SAT 088

Development of Novel ELISAs for Human GDF-9 and BMP-15 homodimers and the GDF-9:BMP-15 heterodimer*

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OBJECTIVE

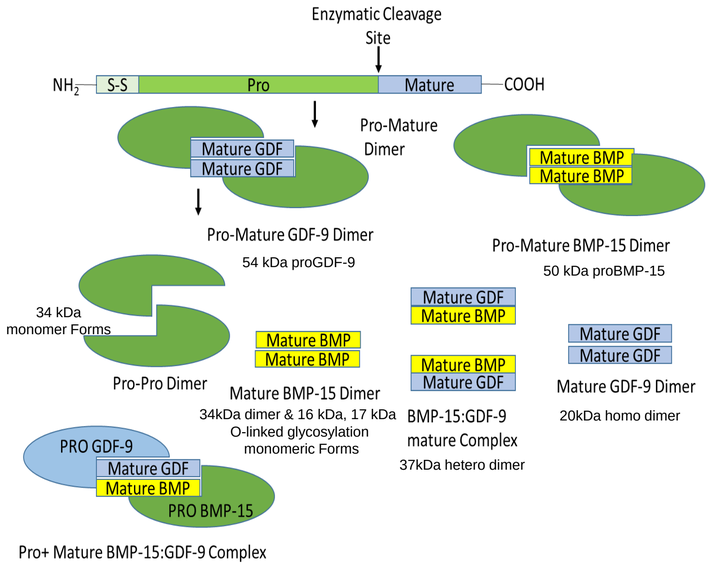
To develop sensitive and specific ELISAs for the quantitative measurement of homodimeric GDF-9 and BMP-15, and for the GDF-9:BMP-15 heterodimer (GDF9:BMP15) and their isoforms in biological fluids.

INTRODUCTION

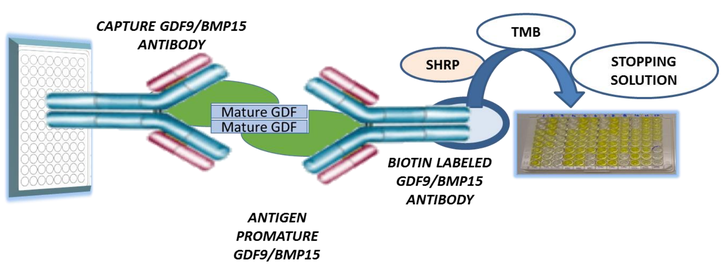
Growth Differentiation Factor 9 (GDF-9) and its closest paralog Bone Morphogenetic Protein 15 (BMP15; also known as GDF-9B) are oocyte-specific growth factors and distinguish themselves from granulosa cell-derived markers of ovarian function (such as AMH, inhibin A, inhibin B, E2) and thus may provide a more direct assessment of oocyte function competence. The mature peptide portions of GDF-9 and BMP-15 can form non-covalent bioactive homo- and heterodimers, but so far no methods have been available for measuring these different forms in biological fluids. BMP-15 and GDF-9 are synthesized as precursors with 249-295 amino acid N-terminal propeptides and 125-139 amino acid mature domains. GDF-9 and BMP-15 form 40 kDa and 34 kDa homodimers respectively and 37 kDa heterodimers. Recent evidence shows that the GDF-9:BMP-15 heterodimer is a highly active GDF-9-like superagonist being up to 1000-fold more potent than homodimeric GDF-9 itself.

This heteromeric GDF-9:BMP-15 appears to be the biologically most relevant form of the dimers. Like GDF9, it induces SMAD 2/3 phosphorylation and, does not activate BMP-like SMAD 1/5/8 signaling, in line with the observation that SMAD 2/3 knockout mice have severely impaired fertility.

Various combinations of GDF9 and BMP15 Molecular Forms



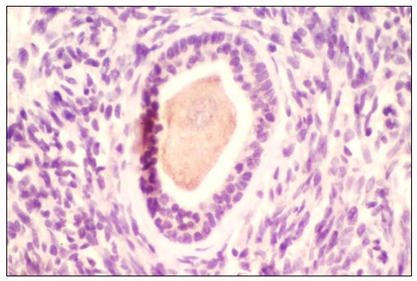
METHOD



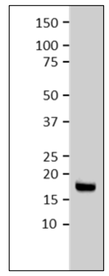
ELISA METHODS TABLE

ELISA Reagents	Capture Antibody	Detection antibody	Cross-Reactivity	Species Cross-Reactivity
AL-176	AG018 Mature GDF-9	AG011 Mature GDF-9	Pro + Mat GDF-9, Mat GDF-9, GDF-9:BMP-15	Human
AL-177	AG029 Pro-GDF-9	AG010 Pro-GDF-9	Pro + Mature GDF-9, GDF9:BMP-15	Human, Canine
AL-178	AG029 Pro-GDF-9	AG011 Mature GDF-9	P+M GDF-9, Mat GDF-9, GDF-9:BMP-15	Human, Canine, Ovine, Bovine
AL-179	AB020 Mature BMP15	AB020 Mature BMP15	Pro +Mat BMP-15, Mat GDF9	Human
AL-180	AB015 Pro-BMP15	AB021 Mature BMP15	Pro +Mat BMP-15	Human
Al-181	AG027 GDF-9	AB020 BMP15	Pro +Mat BMP-15, Mat BMP-15, GDF-9:BMP-15	Human
Al-182	AB020 BMP15	AG027 GDF-9	Pro +Mat BMP-15, Mat BMP-15, GDF-9:BMP-15	Human

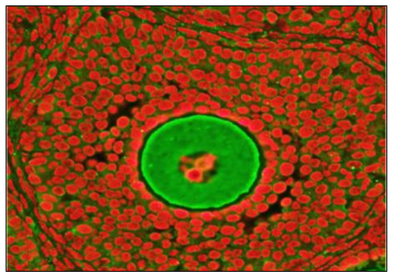
Western Blot and Immunostaining Analysis using GDF-9 Monoclonal Antibodies



AB-325-AG013 Immunohistochemistry
Formalin-fixed human ovary section stained with 10 ug/ml GDF-9 monoclonal antibody.

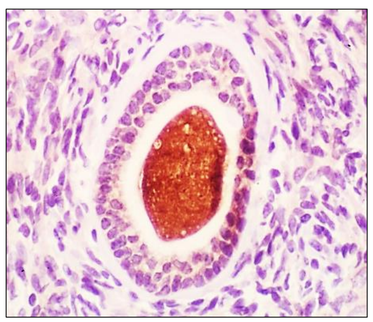


AB-325-AG012 Immunoblot of 16 kDa GDF-9 mature monomer protein purified from CHO cells.

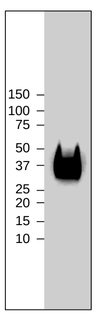


AB-325-AG012 Immunofluorescence
Paraffin-embedded mouse ovary section stained with 5 ug/ml GDF-9 monoclonal antibody showing oocyte-specific staining (green) of preantral folllicle.

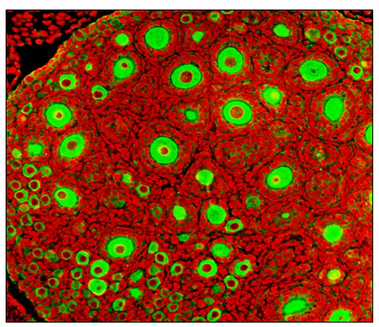
Western Blot and Immunostaining Analysis using BMP-15 Monoclonal Antibodies



AB-324-AB022 Immunohistochemistry
Formalin-fixed human ovary section stained with 10 ug/ml BMP-15 monoclonal antibody.



AB-324-AB021 Immunoblot of BMP-15 pro-mature protein under non-reducing conditions.



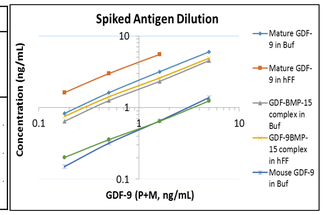
AB-324-AB016 Immunofluorescence
Paraffin-embedded 1 week old mouse ovary section stained with 5 ug/ml BMP-15 monoclonal antibody showing oocyte-specific staining (green) of follicles.

Analytical Sensitivity: The analytical sensitivity in the AL-176, GDF-9 assay as calculated by the interpolation of mean plus two standard deviation of 16 replicates of calibrator A (0 pg/mL) and calibrator B (30 pg/mL) is 7 pg/mL.

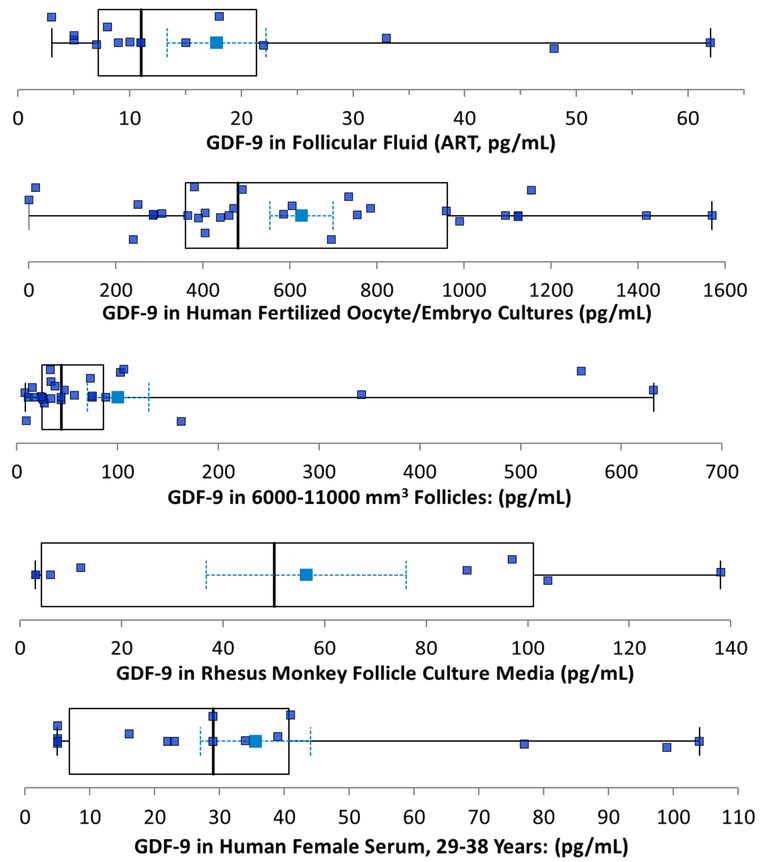
Imprecision

GDF-9		Within run		Total	
#	Mean (pg/mL)	SD (pg/mL)	% CV	SD (pg/mL)	% CV
1	579.2	16.8	2.9%	16.8	2.9%
2	1514.0	49.3	3.3%	49.3	3.3%
3	224.9	14	6.2%	14.6	6.5%
4	1065.1	16	1.5%	17.0	1.6%

Linearity of Dilution



Expected GDF-9 Levels by AL-176 ELISA



CONCLUSIONS

A portfolio of well-characterized assays for isoforms of GDF-9, BMP-15 and the most biologically potent GDF-9:BMP-15 complex are available to reliably quantitate these important endocrine and local regulators in physiological and pathophysiological studies of human folliculogenesis.

ACKNOWLEDGEMENTS

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