Why are commercial oocyte-secreted factors not helpful in IVF/IVM?

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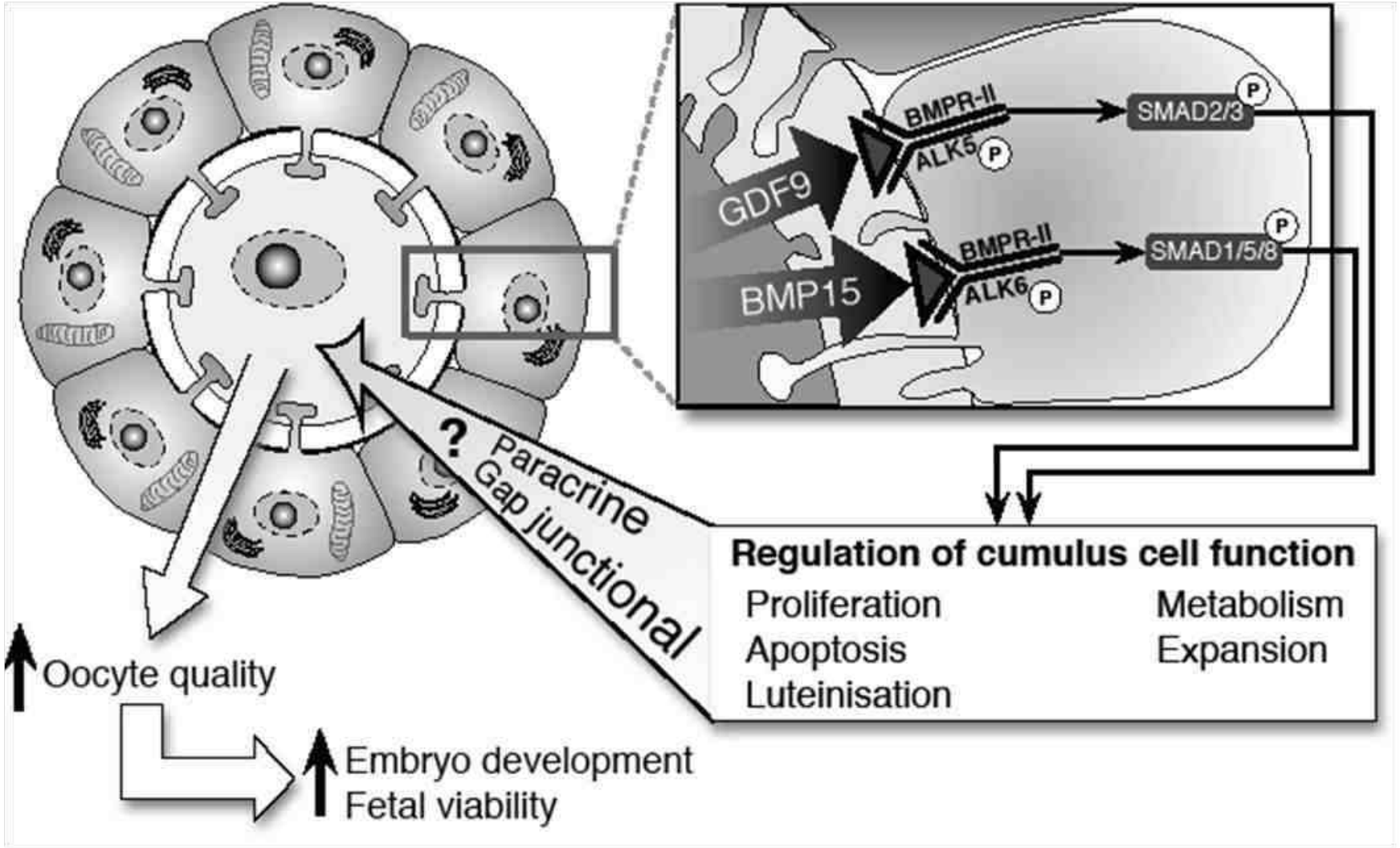
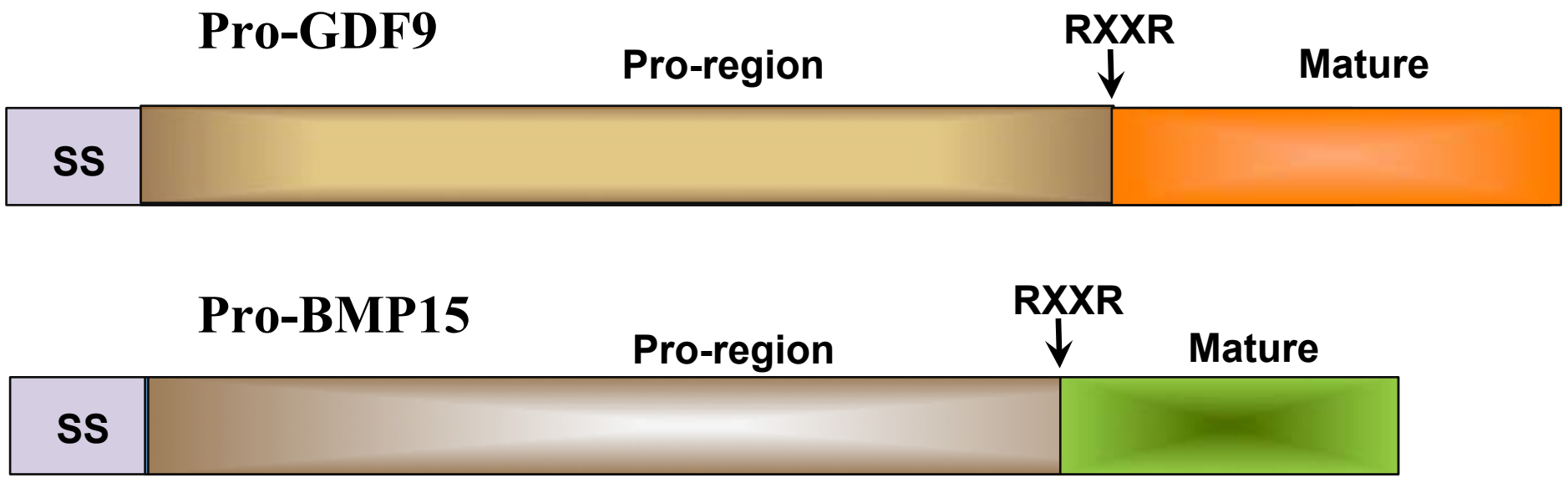
Oocyte-Secreted Factors GDF9 & BMP15 Regulate Granulosa Cells and Oocyte Developmental Competence



Gilchrist RB (2011) *Reprod. Fert. Dev.* 23:23-31

GDF9 and BMP15 as TGF-β family members



- TGF-β family mature regions normally form covalent dimers
- GDF9 & BMP15 lack 4th Cys of the 7 conserved Cys usually found in the mature region, hence they form non-covalent dimers

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Oocyte-secreted factors enhance oocyte developmental competence

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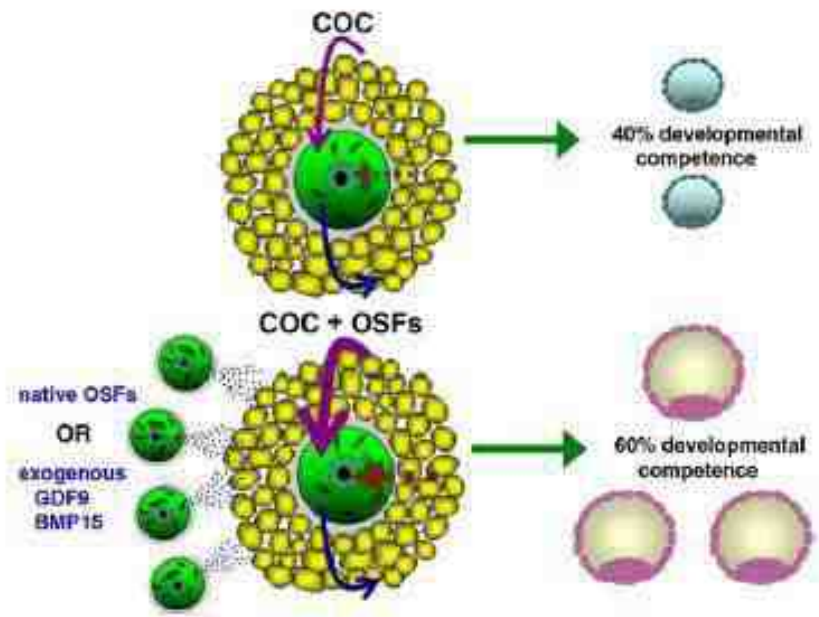
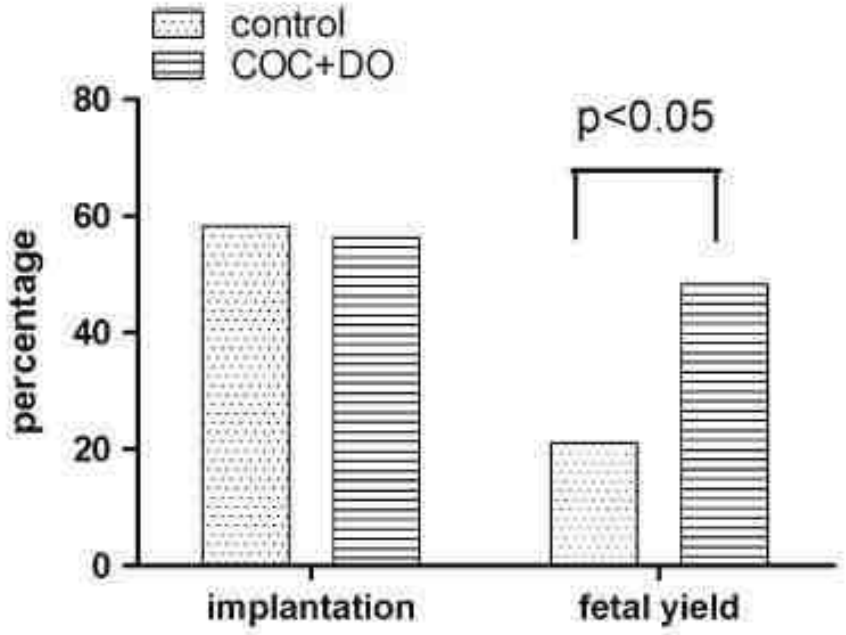


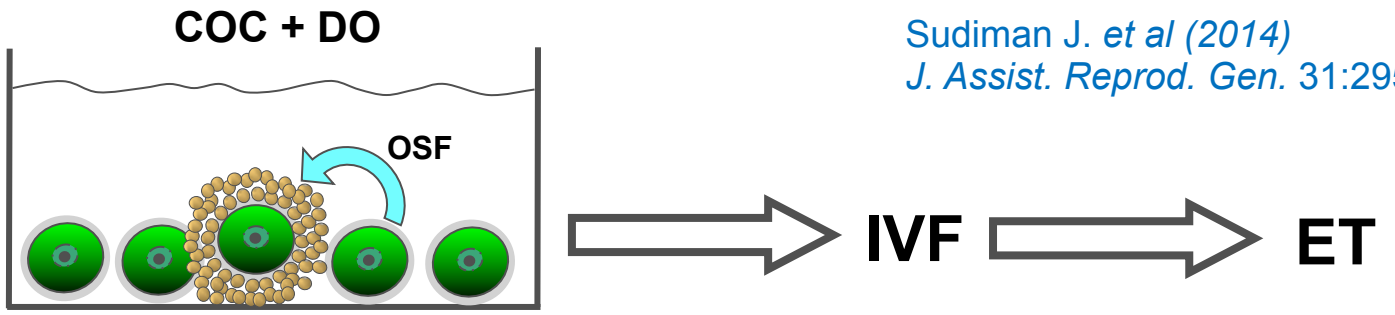
"Native" oocyte-secreted factors improve oocyte quality/ embryo/fetal development

- Bovine:
  - *Hussein TS et al 2006*
  - *Hussein TS et al 2011*
  - *Dey SR et al 2012*
  - *Sugimura S et al 2014*
  - *Su J et al 2014*
- Murine: *Sudiman J et al 2014*
- Caprine: *Romaguera R et al 2010*
- Porcine: *Gomez MNL et al 2012*



Fetal yield improvement in a mouse model

Sudiman J. *et al (2014)*
J. Assist. Reprod. Gen. 31:295-306



Commercial forms of GDF9 and/or BMP15 do not improve oocyte quality

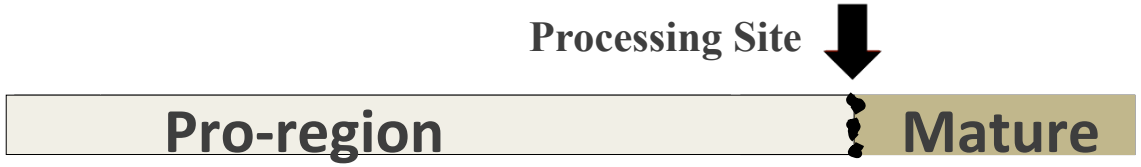
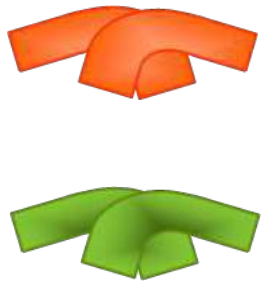


Table 3 Effect of graded doses of recombinant GDF9 during IVM on subsequent embryo development

Treatment	GDF9 (ng/ml)	Number of oocytes	Cleavage[a]	Blastocyst on day 6[b]	Hatching blastocyst on day 6[c]
Control	0	152	82.9±3.3	81.7±4.4	62.6±8.6
GDF9	50	118	74.6±5.2	72.6±8.9	61.8±8.9
GDF9	100	118	74.8±9.6	82.6±7.8	59.1±7.6
GDF9	200	147	86.1±4.7	82.5±8.3	64.3±7.1

Table 4 Effect of graded doses of BMP15 during IVM on subsequent embryo development

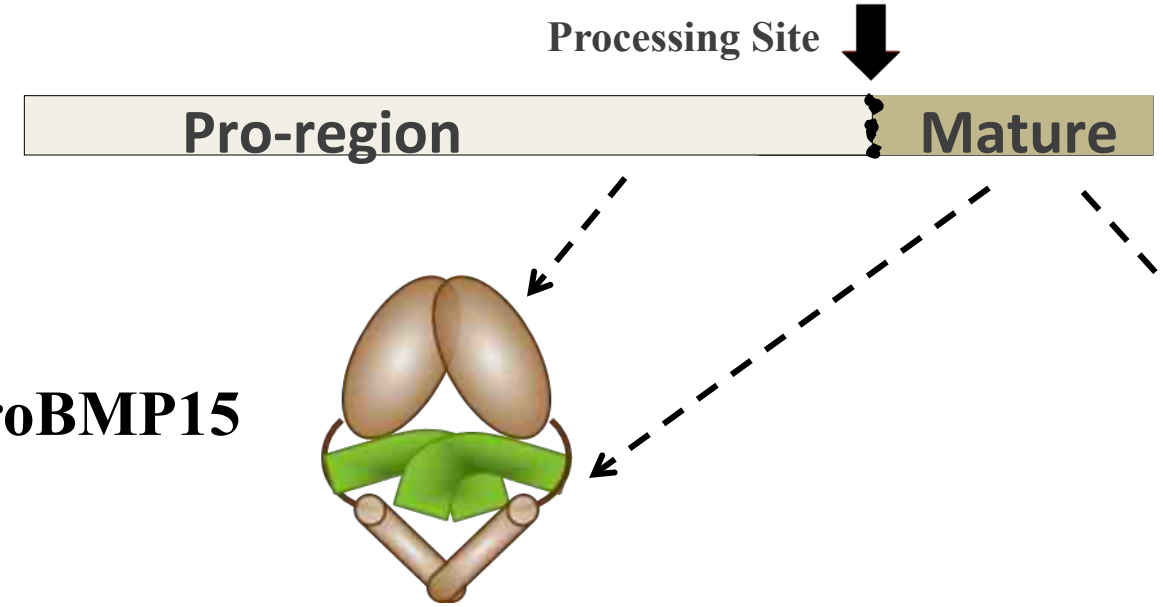
Treatment	BMP15 (ng/ml)	Number of oocytes	Cleavage[a]	Blastocyst on day 6[b]	Hatching blastocyst on day 6[c]
Control	0	241	66.6±8.6	64.8±8.0	44.3±8.2
BMP15	50	193	77.7±4.7	56.4±8.8	36.5±9.5
BMP15	100	200	75.8±8.5	65.1±7.9	45.0±9.1
BMP15	200	187	76.3±7.1	63.4±8.1	43.1±8.8



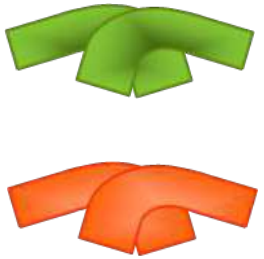
GDF9 or BMP15 mature region dimer
(R&D Systems)

Murine: Sudiman J. *et al* (2014) *J. Assist. Reprod. Gen.* 31:295-306

Pro-mature BMP15 improves oocyte quality



proBMP15

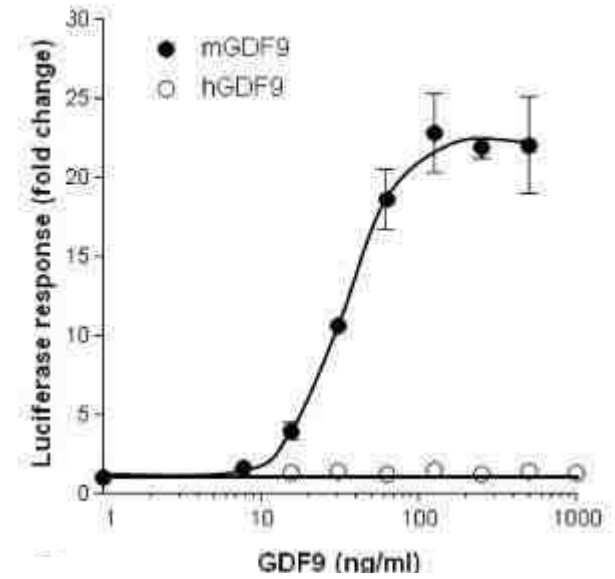


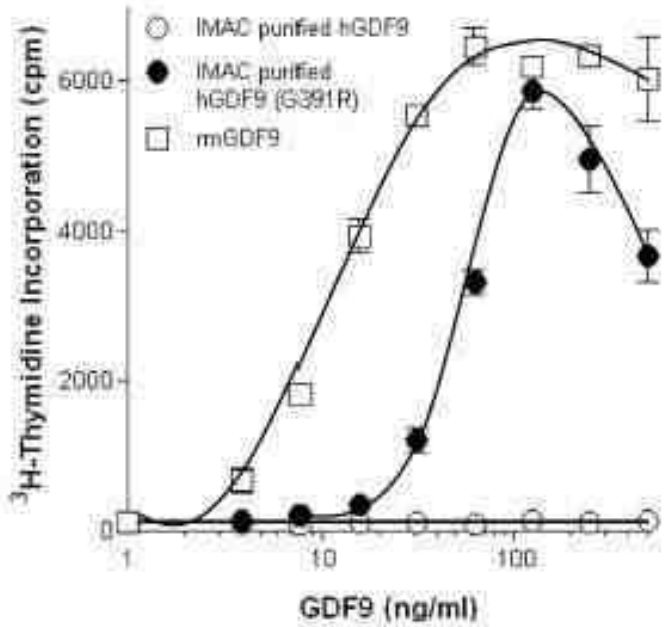
BMP15 or GDF9 mature region dimer (R&D Systems)

	Form	Cleavage (%)	Blastocyst (%)
Control	-	94	43[a,b]
GDF9	mature	89	36[a]
BMP15	mature	92	50[b,c]
proBMP15	pro-mature	89	58[c]

Bovine: Sudiman J. *et al* (2014) PLoS One 9(7):e103563

Human GDF9 is naturally latent (needs activation)

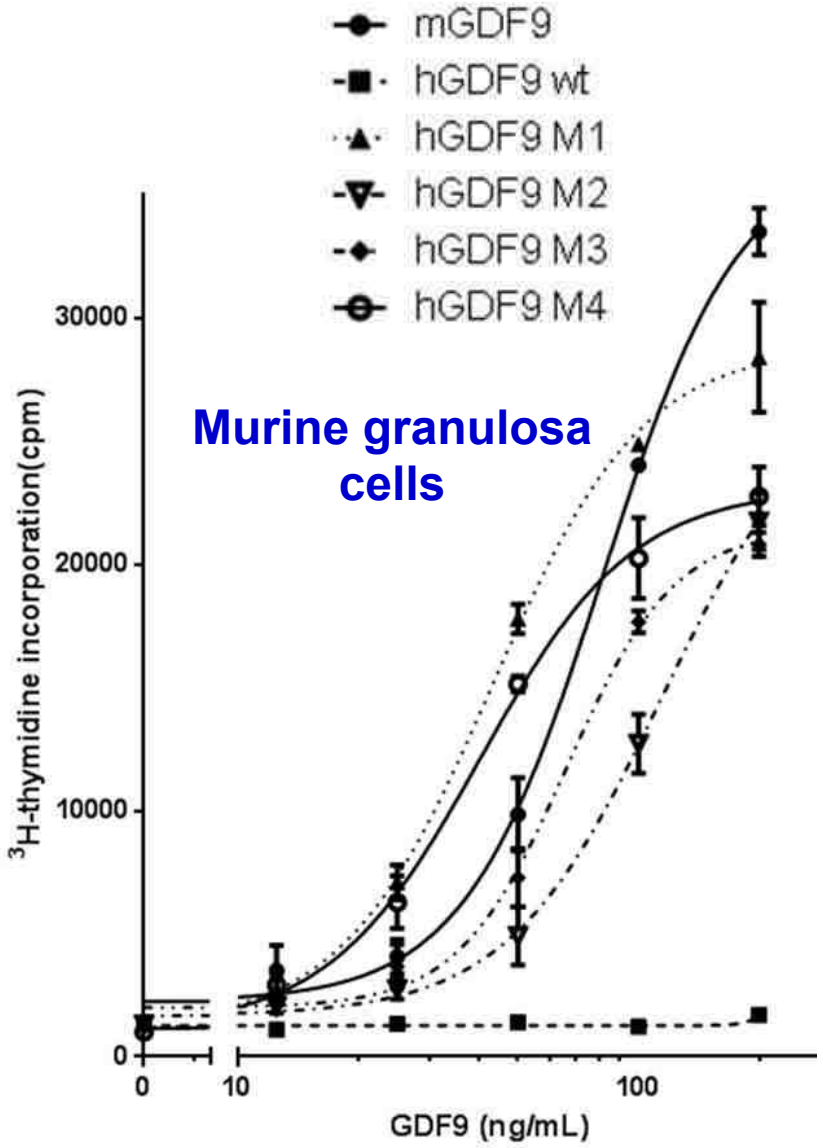




Simpson CM *et al* (2012) *Endocr.* 153:1301-1310

	Pre-helix loop
CHICK	CKGDCPRVVGHRYGSP
ALLIGATOR	CKGDCPRVVGHRYGSP
MOUSE	CKGDCPRAVRHRYGSP
RAT	CKGDCPRAVRHRYGSP
HAMSTER	CKGDCPRAVRHRYGSP
RABBIT	CKGDCPRAVGHRYGSP
SHEEP	CKGDCPRAVGHRYGSP
GOAT	CKGDCPRAVGHRYGSP
BOVINE	CKGDCPRAVGHRYGSP
YAK	CKGDCPRAVGHRYGSP
BUFFALO	CKGDCPRAVGHRYGSP
DEER	CKGDCPRAVGHRYGSP
PIG	CKGDCPRAVGHRYGSP
PANDA	CKGNCPRAVGHRYGSP
DOG	CKGDCPRAVGHRYGSP
CAT	CKGDCPRALGHRYGSP
BAT	CKGDCPRAVGHRYGSP
ARMADILLO	CKGDCPRAVGHRYGSP
HUMAN	CKGDCPRAVGHRYGSP
BABOON	CKGDCPRAVGHRYGSP
MARMOSET	CKGDCPRAVGYRYGSP
SHREW	CKGDCPRAVGHRYGSP
GALAGO	CKGDCPRAVRHRYGSP
POSSUM	CKGVCPRLVRHRYGSP
ZEBRAFISH	CKGSCPRNVGFMYGSP
EEL	CKGICPRAMGFIYGSP
SEABASS	CRGICPRTVGFIYGSP
	: *** : . ****

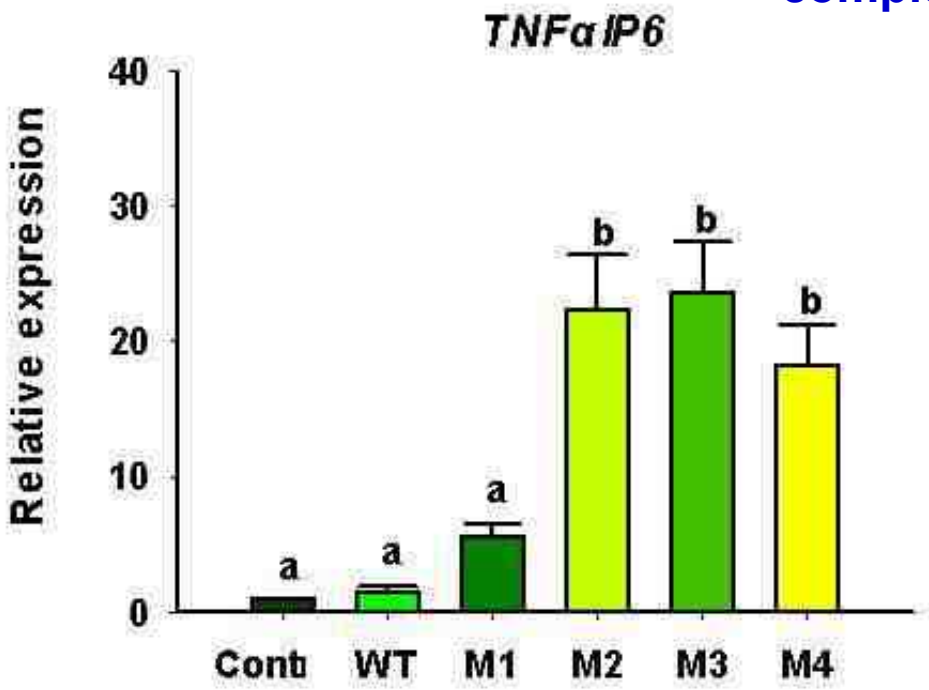
Bioactivity of chimeric forms of hGDF9



Li JJ *et al* (2015) *Mol. Endocr.*

GDF9 chimera	mutated AA residues
M1	**G391R**
M2	S325R, **G391R**, S412P, K450R
M3	S325R, **G391R**, S412P
M4	S325R, **G391R**, S412P, A422G, K450R

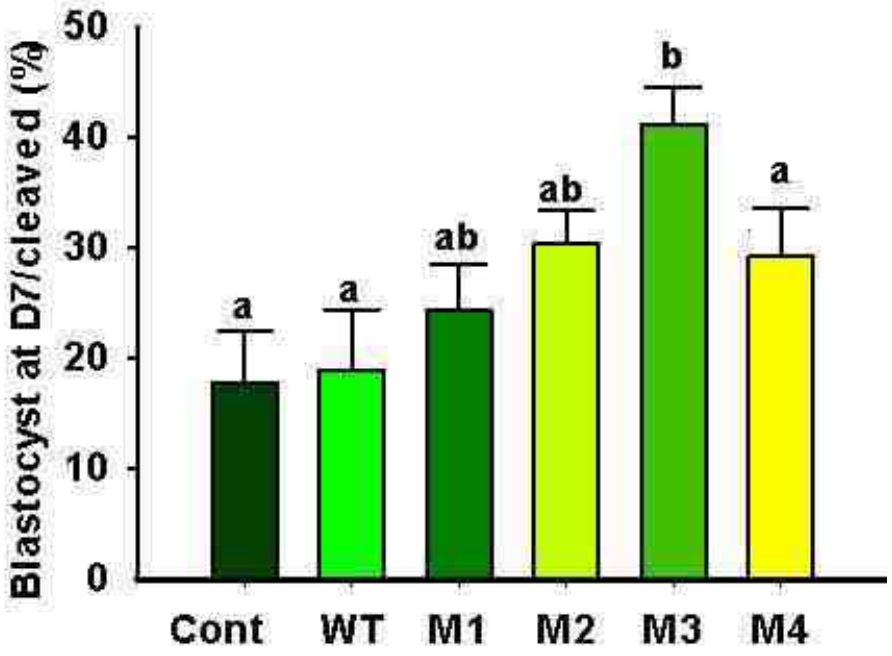
Porcine cumulus oocyte complexes

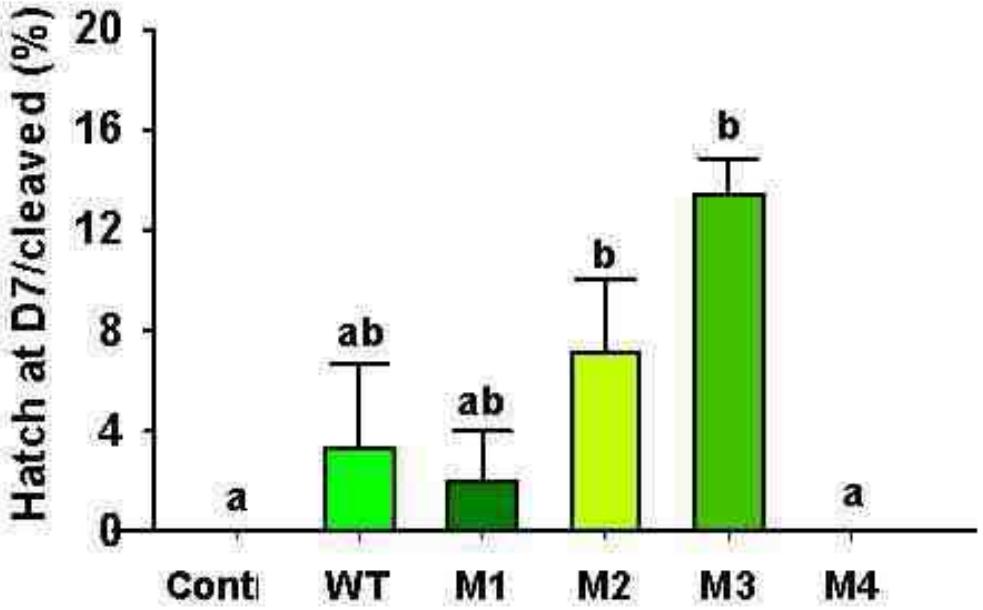


Chimeric forms of hGDF9 that improve IVM

Porcine cumulus oocyte complexes

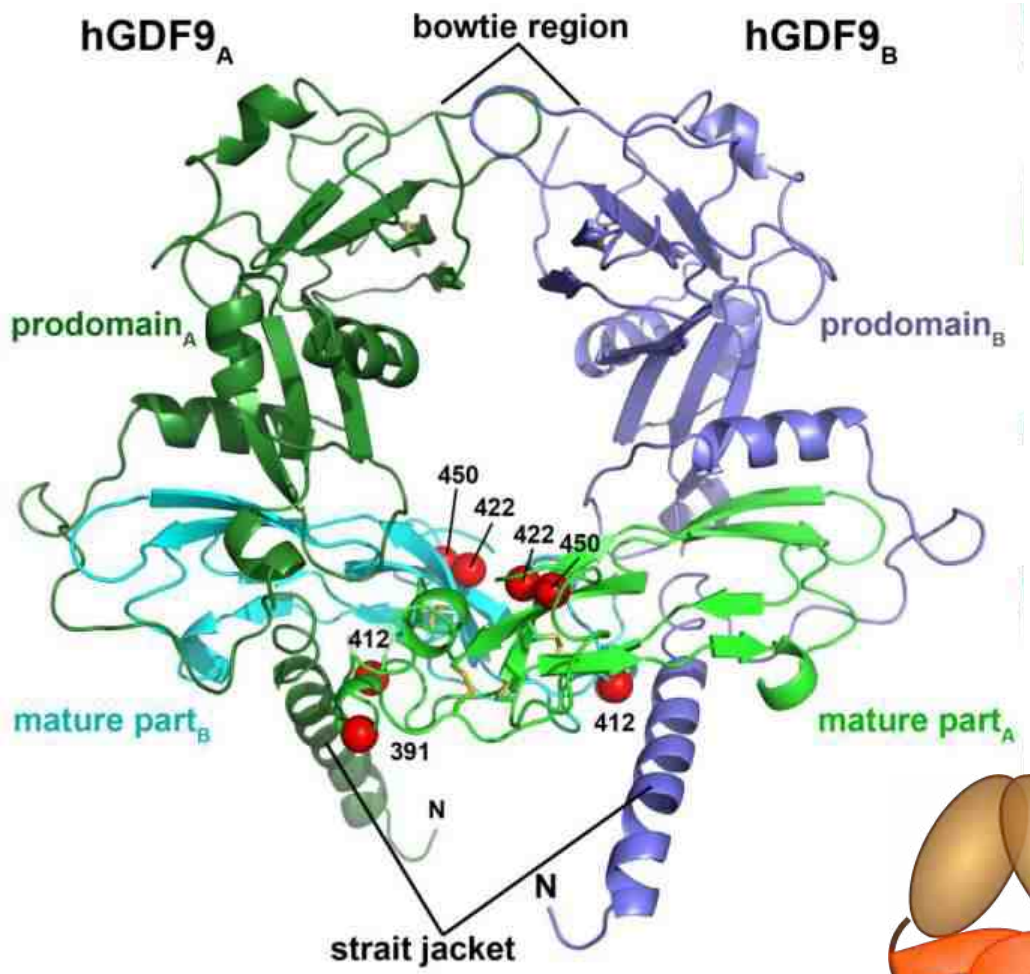
GDF9 chimera	mutated AA residues
M1	**G391R**
M2	S325R, **G391R**, S412P, K450R
M3	S325R, **G391R**, S412P
M4	S325R, **G391R**, S412P, A422G, K450R



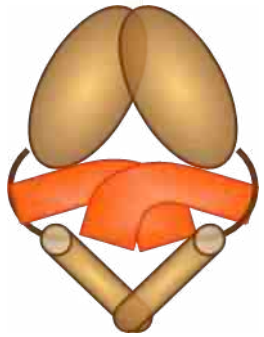


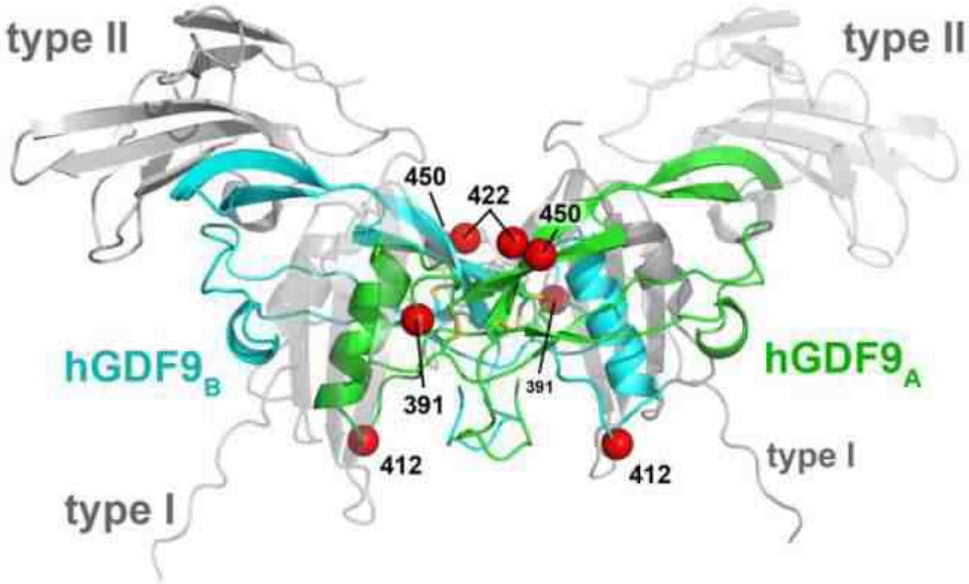
Li JJ *et al* (2015) *Mol. Endocr.*

Modelling of chimeric forms of hGDF9



pro/mature complex of hGDF9





hGDF9 mature region/receptor complex

- Ser412Pro mutation may decrease the affinity of the GDF9 mature region for the pro-region
- Gly391Arg may increase hGDF9 receptor binding affinity

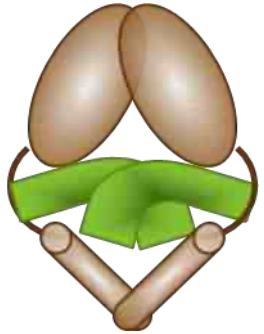
Li JJ *et al* (2015) *Mol. Endocr.*

Summary

We have found the following purified human proteins to be active in stimulating developmental competence:

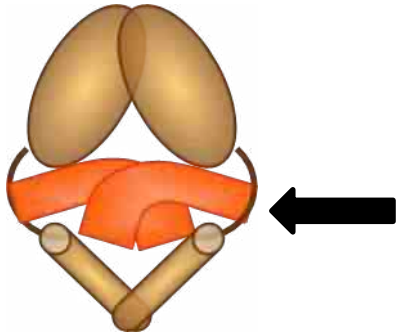
- **BMP15 in a bovine model.**

  Sudiman J. *et al* (2014)



- **GDF9 M3 mutant, in a low competence porcine model.**

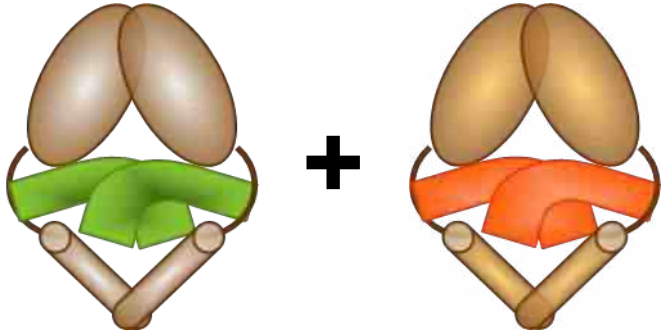
  Li JJ. *et al* (2015)



Ser325Arg
Gly391Arg
Ser412Pro

- **GDF9 + BMP15, in a low competence porcine model**

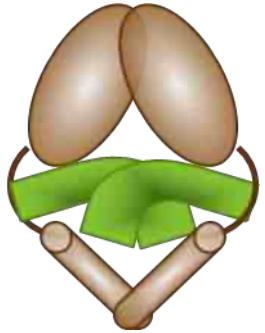
  Sugimura S *et al* (2015)



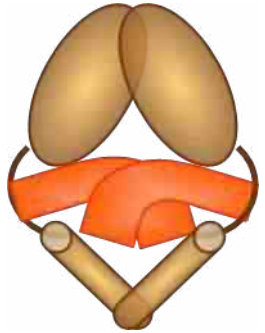
Conclusion

Concerning developmental competence

Active →



BMP15



GDF9

Depending on particular IVM model and form of OSF

Not active →

Commercial forms

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