Multiple Protein Markers for Diagnosis and Prognosis of Male Fertility

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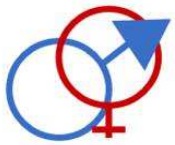
Importance of Male Fertility Determination



- Approximately 15% of all couples are infertile, and 50% of disintegration of fertilization comes from male factor.
- Male infertility lead to large economic losses in Animal industry.
- In bovine, more than 70% of cows are bred by artificial insemination, but only 50% of successful full-term pregnancy rates per insemination
- Therefore, the ability to predict fertility potential in both humans and animals is of critical importance.

Importance of Male Fertility Determination

- In last decades, several semen analyses have been developed as male fertility evaluation.
  - Sperm morphology, motility, cervical mucus penetration test, sperm-zona interaction etc..

- While these tools essential to provide initial quantitative information on semen, their clinical value of predicting fertility is debated.

What's the Future for the Diagnosis of Sperm Dysfunction and Male Infertility?

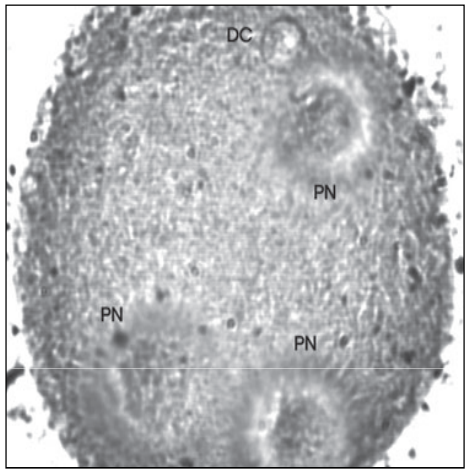
- First, an accurate assessment is the keystone of male fertility and all efforts should be made to improve standard of assessment.
- Secondly, comprehensive genomic and proteomic approaches on male fertility are timely and now required.
- Thirdly, functions of novel genomes and proteomes related with male fertility need to be elucidated.
- Finally, rapid development of microarray and proteomic chip based diagnostics will be made.

(Modified from Corner and Barratt. 2006).

Development of Male Fertility Prediction Tools: Sperm Penetration Assay I

Correlation between Sperm Fertility Index and Litter size of 24 Boar

	Litter size≥ 10	Litter size < 10
SFI ≥ 2.5	6	0
SFI < 2.5	1	17
Sensitivity	85.71%	
Specificity	100.00%	
Positive predictive valu e	100.00%	
Negative predic e		
Overall ac		



International Journal of Andrology
international journal of andrology ISSN 0105-6263

ORIGINAL ARTICLE

The sperm penetration assay predicts the litter size in pigs

Shin-Ae Oh, Young-Ah You, Yoo-Jin Park and Myung-Geol Pang

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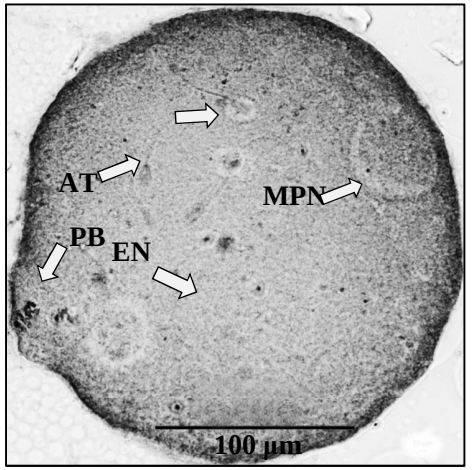
Development of Male Fertility Prediction Tools: Sperm Penetration Assay I

Correlation between Sperm Fertility Index and 46 Bull Fertility

	NRR ≥70%	NRR < 70
SFI ≥ 2.55	21	1
SFI < 2.55	1	23
Sensitivity	95.46%	
Specificity	95.83%	
Positive predictive value	95.46%	
Negative predictive value	95.83%	

Over



Journal of Reproduction and Development, Vol. 58, No 4, 2012

—Original Article—

Sperm Penetration Assay as an Indicator of Bull Fertility

Yoo-Jin PARK[1], El-Sayed A. MOHAMED[1], Shin-Ae OH[1], Sung-Jae YOON[1], Woo-Sung KWON[1], Heung-Ruil KIM[2], Myeung-Sik LEE[3], Kichoon LEE[4] and Myung-Geol PANG[1]

Development of Male Fertility Prediction Tools: Sperm Penetration Assay II

- Although this system is highly accurate in determining male fertility, the methodology is complicated, time-consuming, and expensive.

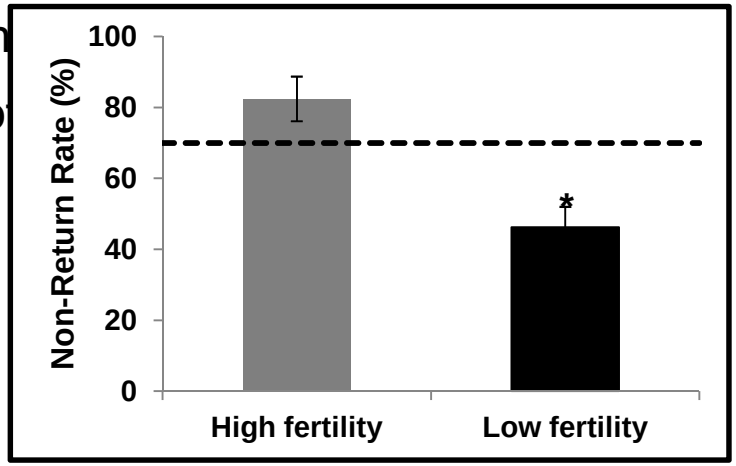
- Thus, new methods will need to be developed to ensure easier and more accurate assessments.

Development of Male Fertility Prediction Tools: Proteomic Approaches I

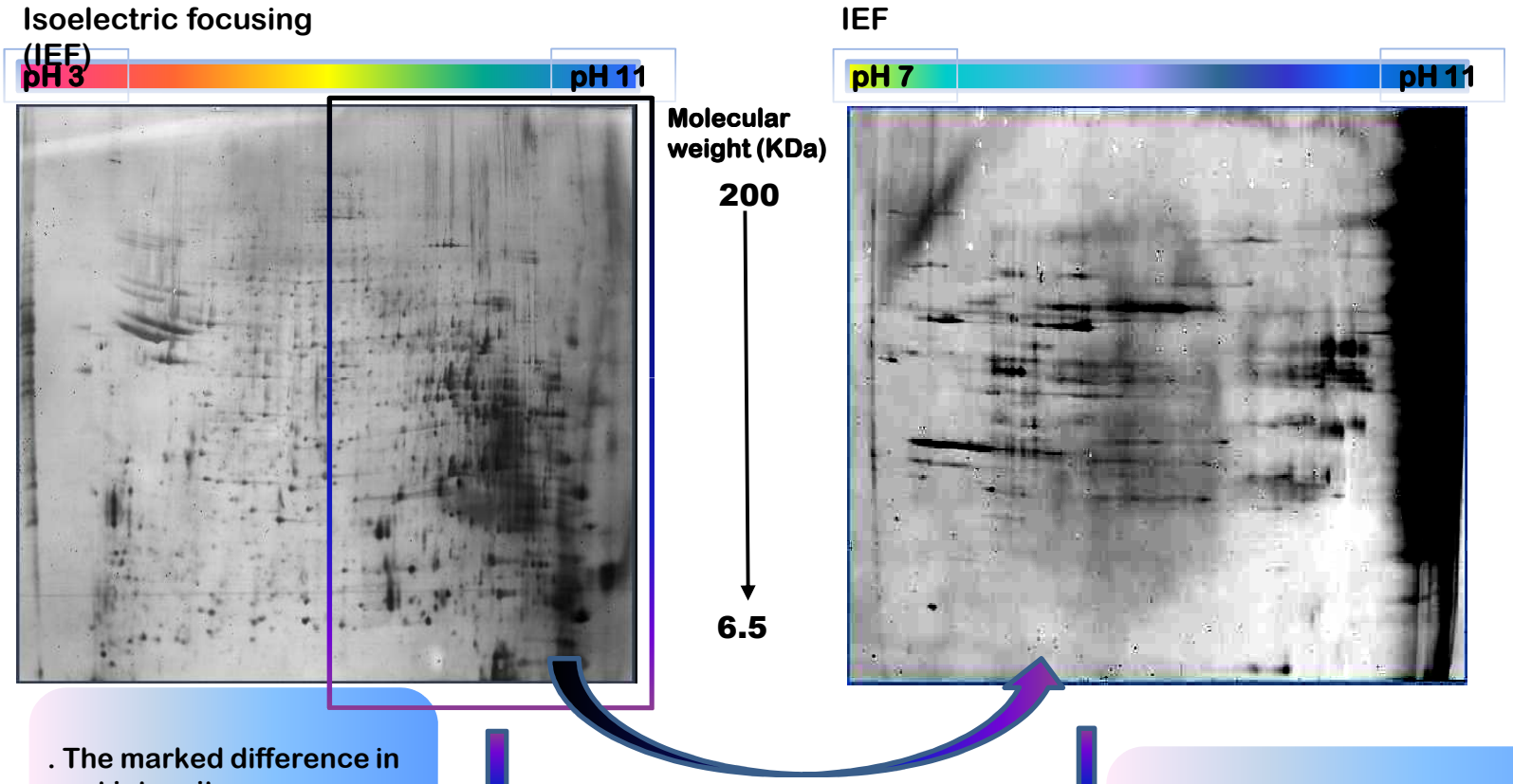
- Proteomic analysis using spermatozoa is acceptable to understand comprehensive process of sperm molecular function related with fertility.
- In last few years, proteomics and genomics were applied to identify the proteins related to male fertility.
- Despite these recent efforts, it is difficult to define the relationship between proteome and sperm fertility after proteomic study.

Development of Male Fertility Prediction Tools: Proteomic Approaches II

- Bovine spermatozoa are an ideal model for male fertility
  - Good breeding records, fertility data, and progeny records
  - Ample semen sample
- Semen source and classification of fertility
  - Six frozen Hanwoo semen were obtained from Hanwoo Improvement in NACF.
  - **Non-return rate (NRR)** was determined as the ratio of the number of cows that did not show an **insemination** divided by to



Development of Male Fertility Prediction Tools: Proteomic Approaches III



. The marked difference in spot intensity was arbitrarily set as **a >3-fold difference.**

Western blotting
With spermatozoa from 20bulls

Protein Identification
Liquid Chromatography – Mass spectrometry/Mass spectrometry (LC/ESI-MS/MS)

Differentially Expressed Proteins of Spermatozoa from High and Low Fertility Bulls I

Experimental comparison	Total No. of spots detected in reference gel	No. of spots with increased expression on one gel compared with the other	No. of spots with decreased expression on one gel compared with the other
Low fertility	287.56 ±20.07	14	5
High fertility	301.43 ±25.33	5	14

Development of Male Fertility Prediction Tools: Proteomic Approaches IV

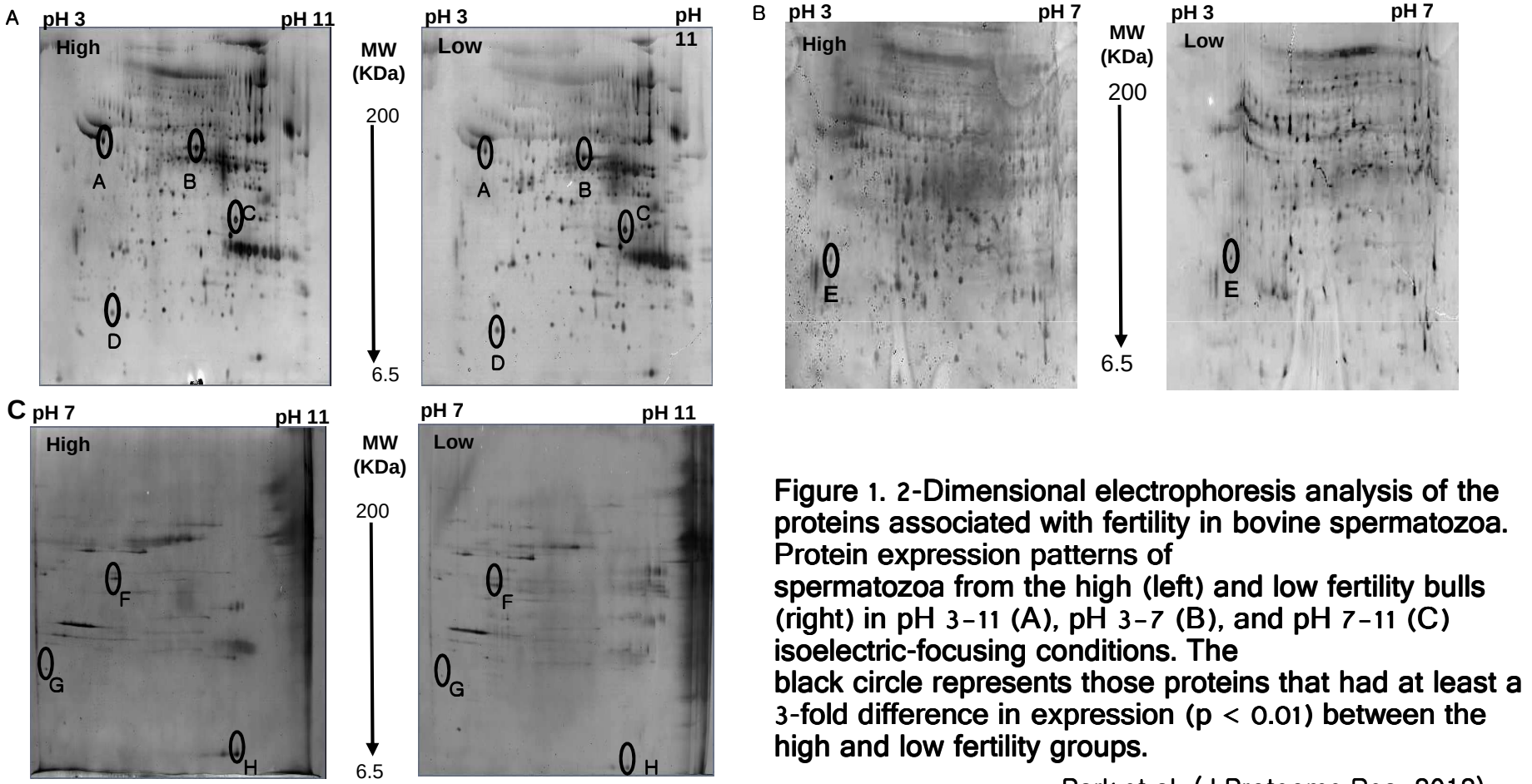


Figure 1. 2-Dimensional electrophoresis analysis of the proteins associated with fertility in bovine spermatozoa. Protein expression patterns of spermatozoa from the high (left) and low fertility bulls (right) in pH 3-11 (A), pH 3-7 (B), and pH 7-11 (C) isoelectric-focusing conditions. The black circle represents those proteins that had at least a 3-fold difference in expression ($p < 0.01$) between the high and low fertility groups.

Park et al. (J Proteome Res, 2012)

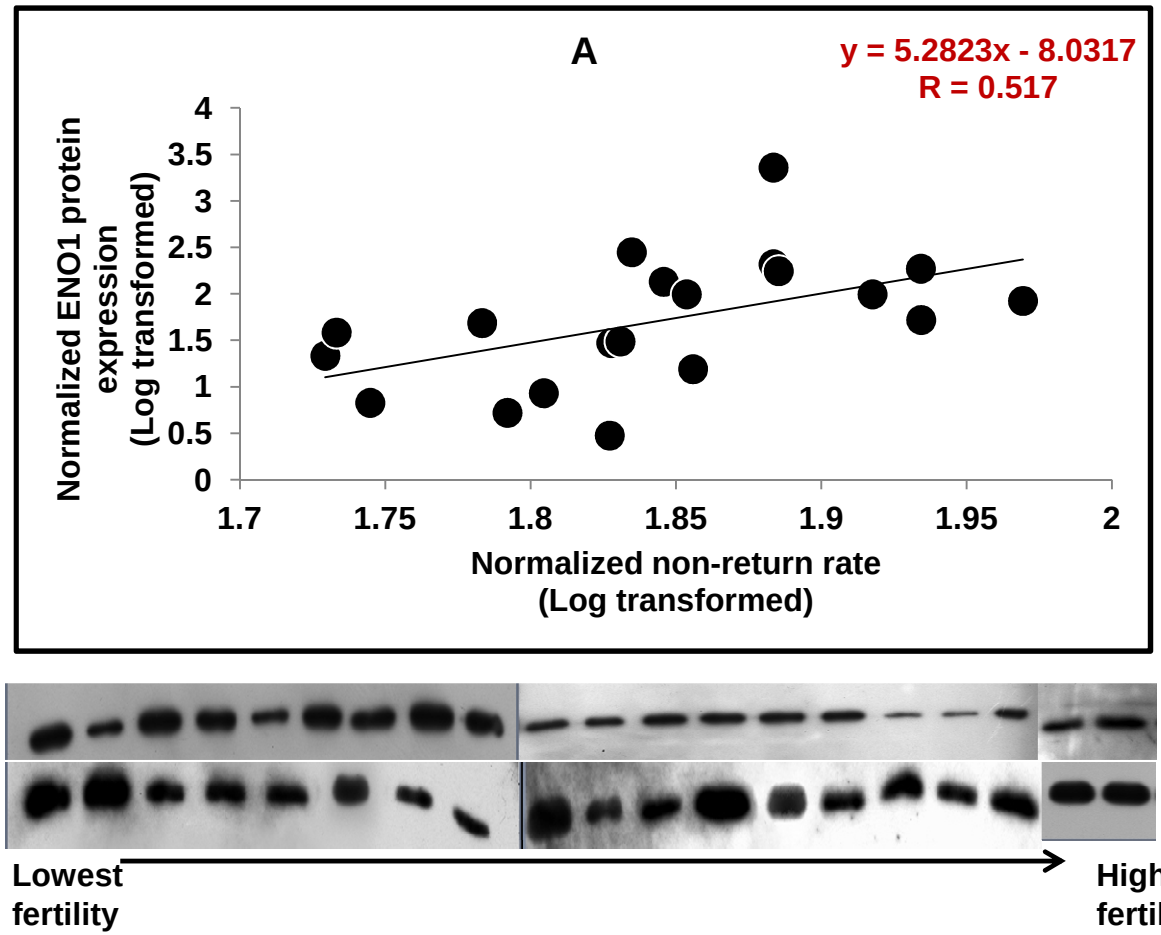
Differentially Expressed Proteins of Spermatozoa from High and Low Fertility Bulls II

Protein description	Symbol	NCBI No	Spot No	MASCOT score [a)]
ATP synthase, H+ transporting, mitochondrial F1 complex, beta subunit	ATP5B	28461221	A	612
Enolase 1	ENO1	87196501	B	208
Voltage dependent anion channel 2	VDAC2	62177148	C	138
Ropporin-1	Ropporin-1	115495919	D	183
Apoptosis-stimulating of p53 protein 2	ASPP2	33860140	E	48
Ubiquinol-cytochrome-c reductase complex core protein 2	UQCRC2	P23004	F	145
Phospholipid hydroperoxide glutathione peroxidase, mitochondrial	GPx4	Q9N2J2	G	315
Alpha-2-HS-glycoprotein	AHSG	P12763	H	59

[a)] MASCOT score is −10 log (p), where p is the probability that the observed match is a random event. Scores >55 indicate identity or extensive homology ($p < 0.05$).

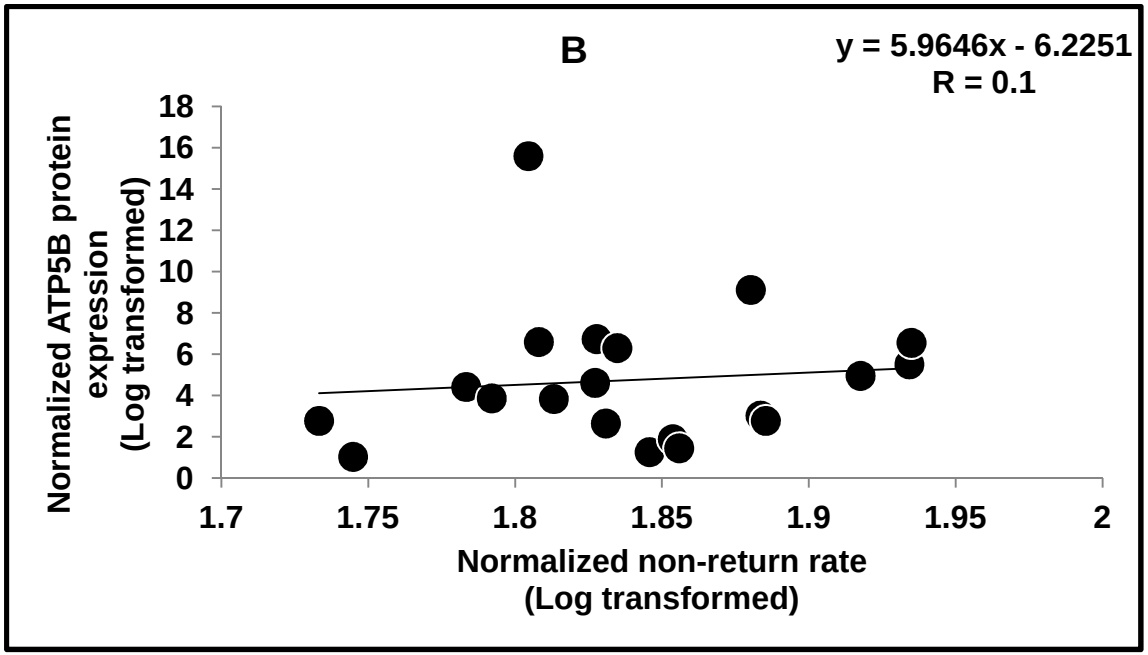
Park et al. (J Proteome Res, 2012)

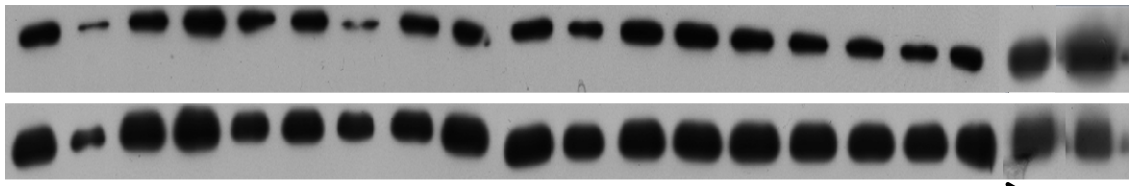
Marked correlation between protein expression and fertility : ENO1



Park et al. (J Proteome Res, 2012)

Marked correlation between protein expression and fertility: ATP5B

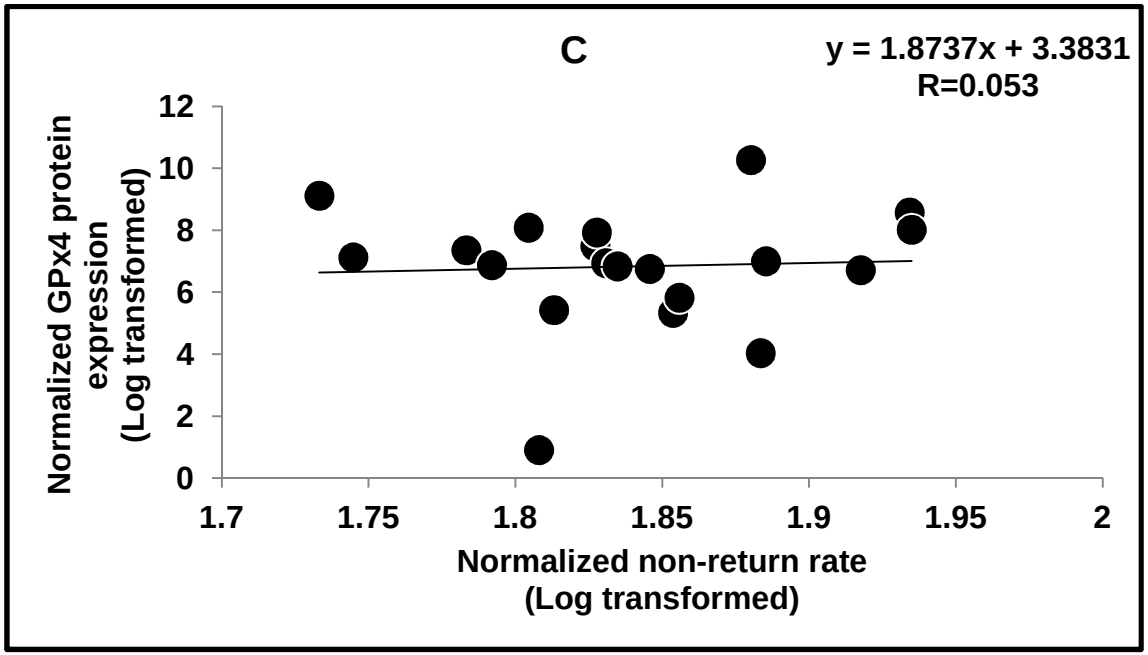


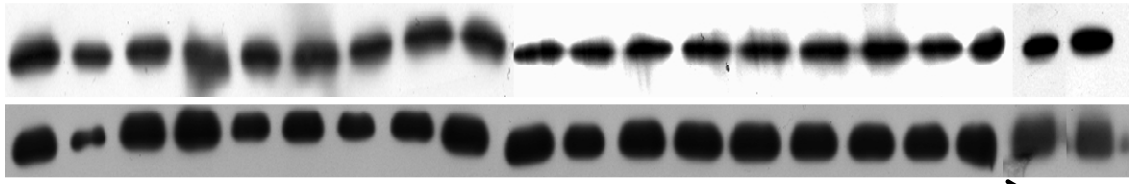


Lowest fertility → Highest fertility

Park et al. (J Proteome Res, 2012)

Marked correlation between protein expression and fertility : GPx4

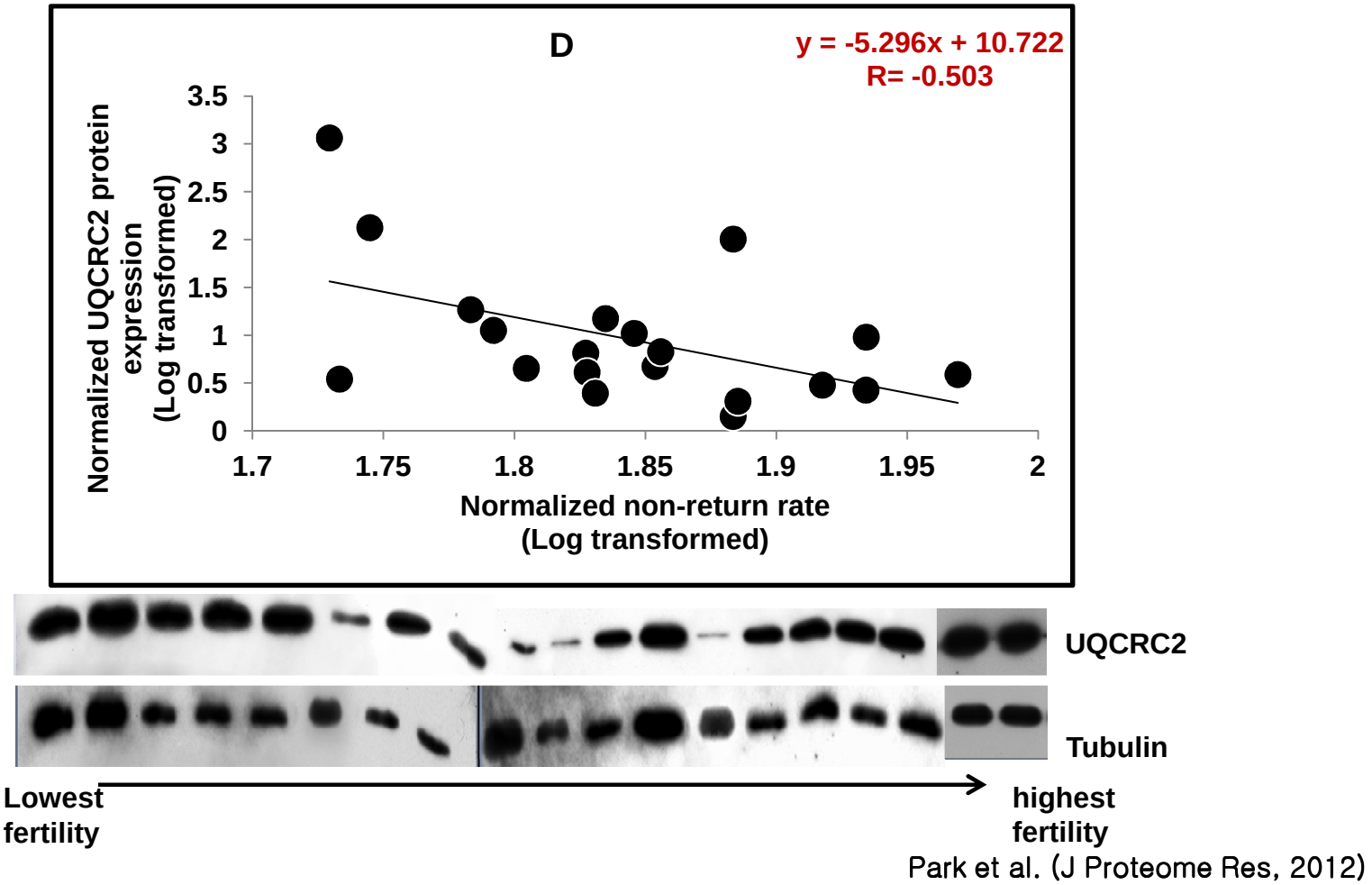




Lowest fertility → Highest fertility

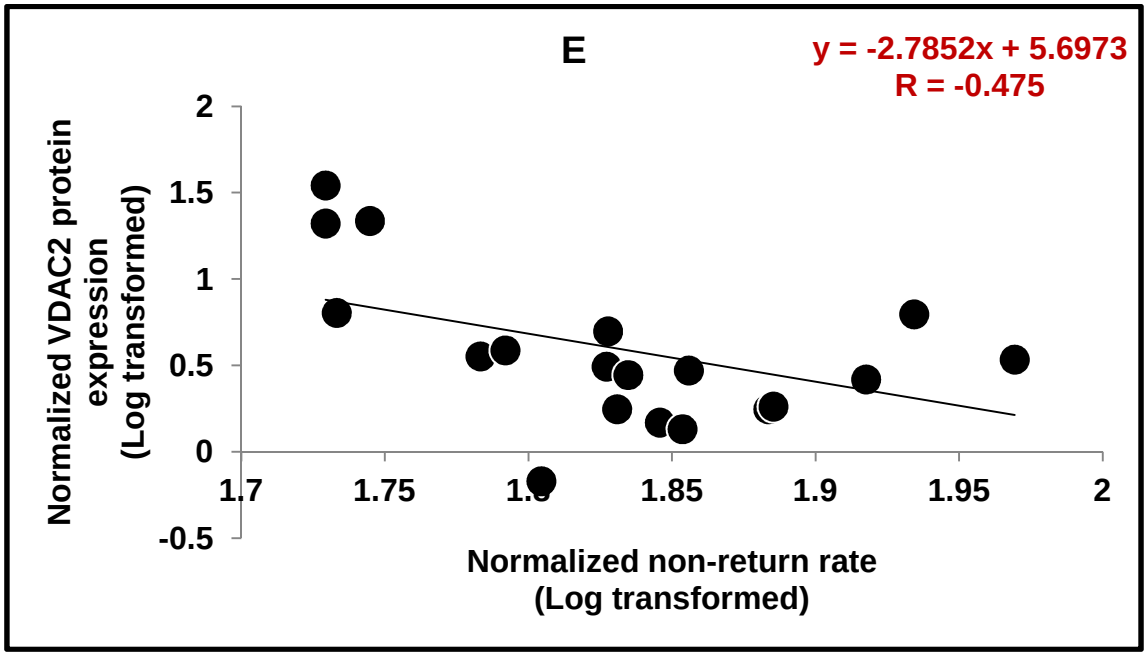
Park et al. (J Proteome Res, 2012)

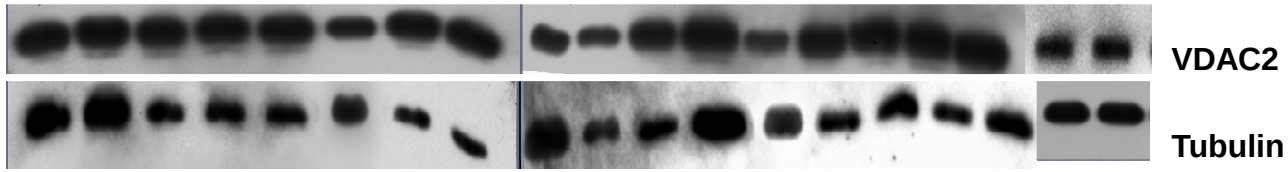
Marked correlation between protein expression and fertility: UQCRC2



Park et al. (J Proteome Res, 2012)

Marked correlation between protein expression and fertility: VDAC2

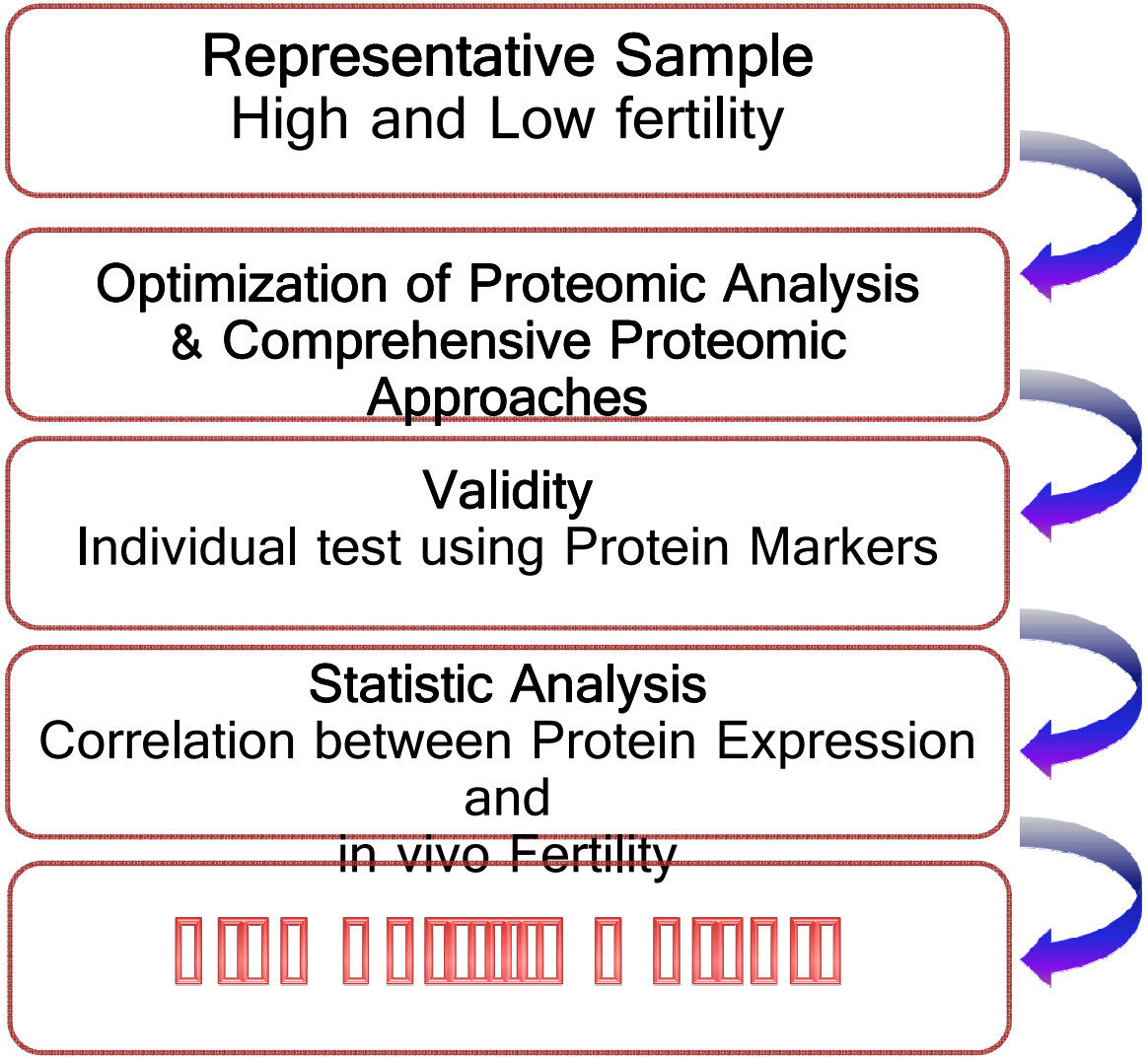




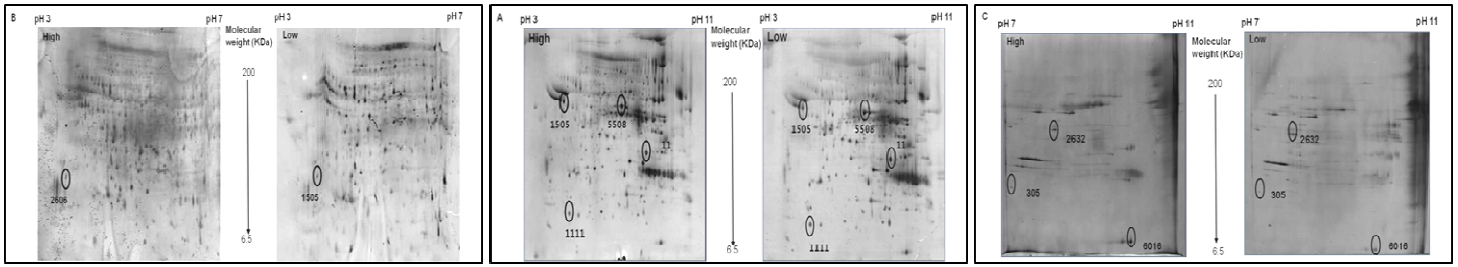
Lowest fertility → highest fertility

Park et al. (J Proteome Res, 2012)

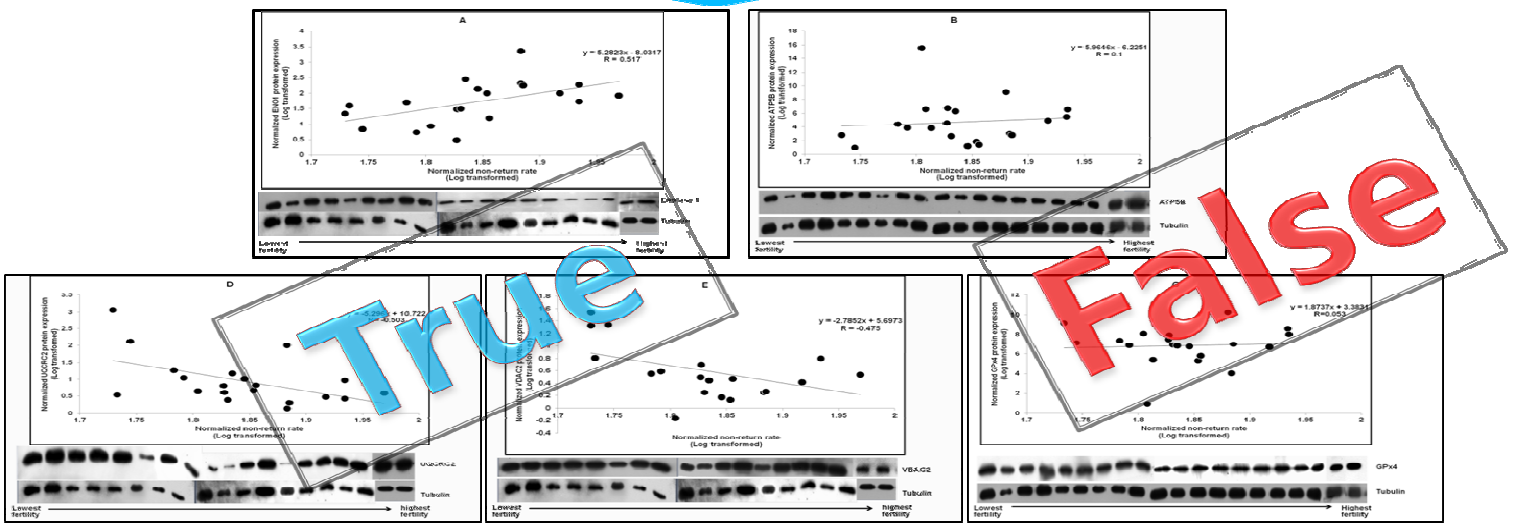
And Then What are the True Fertility Markers??



And Then What are the True Fertility Markers??



What are true fertility markers among these??



Park et al. (J Proteome Res, 2012)

What is the Further Strategies?

- Difference of protein expression in capacitated sperm cells from high and low fertility.
- Understanding of the mechanisms of fertility-related protein markers.
- Development of proteomic chip and microarray.
- Animal industry

	Net Profit (Won)
Porcine: MSY ≥ 20	2,440,000 million
Bovine: 10% improve the Fertility	70,000 million

- New targets for male contraceptives.

감사합니다.

Acknowledgement

This work was carried out with the support of a grant from the Cooperative Research Program for Agriculture Science & Technology Development (Project No. PJ008415 and PJ907170)

Rural Development Administration, Republic of Korea