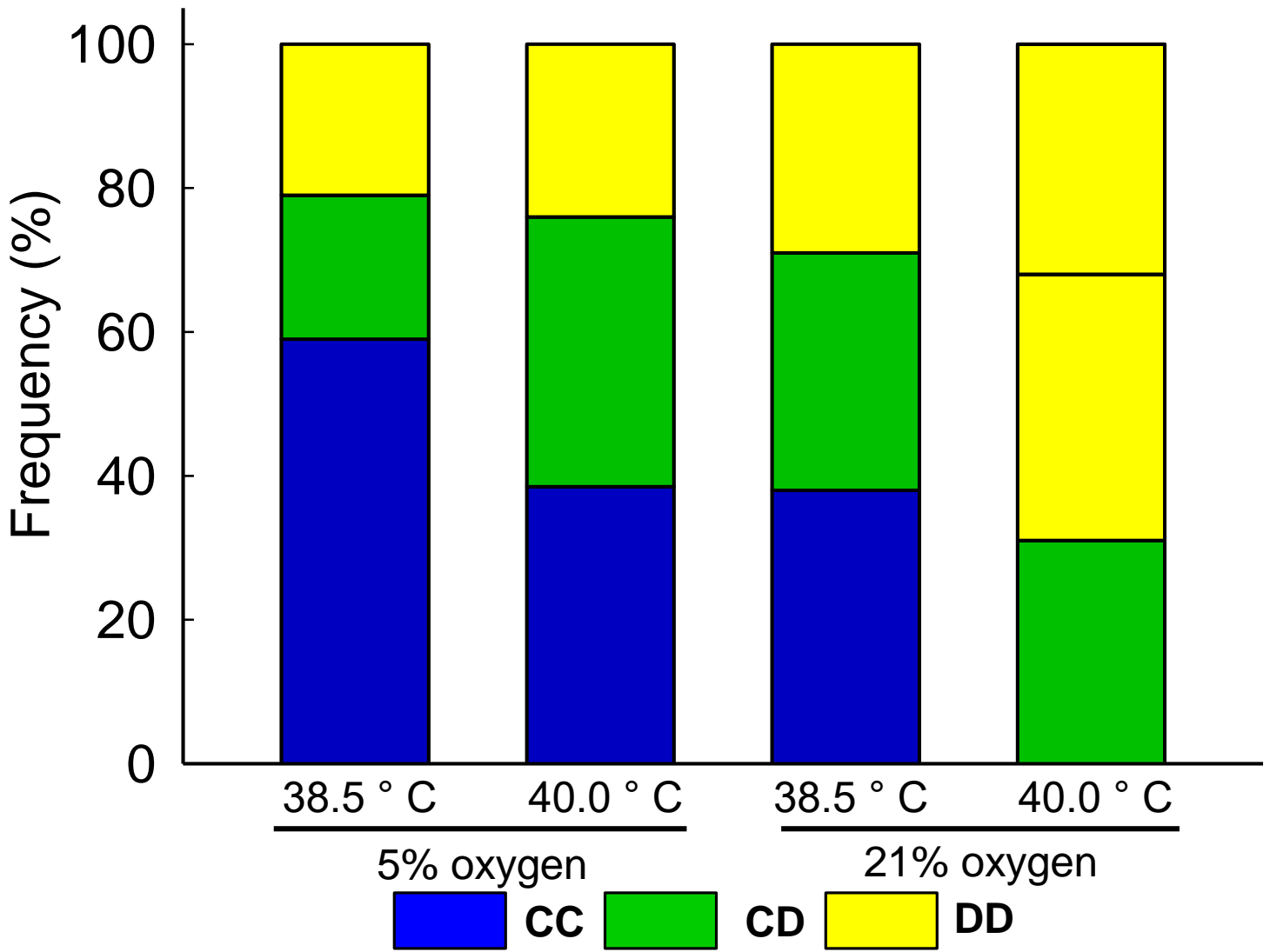
542 embryos across 8 replicates   gen. x temp. x oxygen P=0.0054

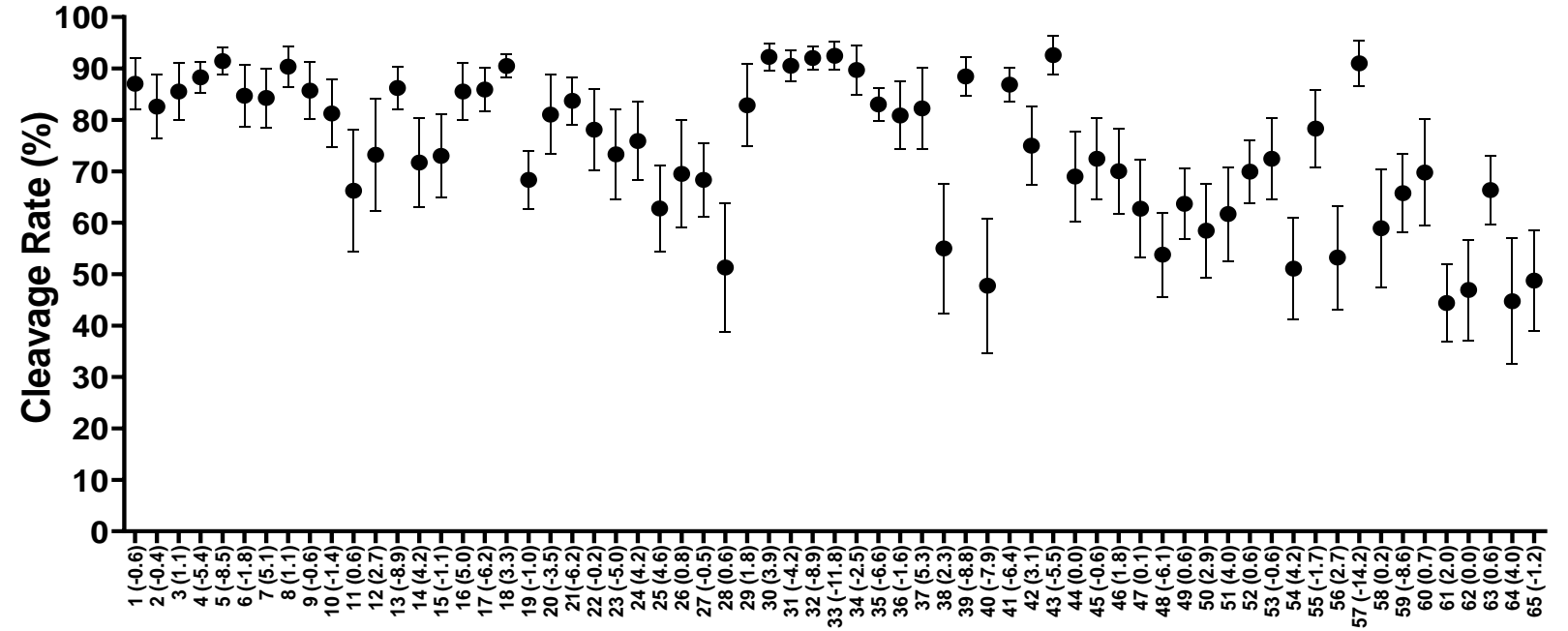# Can we find markers in association with early embryonic development?

1. Why is this important? Fertility → maintaining a functional herd

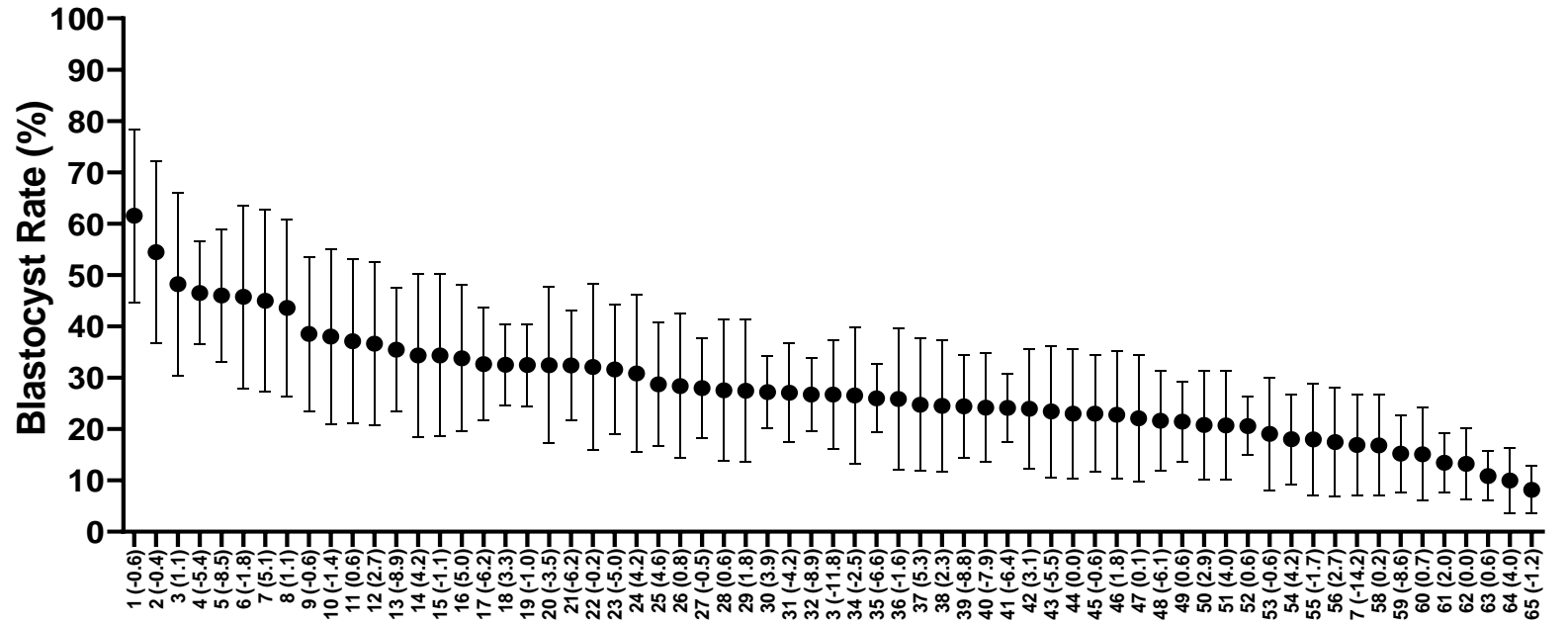2. Adoption of reproductive biotechnologies to propagate more fertile animals
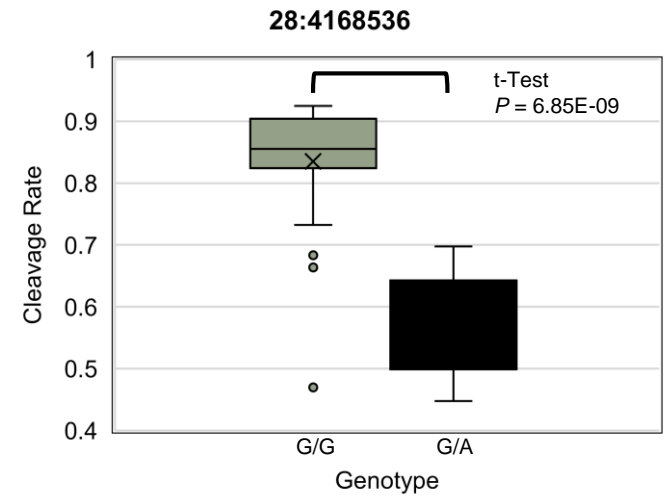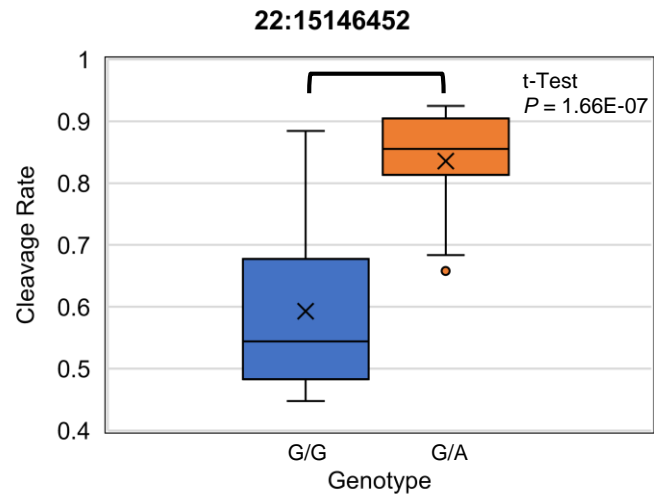   - Embryo donors, nucleus herds

3 IVP runs per sire

n = 65 sires

A

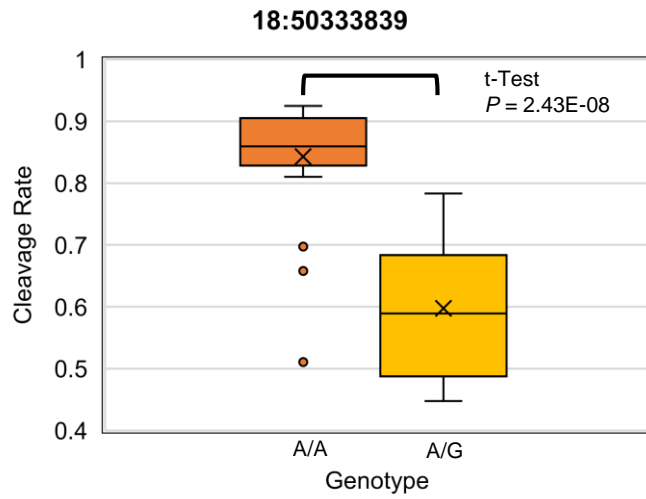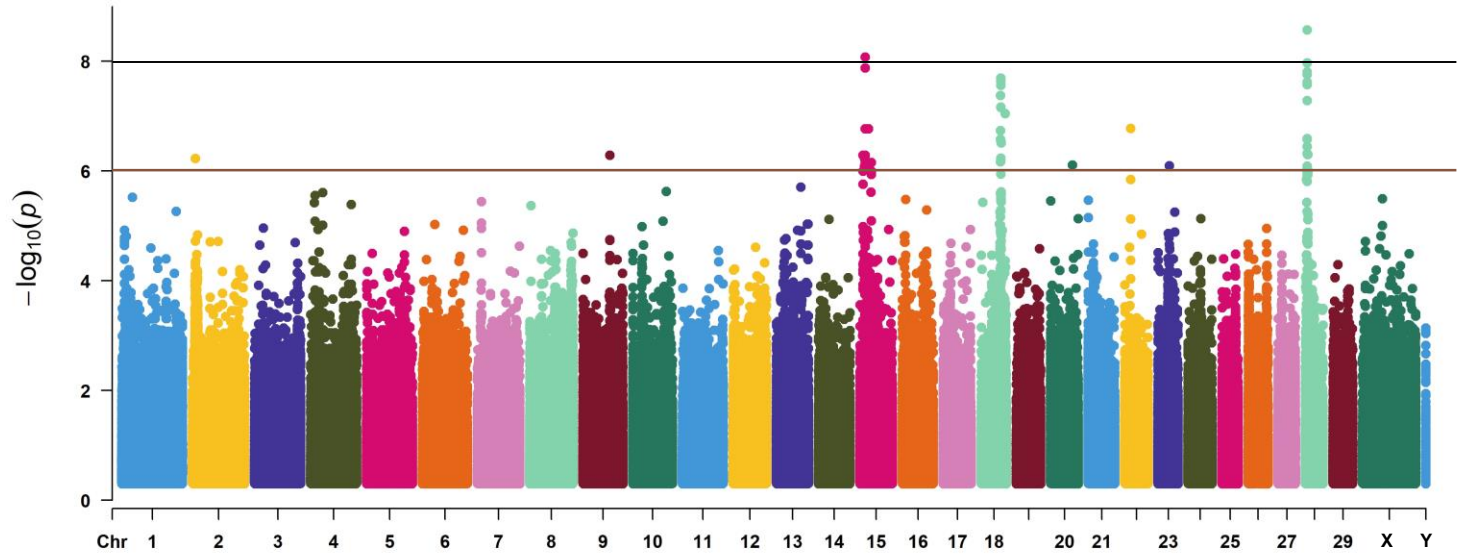**B**

Lockhart et al. (submitted)

Cleavage Rate
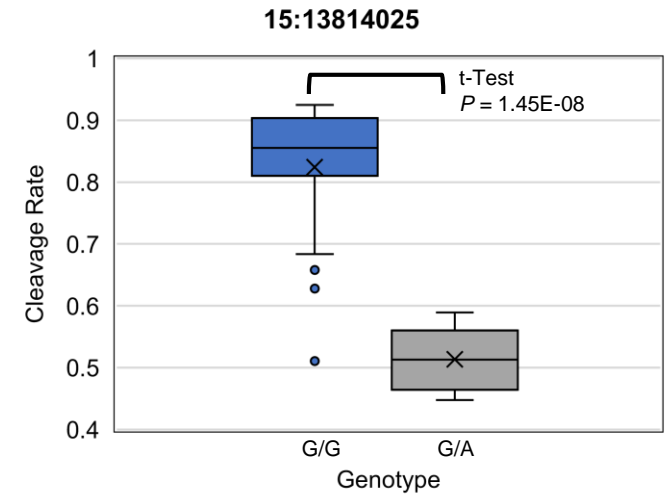
Genome wide association with embryo phenotype

Blastocyst Rate

Table 1. The lead significant SNP per chromosome and the regions containing significant SNPs associated with cleavage rate, genes and relevant quantitative trait loci (QTLs) within 10kb of the significant region and references for each QTL.

Chr	Position	P-Value	SNP ID	Region*	Genes Within Region ± 10kb**	Relevant QTLs Within Region ± 10kb**	QTL References**
2	7,432,127	5.97E-07	rs379842254	7,379,901 – 7,473,280	*COL3A1*	None	None
9	70,595,815	5.15E-07	rs470167401	70,595,815 – 70,595,825	None	None	None
15	13,814,025	8.44E-09	N/A	8,297,020 – 15,65,2425	*AMOTL1, ARHGAP42, CCDC82, CEP57, CNTN5, CWC15, ENDOD1, FAM76B, JRKL, MAML2, MIR1260B, MTMR2, SESN3, TRNAW-CCA*	Conception rate[1,2], daughter pregnancy rate[3], first service conception[2], interval to first estrus after calving[4], length of productive life[3], lifetime profit index[5], net merit[3], sexual precocity[6]	[1]Parker Gaddis et al., 2016; [2]Kiser et al., 2019; [3]Cole et al., 2011; [4]Liu et al., 2017; [5]Nayeri et al., 2017; [6]Melo et al., 2018
18	50,333,839	4.21E-08	N/A	50,318,373 – 51,937,613 62,740,070 – 62,740,086	*ARHGEF1, ATP1A3, AXL, B3GNT8, B9D2, BCKDHA, BSP1, BSP3, BSP5, CADM4, CCDC97, CD177, CD79A, CEACAM1, CIC, CNFN, CXCL17, CYP2B6, CYP2S1, DEDD2, DMAC2, DMRTC2, ERF, ERICH4, ETHE1, EXOSC5, GRIK5, GSK3A, HNRNPUL1, IRGC, IRGQ, LIPE, LYPD3, LYPD4, MEGF8, PAFAH1B3, PHLDB3, PINLYP, PLAUR, POU2F2, PRR19, RABAC1, RPS19, SMG9, SRRM5, TEX101, TGFB1, TMEM145, TMEM91, TRNAA-AGC, XRCC1, ZNF428, ZNF526, ZNF574, ZNF575, ZNF576*	Calving to conception interval[1], conception rate[2,3,4], daughter pregnancy rate[4,5,6], inseminations per conception[1,3,7], length of productive life[5,8], net merit[5], twinning[9]	[1]Ortega et al., 2017; [2]Jiang et al., 2019; [3]Kiser et al., 2019; [4]Ortega et al., 2016; [5]Cole et al., 2011; [6]Cochran et al., 2013; [7]Liu et al., 2017; [8]Nayeri et al., 2017; [9]Kim et al., 2009
20	57,348,543	7.76E-07	rs381921249	57,348,543 – 57,348,548	*FBXL7*	None	
22	15,146,452	1.68E-07	rs446062139	15,146,407 – 15,146,536	None	None	None
23	29,358,587	8.01E-07	rs207934314	29,358,587	*OR12D3*	Calving to conception interval[1], interval to first estrus after calving[1,2]	[1]Müller et al., 2017; [2]Tenghe et al., 2016
28	4,168,536	2.68E-09	rs385003959	4,126,277 – 6,030,617	*DISC1, EGLN1, MAP10, NTPCR, PCNX2, SIPA1L2, SNORD16, TRNAC-GCA, TRNAG-GCC, TRNAG-UCC, TSNAX*	Conception rate[1], length of productive life[2], net merit[2]	[1]Kiser et al., 2019; [2]Cole et al., 2011

Concluding remarks

- Inclusion of markers to improve current fertility traits and substitute less informative markers.

- Adapt management according to the animal genetic merit.

- Functional studies involving genetic engineering to understand the biological role of genetic variants in the regulation of reproductive function in cattle.

- **Re-introduction** of desirable genetic variants associated with increased performance in functional traits.

Genasci Dairy

PH Ranch 1

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SELECT SIRES
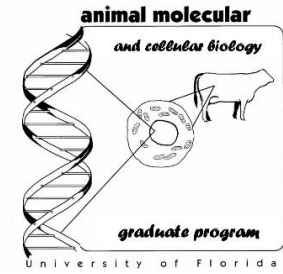
Simplot
DeSoto Biosciences
Missouri Prime Beef Packers

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