Novel *BMP15* Mutations Responsible for an Atypical Hyperprolificacy Phenotype in Sheep

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Sheep as a valuable model to study prolificacy

Gene (Chromosome)	Mutation	Phenotype			Founder Breed	Reference
		Name (Fecundity)	Heterozygous	Homozygous		
***BMP15* (OARX)**	V299D	Inverdale, *FecX*^{I}	Hyper prolific	Sterile	Romney	*Galloway et al.*
	Q291ter	Hanna, *FecX*^{H}	Hyper prolific	Sterile	Romney	*Galloway et al.*
	S367I	Belclare, *FecX*^{B}	Hyper prolific	Sterile	Belclare	*Hanrahan et al.*
	Q239ter	Galway, *FecX*^{G}	Hyper prolific	Sterile	Belclare and Cambridge	*Hanrahan et al.*
	C321Y	Lacaune, *FecX*^{L}	Hyper prolific	Sterile	Lacaune	*Bodin et al.*
	ΔP154S159	Rasa Aragonesa, *FecX*^{R}	Hyper prolific	Sterile	Rasa Aragonesa	*Martinez-Royo et al.; Monteagudo et al.*
***BMPR1B* (OAR6)**	Q249R	Booroola, *FecB*^{B}	**Hyper prolific**	**Hyper prolific**	Booroola Merino, Garole and Javanese	*Mulsant et al.; Souza et al.; Wilson et al.*
***GDF9* (OAR5)**	S395F	High Fertility, *FecG*^{H}	Hyper prolific	Sterile	Belclare and Cambridge	*Hanrahan et al.*
	S427R	Thoka, *FecG*^{T}	Hyper prolific	Sterile	Icelandic	*Nicol et al.*
	F345C	Embrapa, *FecG*^{E}	**Hyper prolific**	**Hyper prolific**	Santa Inês	*Silva et al.*
***β4GALNT2* (OAR11)**	g.36938224T>A g.37034573A>G	Lacaune, *FecL*	**Hyper prolific**	**Hyper prolific**	Lacaune	*Drouilhet et al.*

Sheep as a valuable model to study prolificacy

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		Name (Fecundity)	**Heterozygous**	**Homozygous**		
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	Q291ter	Hanna, $FecX^{H}$	Hyper prolific	Sterile	Romney	*Galloway et al.*
	S367I	Belclare, $FecX^{B}$	Hyper prolific	Sterile	Belclare	*Hanrahan et al.*
	Q239ter	Galway, $FecX^{G}$	Hyper prolific	Sterile	Belclare and Cambridge	*Hanrahan et al.*
	C321Y	Lacaune, $FecX^{L}$	Hyper prolific	Sterile	Lacaune	*Bodin et al.*
	ΔP154S159	Rasa Aragonesa, $FecX^{R}$	Hyper prolific	Sterile	Rasa Aragonesa	*Martinez-Royo et al.; Monteagudo et al.*
***BMPR1B* (OAR6)**	Q249R	Booroola, $FecB^{B}$	**Hyper prolific**	**Hyper prolific**	Booroola Merino, Garole and Javanese	*Mulsant et al.; Souza et al.; Wilson et al.*
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	S427R	Thoka, $FecG^{T}$	Hyper prolific	Sterile	Icelandic	*Nicol et al.*
	F345C	Embrapa, $FecG^{E}$	**Hyper prolific**	**Hyper prolific**	Santa Inês	*Silva et al.*
***β4GALNT2* (OAR11)**	g.36938224T>A g.37034573A>G	Lacaune, *FecL*	**Hyper prolific**	**Hyper prolific**	Lacaune	*Drouilhet et al.*

Description of the study design -1

➢ Populations

- French **Grivette** breed
- Polish **Olkuska** breed

➢ Major gene for ovulation suspected

- Litter Size (LS) measured in Grivette : **High LS≥2.7** *vs.* **Normal LS≤1.8**
- Ovulation Rate (OR) measured in Olskuska : **High OR≥3.0** *vs.* **Normal OR≤2.0**

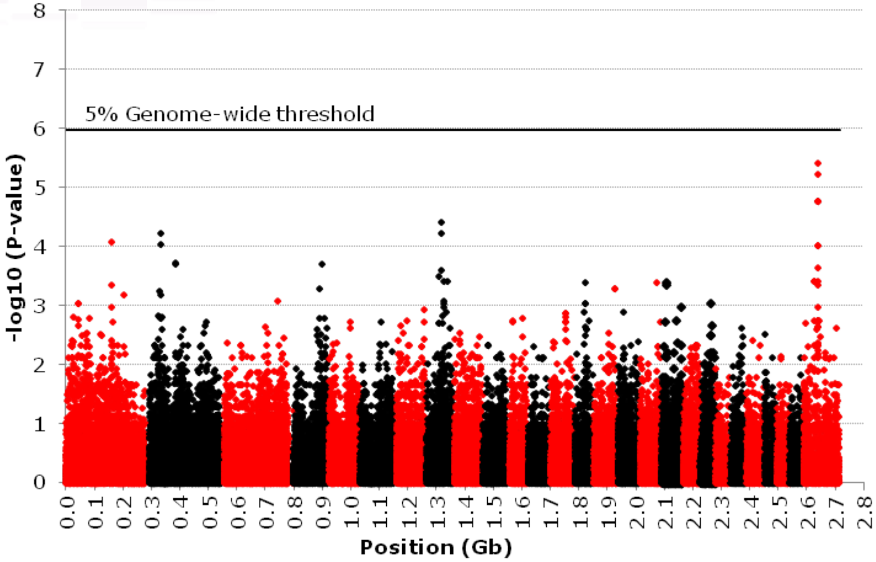
➢ Case (hyper prolific) *vs.* Control (normal) design

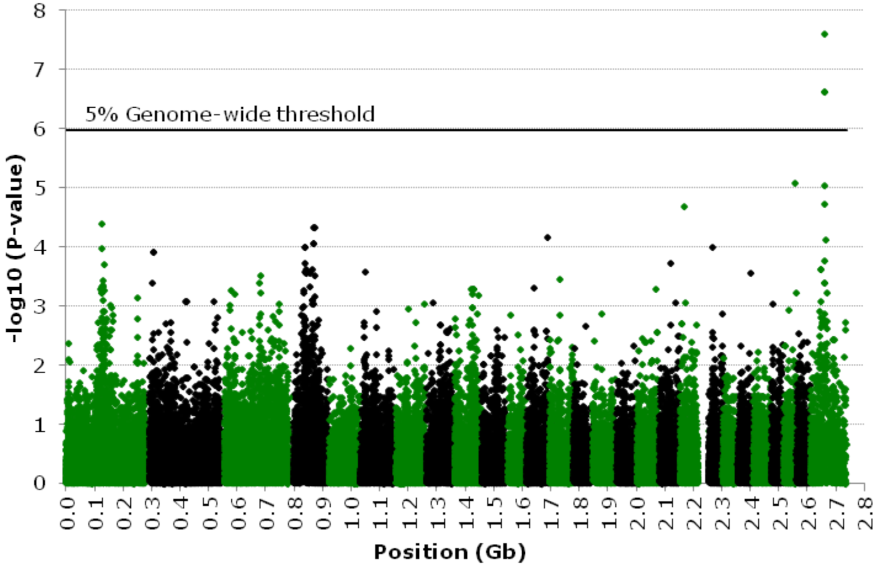
- Genotyping using the **54K SNP array**
- Filtering for Call Rate (>0.99), Call Freq (>0.98), HWE ($\leq 10^{-6}$) and MAF (<0.01)
- Comparison of allelic frequencies

Description of the study design - 2

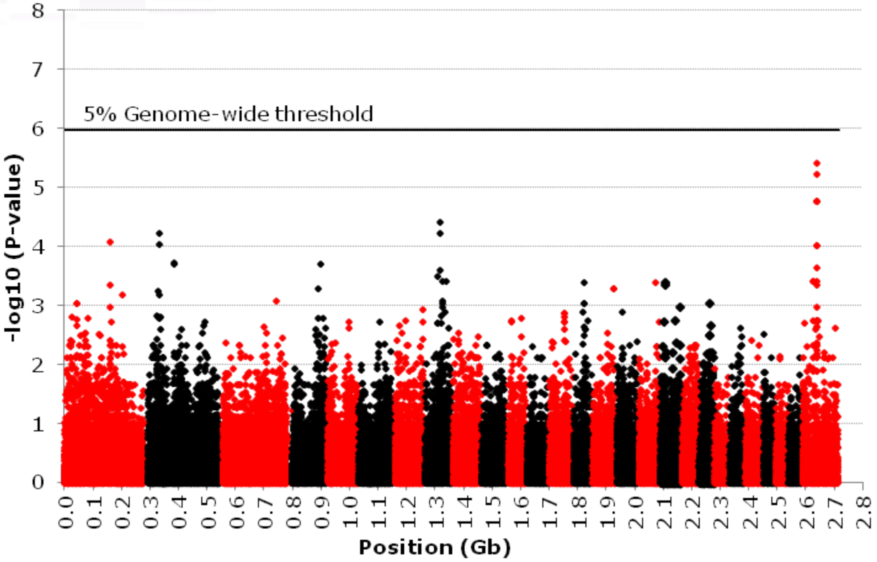
	Initial Design		Final Design	
	Grivette	Olkuska	Grivette	Olkuska
Individuals (n)				
Total	40	64	39	63
Control	11	35	11	34
Case	29	29	28	29
Call Rate < 0,98	1	1		
Phenotype	*Litter Size*	*Ovulation Rate*	*Litter Size*	*Ovulation Rate*
Total	2,75 (0,88)	2,75 (1,40)	2,75 (0,89)	2,77 (1,40)
Control	1,51 (0,27)	1,60 (0,30)	1,51 (0,27)	1,61 (0,30)
Case	3,22 (0,46)	4,14 (0,81)	3,24 (0,46)	4,14 (0,81)
Markers				
Total	54241	54241	47290	46451
Call Freq < 0,98	4142	3595		
Position Pb	84	84		
HWE < 1E-06	0	159		
MAF < 0,01	2725	3952		

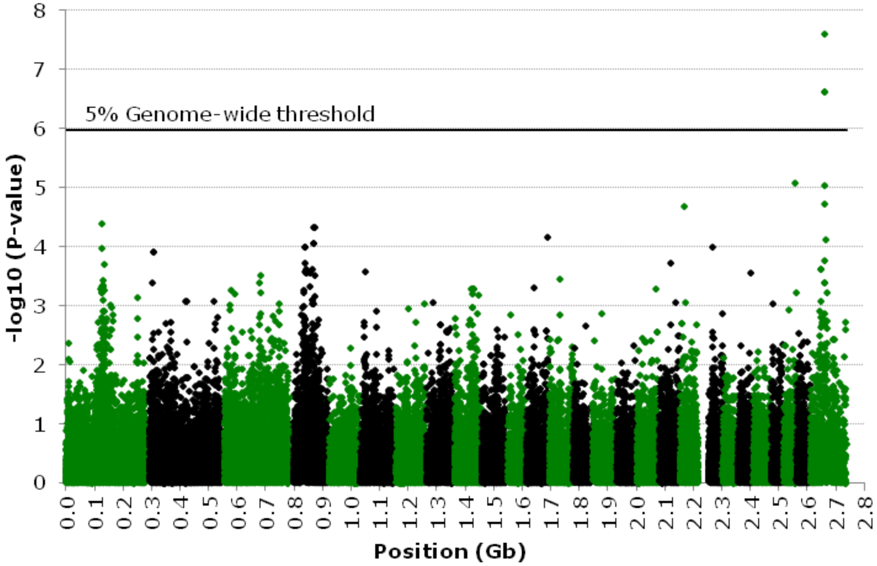
Genome Wide Association Study

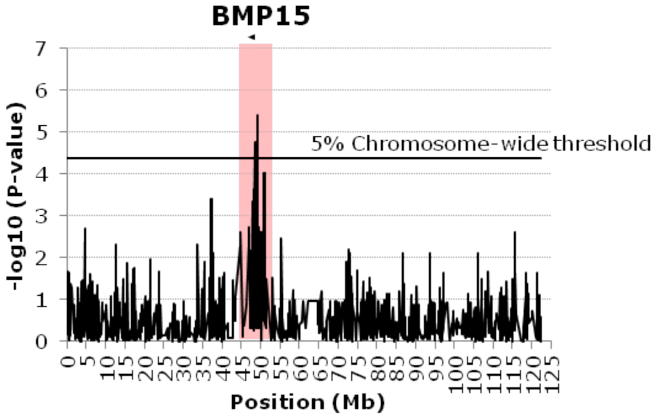


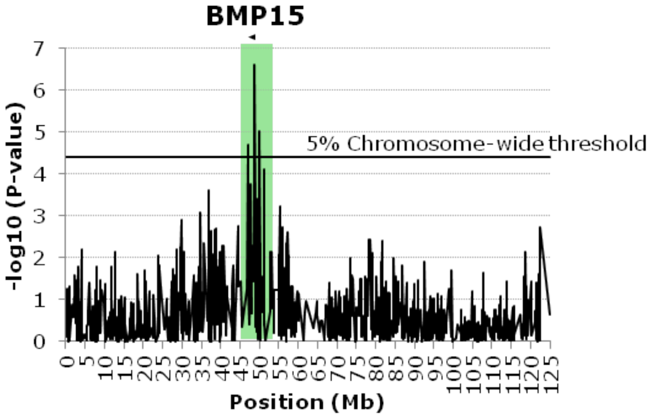


Genome Wide Association Study

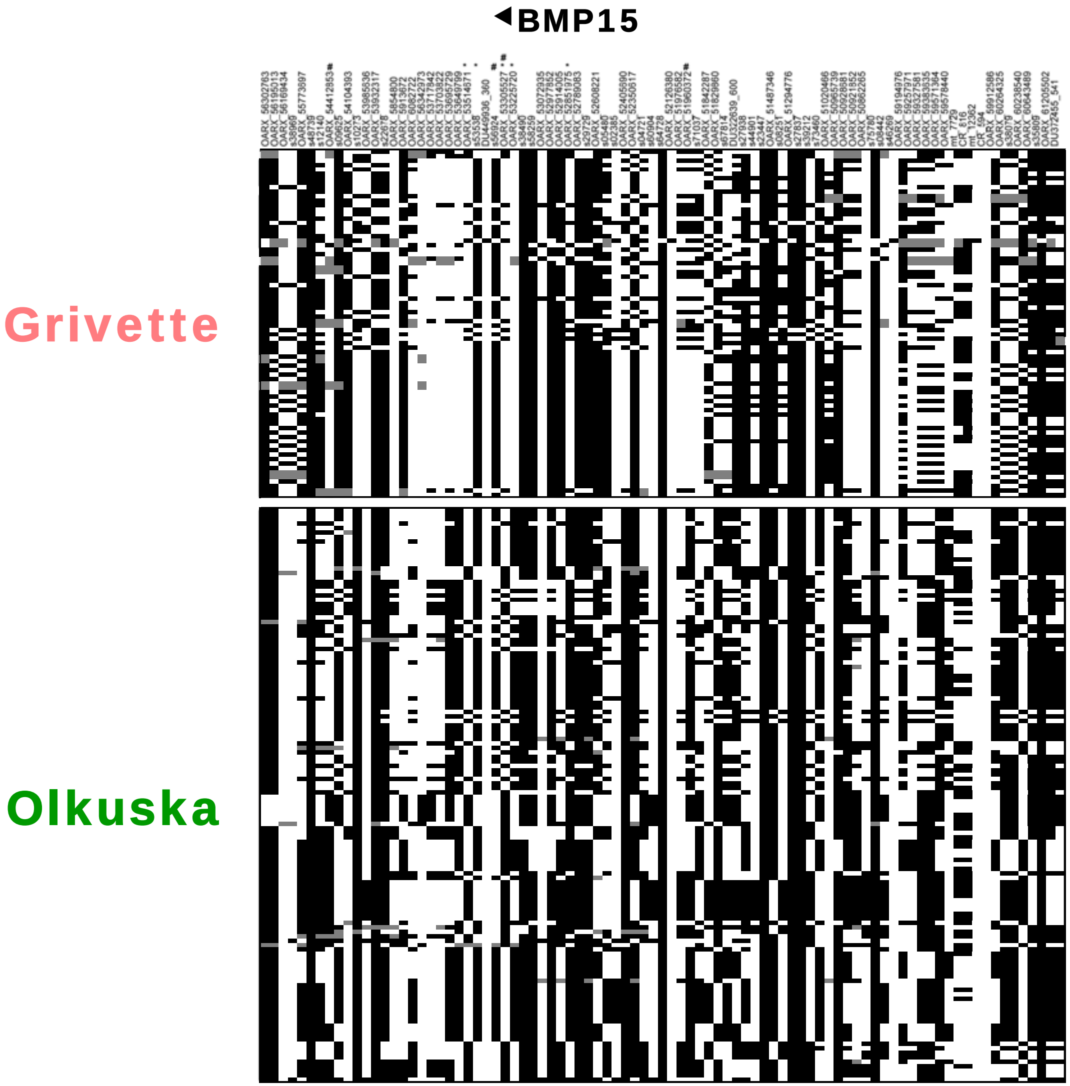








Haplotypic clustering and association



Haplotypic clustering and association

1.3Mb

$Fr_{case}=0.95$ *vs.* $Fr_{control}=0.54$

$p_{corrected}=9.90E^{-03}$

1.3Mb

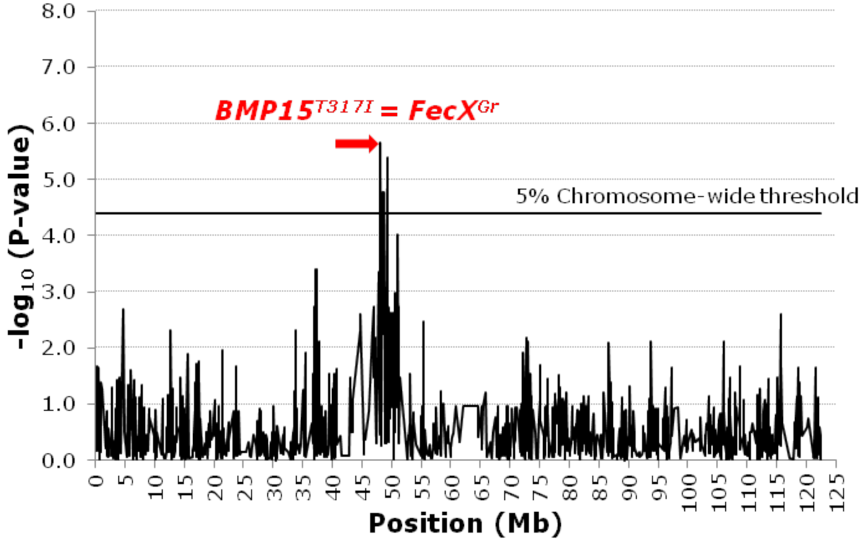
$Fr_{case}=0.76$ *vs.* $Fr_{control}=0.27$

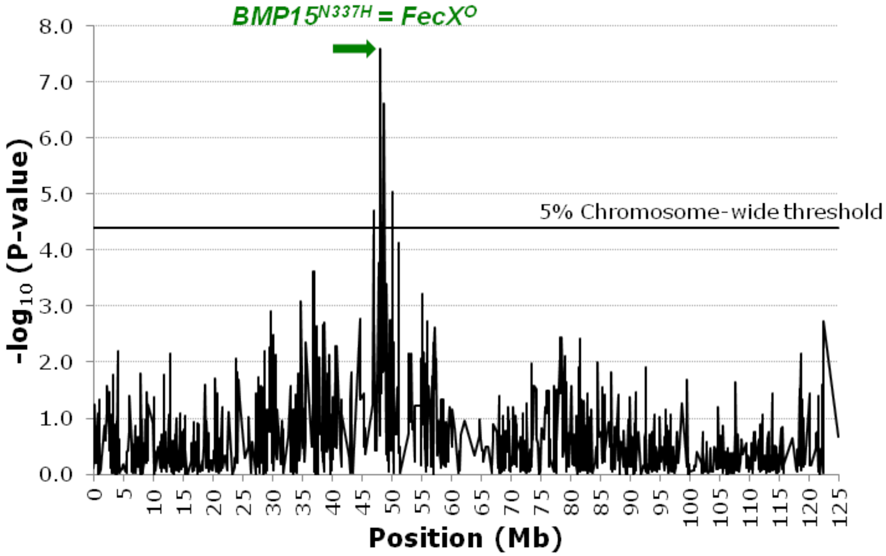
$p_{corrected}=9.90E^{-04}$

BMP15 coding sequence polymorphisms

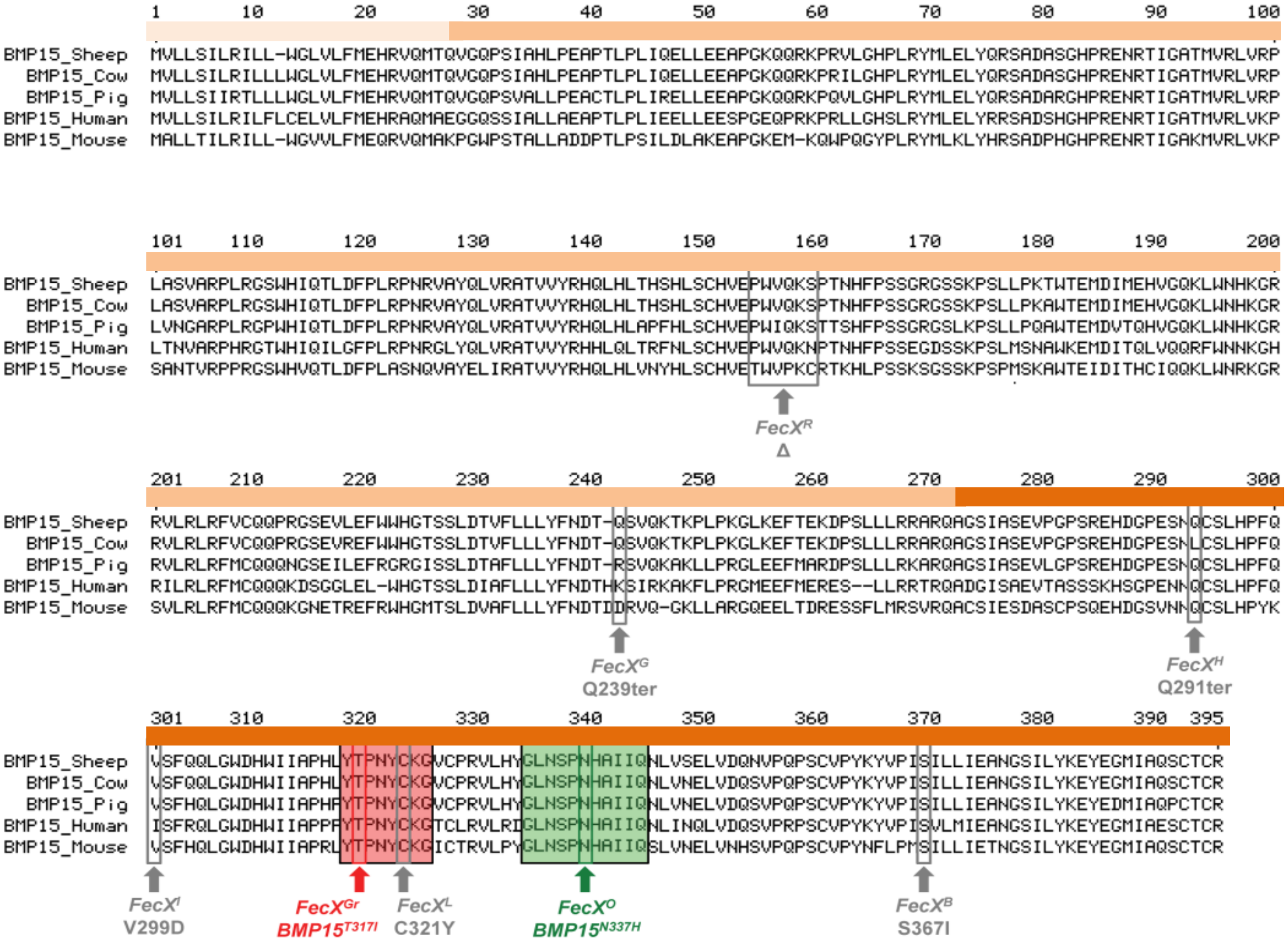
Variant	Base change	Coding Base (bp)	Coding residue (bp)	Amino Acid Change	Genotype					
Grivette						*Controls*			*Cases*	
ΔL10	ΔCTT	28-30	10	Deletion Leu	-/- (6)	-/+ (5)	+/+ (0)	-/- (27)	-/+ (1)	+/+ (0)
P249P	C>T	747	249	Unchanged Pro	C/C (0)	C/T (0)	T/T (11)	C/C (0)	C/T (0)	T/T (28)
T317I=$FecX^{Gr}$	**C>T**	**950**	**317**	**Thr>Ile**	**C/C (2)**	**C/T (6)**	**T/T (3)**	**C/C (0)**	**C/T (2)**	**T/T (26)**
Olkuska										
ΔL10	ΔCTT	28-30	10	Deletion Leu	-/- (0)	-/+ (7)	+/+ (26)	-/- (0)	-/+ (0)	+/+ (29)
V135G	T>G	404	135	Val>Gly	T/T (33)	T/G (1)	G/G (0)	T/T (29)	T/G (0)	G/G (0)
N337H=$FecX^{O}$	**A>C**	**1009**	**337**	**Asn>His**	**A/A (16)**	**A/C (18)**	**C/C (0)**	**A/A (1)**	**A/C (9)**	**C/C (19)**

Novel *BMP15 Fecundity* mutations - 1





Novel *BMP15 Fecundity* mutations - 2



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              1         10        20        30        40        50        60        70        80        90        100
BMP15_Sheep   MVLLSILRILL-WGLVLFMEHRVQMTQVGQPSIAHLPEAPTLPLIQELLEEAPGKQQRKPRVLGHPLRYMLELYQRSADASGHPRENRTIGATMVRLVRP
  BMP15_Cow   MVLLSILRILLLWGLVLFMEHRVQMTQVGQPSIAHLPEAPTLPLIQELLEEAPGKQQRKPRILGHPLRYMLELYQRSADASGHPRENRTIGATMVRLVRP
  BMP15_Pig   MVLLSIIRTLLLWGLVLFMEHRVQMTQVGQPSVALLPEACTLPLIRELLEEAPGKQQRKPQVLGHPLRYMLELYQRSADARGHPRENRTIGATMVRLVRP
BMP15_Human   MVLLSILRILFLCELVLFMEHRAQMAEGGQSSIALLAEAPTLPLIEELLEESPGEQPRKPRLLGHSLRYMLELYRRSADSHGHPRENRTIGATMVRLVKP
BMP15_Mouse   MALLTILRILL-WGVVLFMEQRVQMAKPGWPSTALLADDPTLPSILDLAKEAPGKEM-KQWPQGYPLRYMLKLYHRSADPHGHPRENRTIGAKMVRLVKP

              101      110       120       130       140       150       160       170       180       190       200
BMP15_Sheep   LASVARPLRGSWHIQTLDFPLRPNRVAYQLVRATVVYRHQLHLTHSHLSCHVEPWVQKSPTNHFPSSGRGSSKPSLLPKTWTEMDIMEHVGQKLWNHKGR
  BMP15_Cow   LASVARPLRGSWHIQTLDFPLRPNRVAYQLVRATVVYRHQLHLTHSHLSCHVEPWVQKSPTNHFPSSGRGSSKPSLLPKAWTEMDIMEHVGQKLWNHKGR
  BMP15_Pig   LVNGARPLRGPWHIQTLDFPLRPNRVAYQLVRATVVYRHQLHLAPFHLSCHVEPWIQKSTTSHFPSSGRGSLKPSLLPQAWTEMDVTQHVGQKLWNHKGR
BMP15_Human   LTNVARPHRGTWHIQILGFPLRPNRGLYQLVRATVVYRHHLQLTRFNLSCHVEPWVQKNPTNHFPSSEGDSSKPSLMSNAWKEMDITQLVQQRFWNNKGH
BMP15_Mouse   SANTVRPPRGSWHVQTLDFPLASNQVAYELIRATVVYRHQLHLVNYHLSCHVETWVPKCRTKHLPSSKSGSSKPSPMSKAWTEIDITHCIQQKLWNRKGR

              201      210       220       230       240       250       260       270       280       290       300
BMP15_Sheep   RVLRLRFVCQQPRGSEVLEFWWHGTSSLDTVFLLLYFNDT-QSVQKTKPLPKGLKEFTEKDPSLLLRRARQAGSIASEVPGPSREHDGPESNQCSLHPFQ
  BMP15_Cow   RVLRLRFVCQQPRGSEVREFWWHGTSSLDTVFLLLYFNDT-QSVQKTKPLPKGLKEFTEKDPSLLLRRARQAGSIASEVPGPSREHDGPESNLCSLHPFQ
  BMP15_Pig   RVLRLRFMCQQQNGSEILEFRGRGISSLDTAFLLLYFNDT-RSVQKAKLLPRGLEEFMARDPSLLLRKARQAGSIASEVLGPSREHDGPESNQCSLHPFQ
BMP15_Human   RILRLRFMCQQQKDSGGLEL-WHGTSSLDIAFLLLYFNDTHKSIRKAKFLPRGMEEFMERES--LLRRTRQADGISAEVTASSSKHSGPENNQCSLHPFQ
BMP15_Mouse   SVLRLRFMCQQQKGNETREFRWHGMTSLDVAFLLLYFNDTIDRVQ-GKLLARGQEELTDRESSFLMRSVRQACSIESDASCPSQEHDGSVNNQCSLHPYK

              301      310       320       330       340       350       360       370       380       390  395
BMP15_Sheep   VSFQQLGWDHWIIAPHLYTPNYCKGVCPRVLHYGLNSPNHAIIQNLVSELVDQNVPQPSCVPYKYVPISILLIEANGSILYKEYEGMIAQSCTCR
  BMP15_Cow   VSFQQLGWDHWIIAPHLYTPNYCKGVCPRVLHYGLNSPNHAIIQNLVNELVDQSVPQPSCVPYKYVPISILLIEANGSILYKEYEGMIAQSCTCR
  BMP15_Pig   VSFHQLGWDHWIIAPHRYTPNYCKGVCPRVLHYGLNSPNHAIIQNLVNELVDQSVPQPSCVPYKYVPISILLIEANGSILYKEYEDMIAQPCTCR
BMP15_Human   ISFRQLGWDHWIIAPPRYTPNYCKGTCLRVLRIGLNSPNHAIIQNLINQLVDQSVPRPSCVPYKYVPISVLMIEANGSILYKEYEGMIAESCTCR
BMP15_Mouse   VSFHQLGWDHWIIAPRLYTPNYCKGICTRVLPYGLNSPNHAIIQSLVNELVNHSVPQPSCVPYNFLPMSILLIETNGSILYKEYEGMIAQSCTCR
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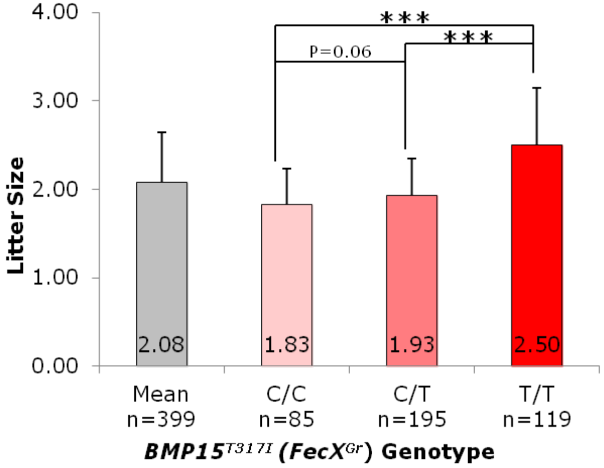
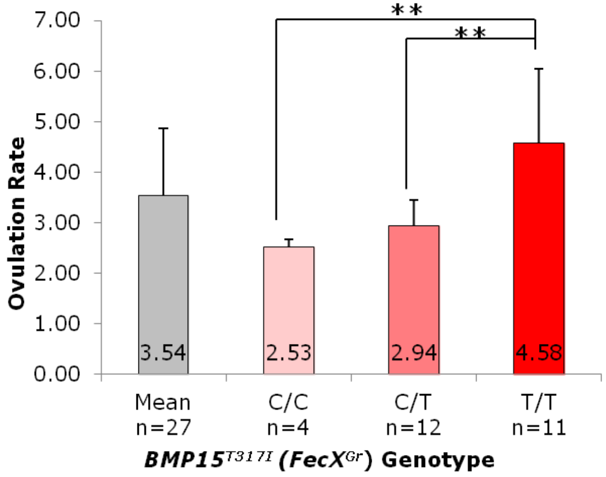
Effect of mutations on the prolificacy





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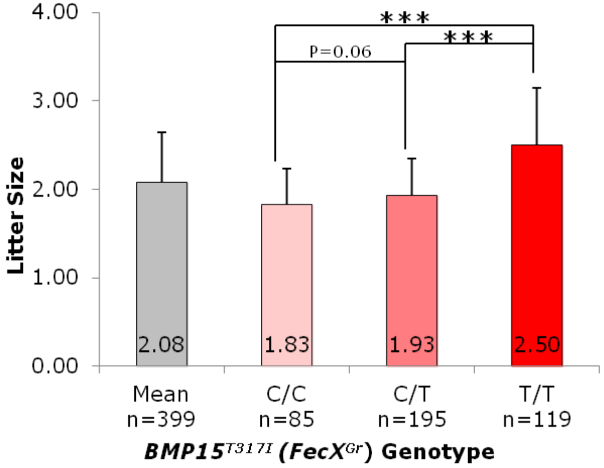
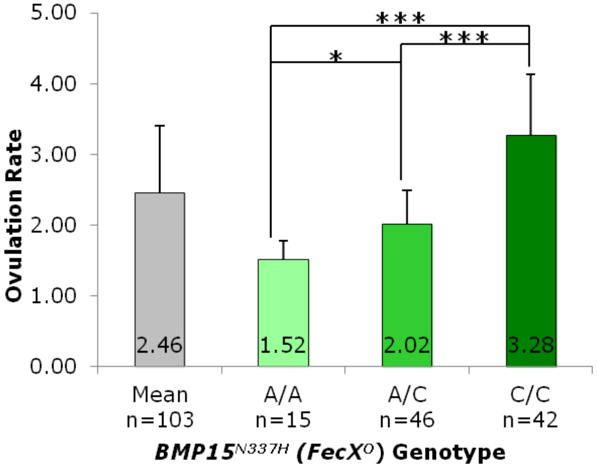
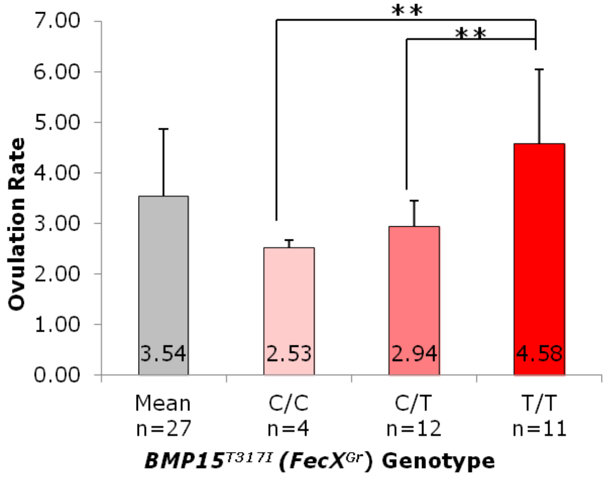
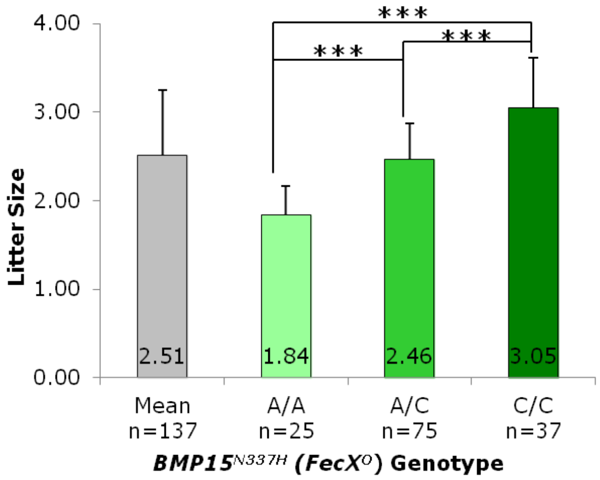
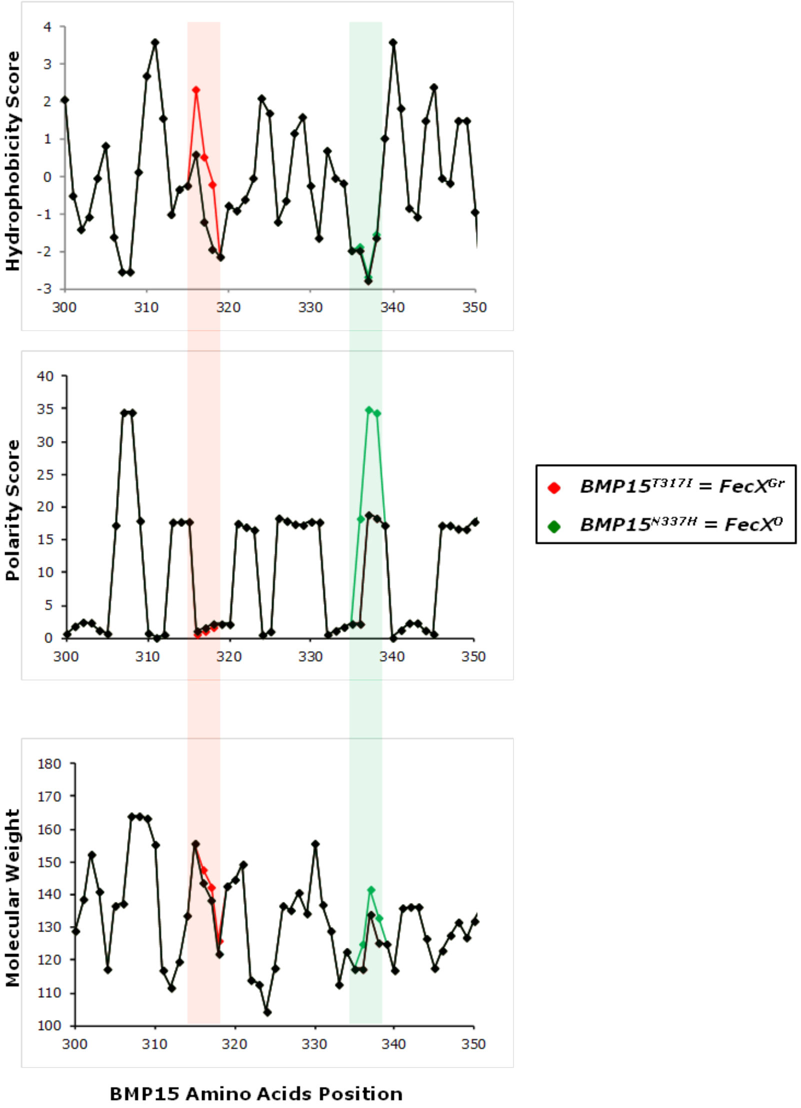




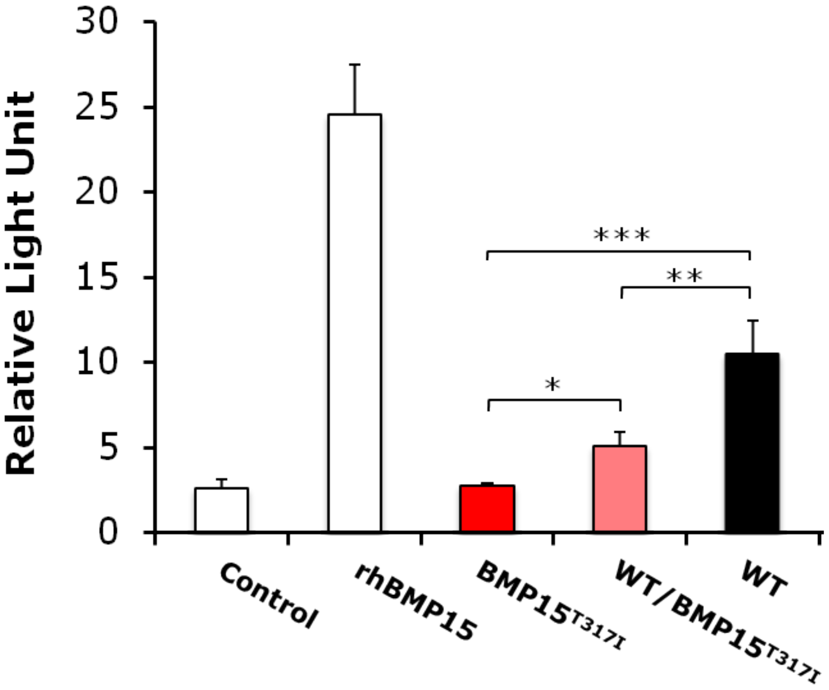




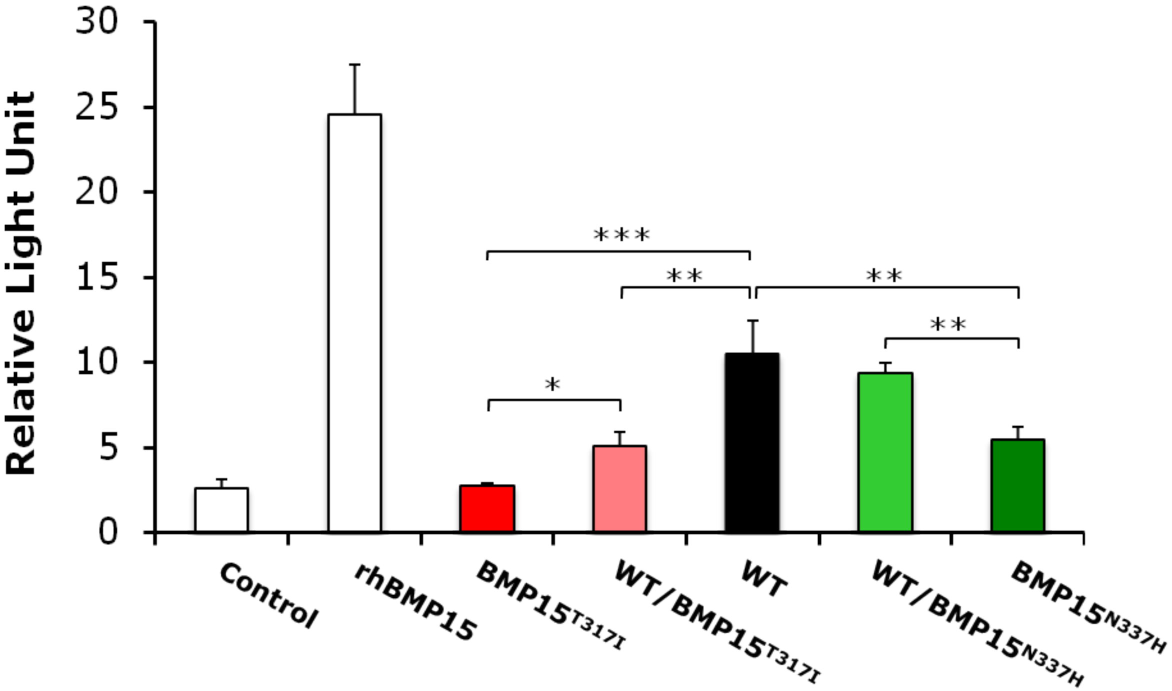
Effect of mutations on the BMP15 protein



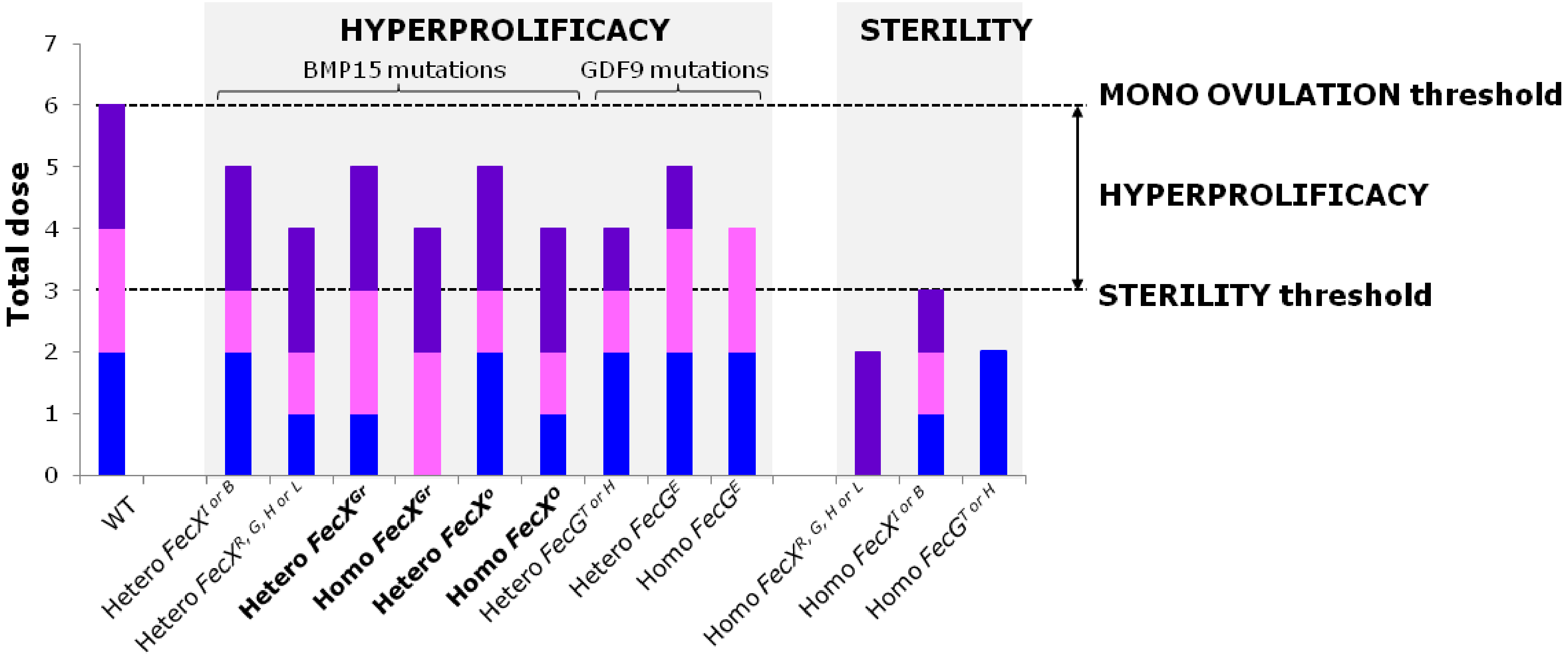
Effect of mutations on the BMP15 signaling



Effect of mutations on the BMP15 signaling



Model of the ovulation quota control



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