GENIFER project:
Fine mapping and effects of QTL affecting fertility in Holstein cattle

LEFEBVRE R., FRITZ S., LEDOUX D., GATIEN J., GENESTOUT L., ROSSIGNOL M.N., GRIMARD B., BOICHARD D., HUMBLOT P., PONSART C.

LEFEBVRE Rachel

EAAP 2013 Nantes, France
29 / 08 / 2013

Context

- High interest in reproductive traits of Holstein breed

- 2006: *CARTOFINE*, QTL mapping granddaughter design

  →16 QTL detected for conception rate

  → Particular emphasis on a fertility QTL on chromosome 3

  (*Druet et al, 2008*)

- *GENIFER* goals:
  - Confirm fertility QTL on an independent data set
  - Fine mapping
  - Characterize more accurately the QTL nature thanks to monitoring events between 0 and 90 days after first AI

Data collection

- A 3-year survey :
  - 3 organizations: INRA, UNCEIA and ENVA
  - French breeding companies and voluntary breeders

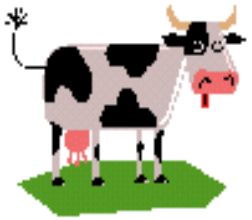
- 12 Holstein sires chosen:
  - Likely heterozygous for QTL on chromosome 3
  - Contemporary
  - Goal = 400 daughters/sire

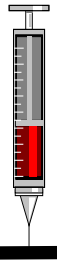
Data collection

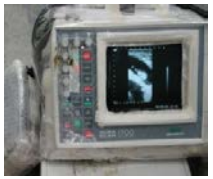
Blood sample (genotyping)

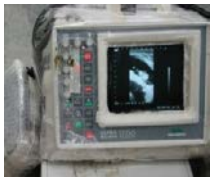
2 milk samples (P4 assay)

2 pregnancy diagnoses









D0 D21-23 D35-45 D80-100

AI1

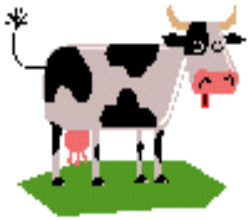
Possible return into estrus

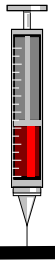
Data collection

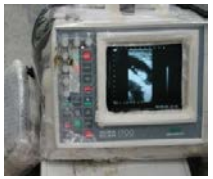
Blood sample (genotyping)

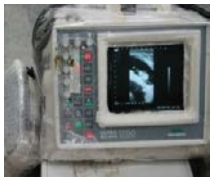
2 milk samples (P4 assay)

2 pregnancy diagnoses





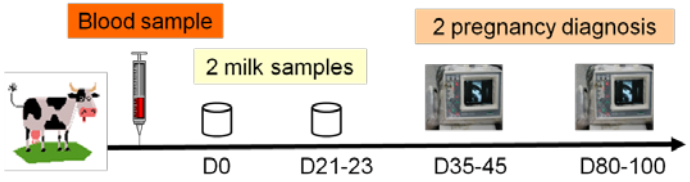




D0 D21-23 D35-45 D80-100

→ 4559 females

Phenotyping



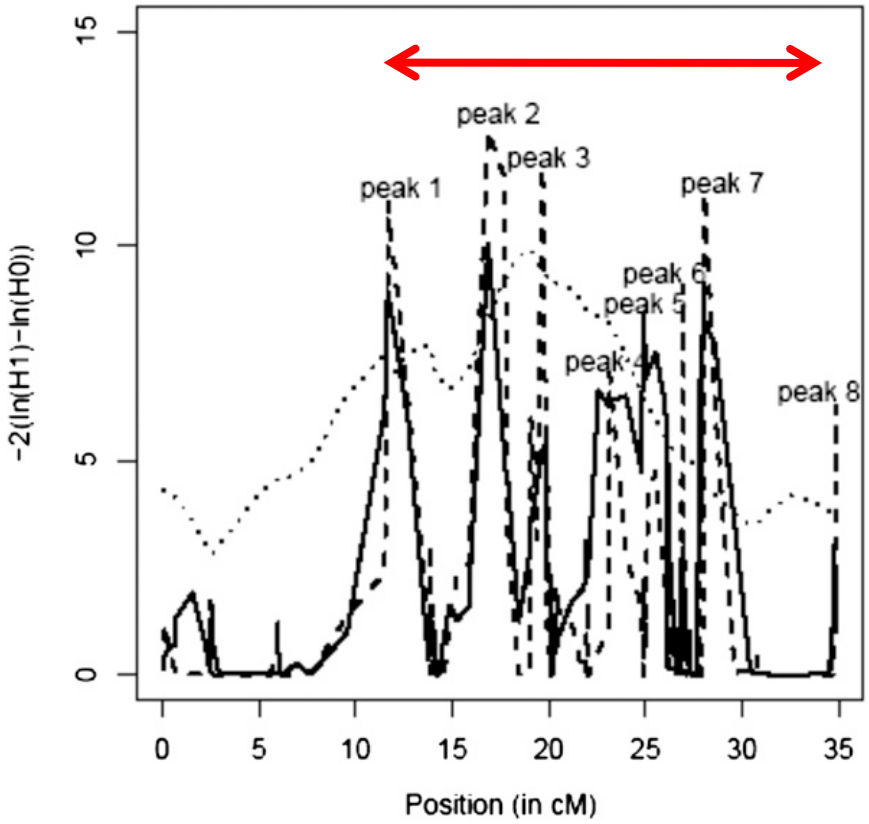
P4 assay		Pregnancy diagnosis		Calving date	Interpretation
D0	D21-23	D40	D90		
+					Insemination Out of Estrus (**IOE**)
-	-				No Fertilization or Early Embryonic Mortality (**NF-EEM**)
-	+	Open			Late Embryonic Mortality (**LEM**)
-	+	Pregnant	Open		Fetal Mortality (**FM**)
-	+	Pregnant	Pregnant	No	Abortion
-	+	Pregnant	Pregnant	Yes	Successful Pregnancy

Genotyping

- Creation of a customized Golden Gate chip in collaboration with LABOGENA

- 353 SNP chip
  - 16 regions of presumed fertility QTL in Holstein breed on 13 chromosomes
  - 120 SNP on chromosome 3

Genotyping



(Druet *et al*., 2008)

CARTOFINE project :

QTL mapping of chromosome 3

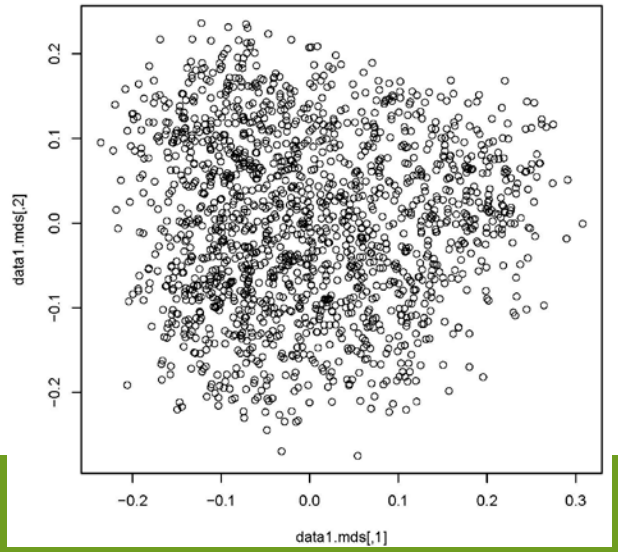
→ 96 SNP in 10-35 cM region

Data Quality Check

Elimination criteria	Threshold	Deleted
MAF	0.03	8
SNP Call Rate	0.95	39
Individual Call Rate	0.95	118
IBS	0.95	27

} SNP used = 306

} Females used = 2669



⇒ No population stratification

QTL detection method

- GenABEL (package of R) → Fasta method
  - o Genome wide association analysis
  - o Marker by marker
  - o Mixed model = environment fixed effects + genetic random effects
  - o Family information

- Linkage Disequilibrium and Linkage Analysis

  → *not shown here*

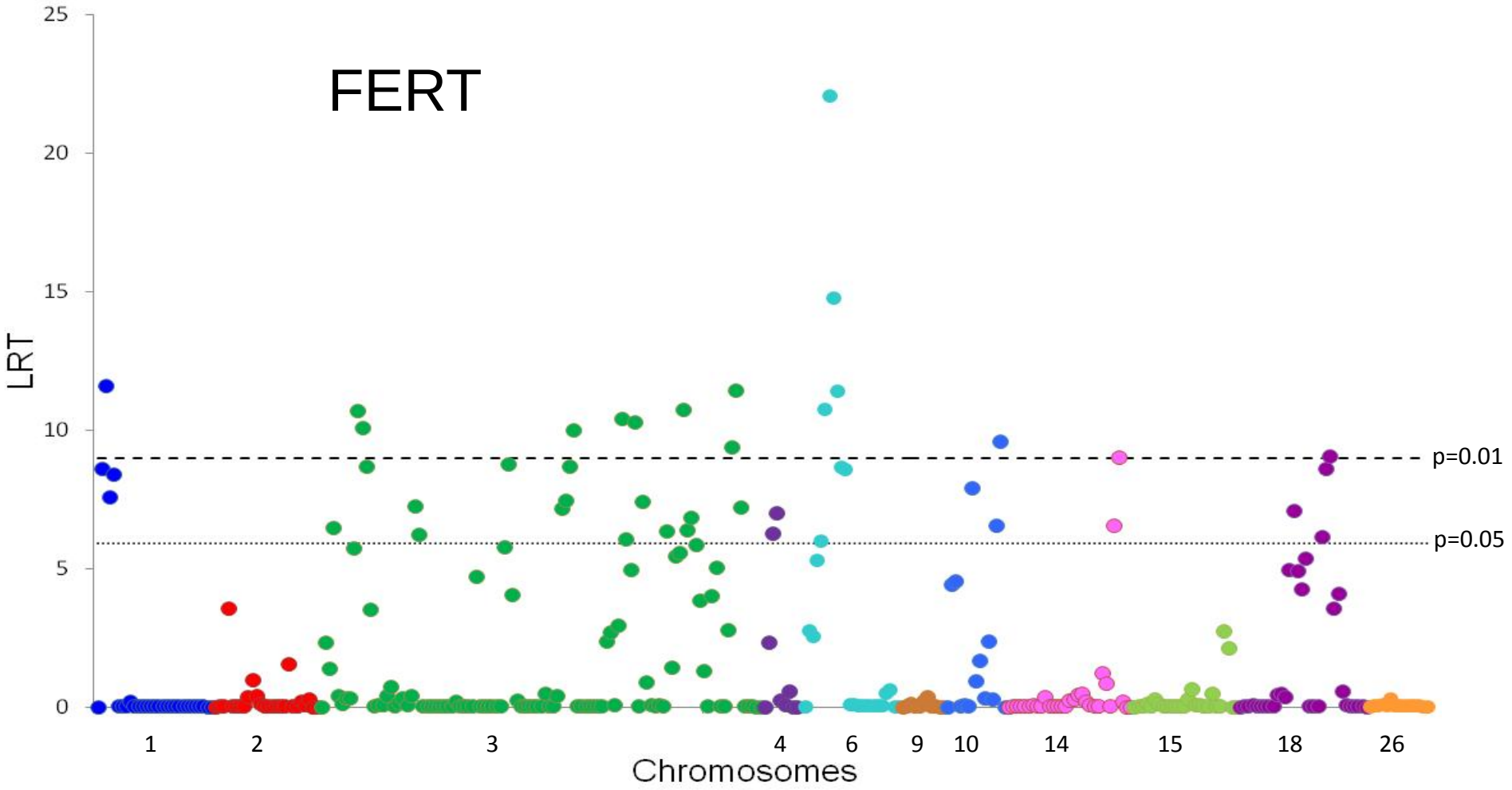
QTL detection method

- One overall trait : FERT
  - o average fertility over all inseminations in the career
  - o derived from the national evaluation (YD)

- 7 analytical traits characterizing the first AI:
  - o IOE, NF-EEM, LEM, FM, Abortion
  - o EM (Total Embryonic Mortality)
  - o Failure (whatever the stage)

→ Correction for herd and cow parity

QTL Validation



→ Presence of QTL confirmed in most regions

Results - Components of the first AI

Trait	N	Heritability	Detected SNP	
			p < 0.05	p < 0.01
Failure	2548	0.020	17	6
IOE	2641	0.003	21	5
NF-EEM	2520	0.023	20	3
LEM	1572	0.031	26	3
EM	2520	0.023	24	8
FM	1222	0.007	16	2
Abortion	1196	0.001	11	3

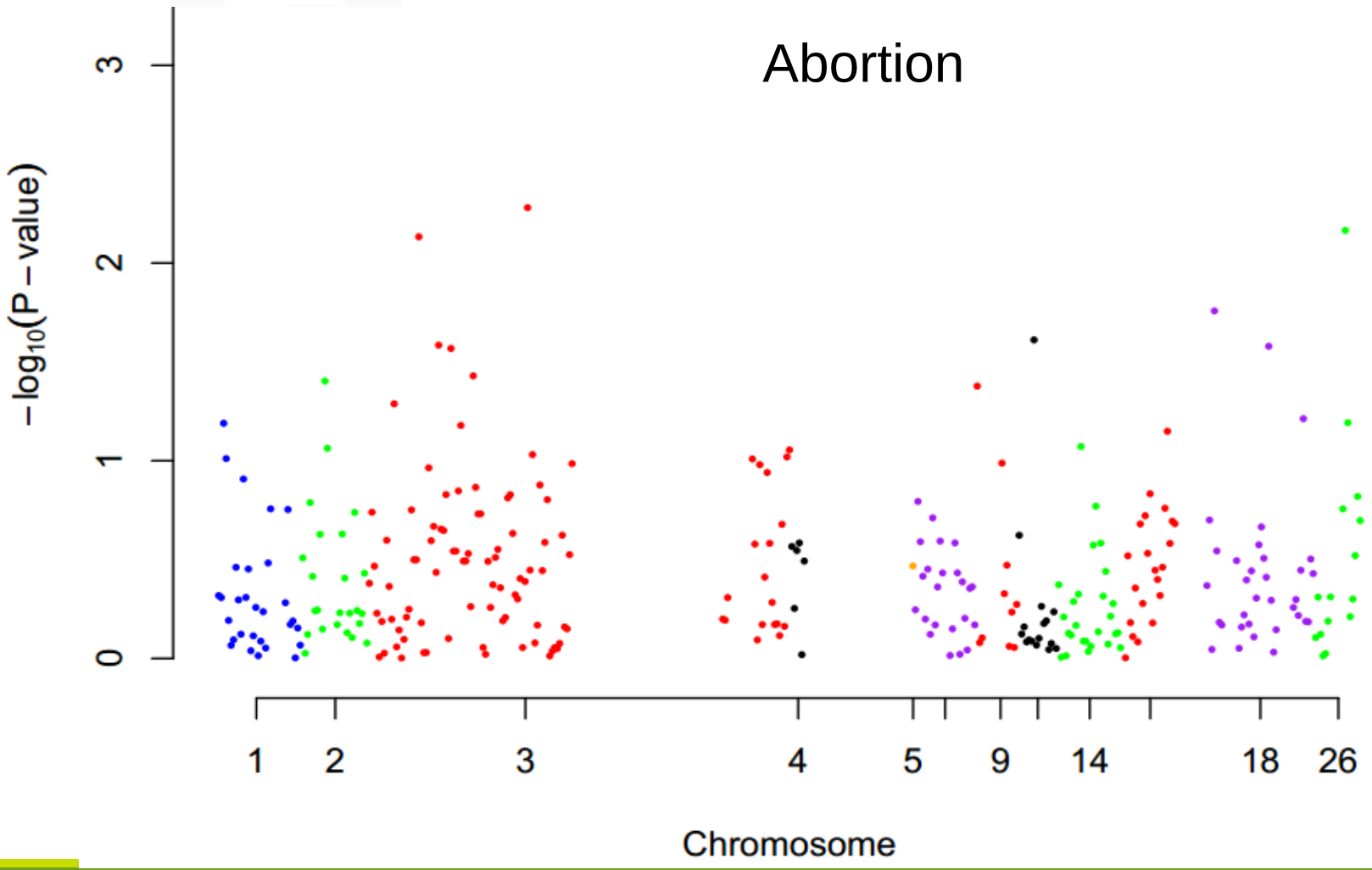
Results - Components of the first AI

BTA	Position (Mb)	Failure	IOE	NF-EEM	LEM	EM	FM	Abortion
1	6	X			x	X		
	8	X			x	X	x	
2	8			X		x		x
3	14-15				x			X
	20-26				X	x		X
	27			X				
	102		X	x	x			
4	68	X	x	x	x	X		
6	93	x		x	X	X	x	
	96-97	X	x	X	x	X	x	
9	67		X					
10	25		X					
14	26		x				X	
	28-30						X	
18	48	X		x		X		
26	40							X

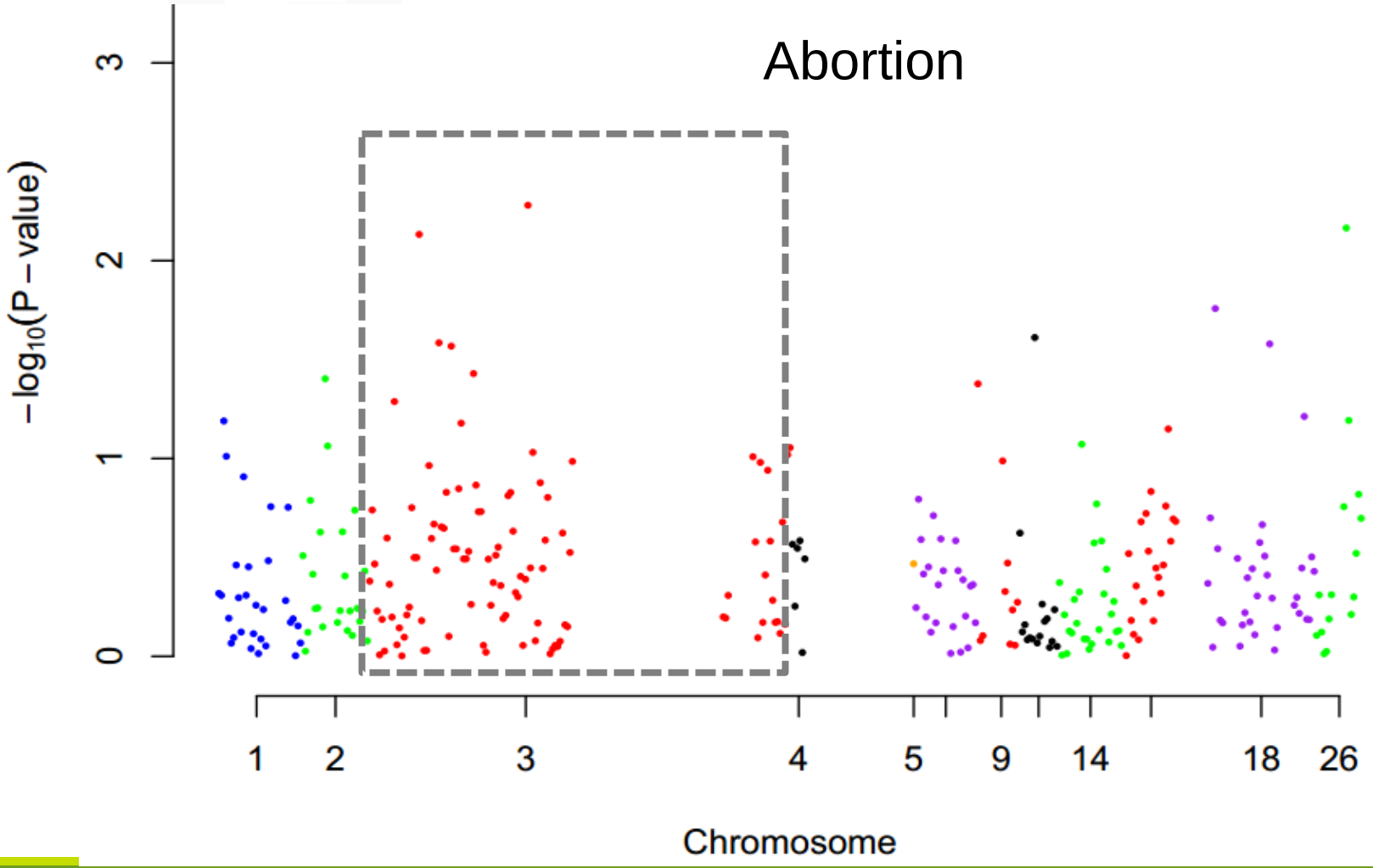
X region detected with p<0.01

x region detected with p<0.05

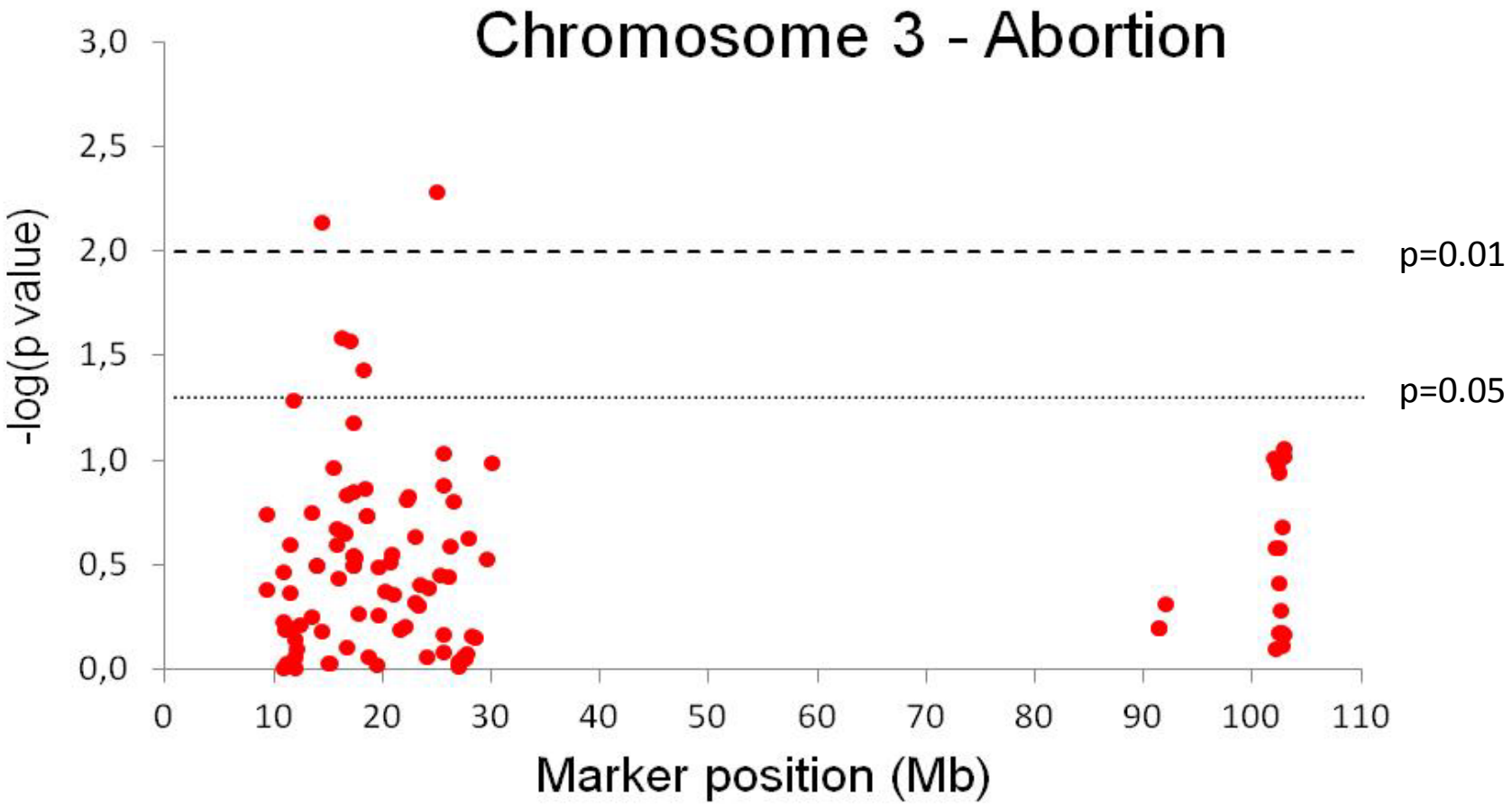
Results - Components of the first AI



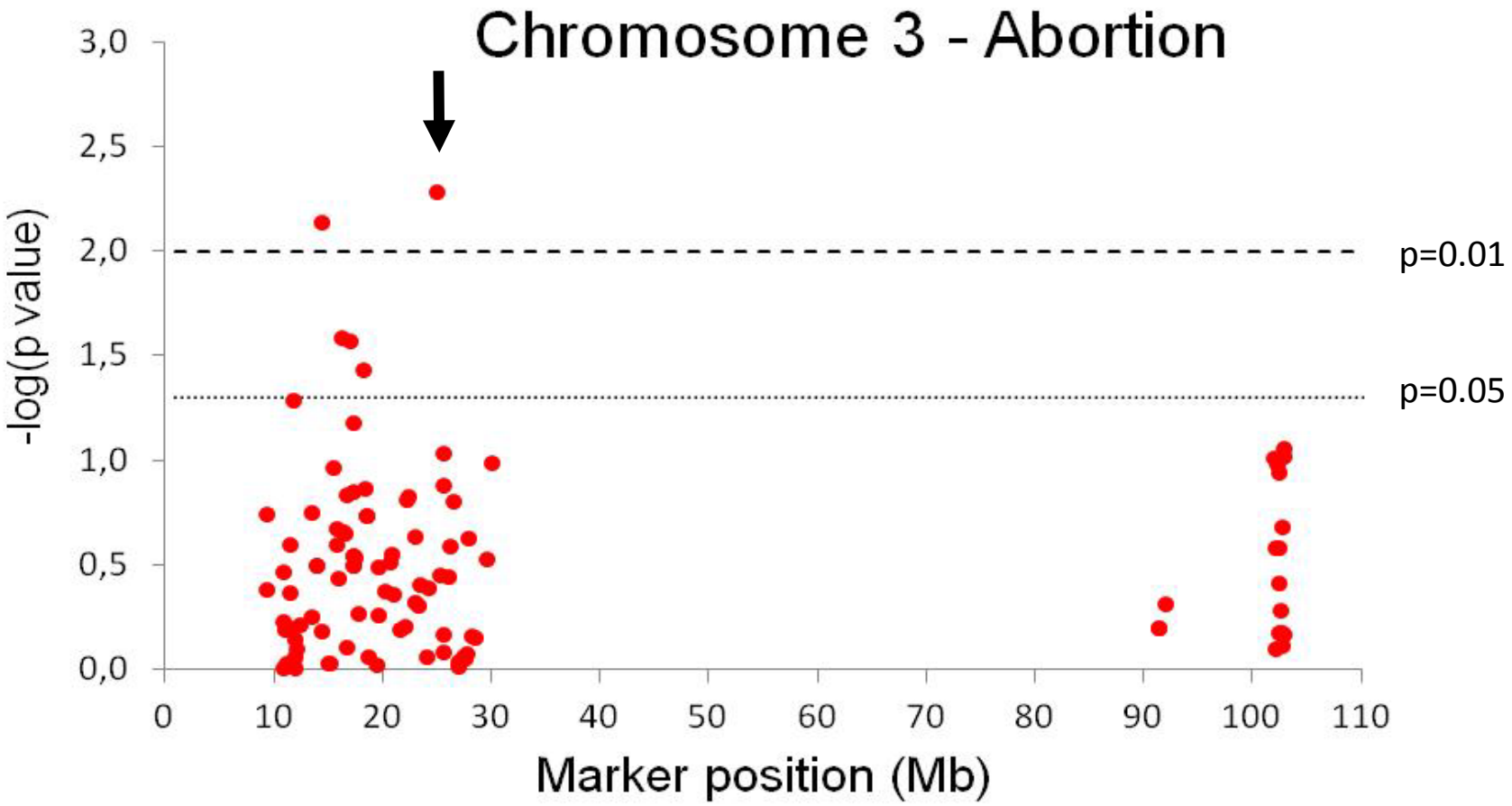
Results - Components of the first AI



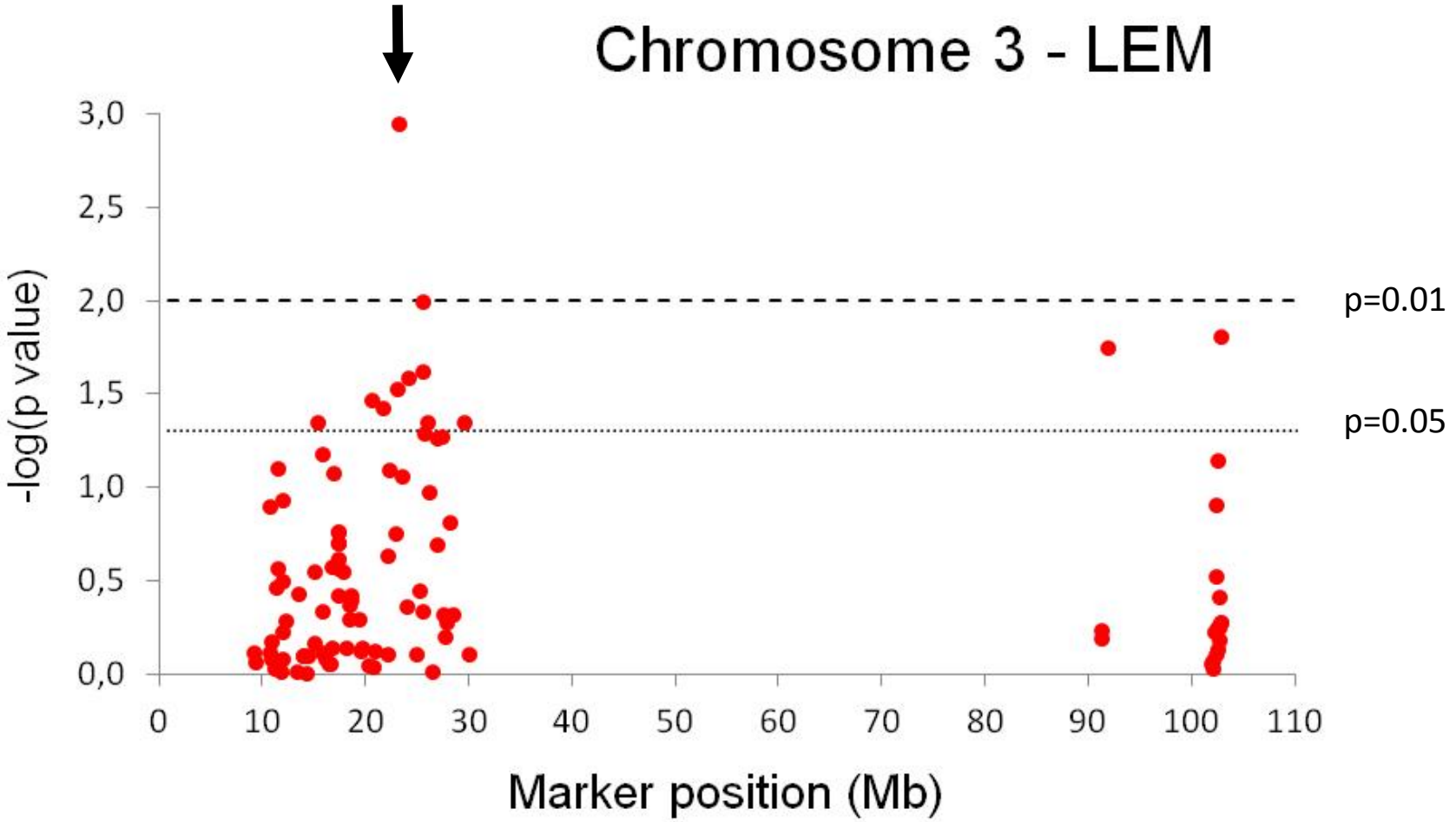
Results - Components of the first AI



Results - Components of the first AI



Results - Components of the first AI



Conclusions

- Previously detected regions are confirmed by our results of FERT

- Haplotype analysis (LDLA) confirmed the results

- For each component trait: 2 to 8 SNP with a significant effect

Conclusions

- Chromosome 3 : likely several different QTL
  - 23-27 Mb: NF-EEM, LEM and Abortion
  - 14 Mb: Abortion
  - 102 Mb: IOE

➢ Next step for fertility studies: Sire sequencing

→ Link with daughters phenotypes?

→ Candidate polymorphisms?

Acknowledgements

- Colleagues who initiate the project

- ANR and Apis-Gene for financial support

INRA
SCIENCE & IMPACT

Thank you for your attention