Proteomic analysis of equine follicular fluid during late follicle development

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Introduction

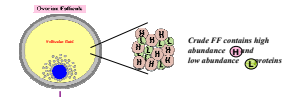
Follicular fluid (FF):

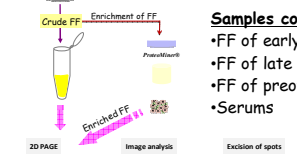
- ✓ accumulates into the antrum of follicles
- ✓ is in part an exudate of serum
- ✓ contains essential substances implicated in oocyte maturation and fertilization

Aim of our study:

- ➢ to investigate the protein content of mare FF
- ➢ to compare FF and serum protein profiles
- ➢ to remove some of the high-abundance serum proteins in order to detect lower abundance ones which may have a key role in follicle development

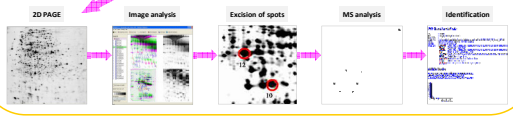
Methods





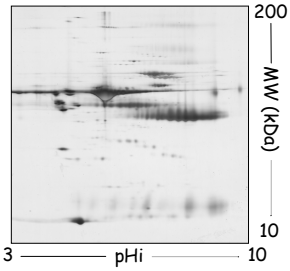
Samples collected:

- •FF of early dominance stage (ED)
- •FF of late dominance stage (LD)
- •FF of preovulatory stage (post LH; PO)
- •Serums



Results

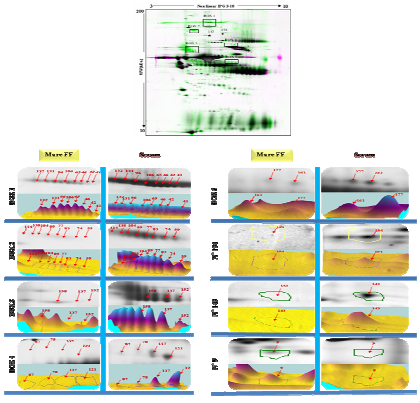
① FF protein content



Silver stained 2D-PAGE (10%) of **crude** FF

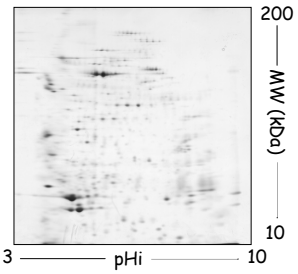
- 459 protein spots detected
- 27 of them identified by MS
- All were classified as high abundance serum proteins (albumin, Ig, Tf, haptoglobine…)
- No differential protein spots between the 3 stages (ED, LD, PO)

② FF compared to serum



- 30 differentially expressed protein spots between FF and serum

③ FF enrichment



Silver stained 2D-PAGE (10%) of **enriched** FF

- 992 protein spots detected
- 30 of them identified by MS
- Only few are high abundance serum proteins
- -Identification of some lower abundance proteins
- Identification of some follicle specific proteins (inhibin…)

Conclusion

- ✓ **Follicular fluid (FF)** contains several high adundance proteins also present in serum.
- ✓ **2D-PAGE** is a suitable method to analyze protein content of FF and serum.
- ✓ **Enrichment** of FF before 2D-PAGE may be a useful approach to identify novel protein candidates that may play a role in follicle development or maturation.

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Abstract

Ovarian follicular fluid is in part an exudate of serum, as surrounding cell layers permit the free diffusion of proteins of up to 500 kDa. This fluid also contains locally produced factors related to the metabolic activity of ovarian cells and it reflects the physiological status of the follicle. Furthermore, it contains essential substances implicated in oocyte maturation and fertilization, granulosa cells proliferation and differentiation, ovulation and luteinization of the follicle. Studies on its components may contribute to understand the mechanisms underlying these processes. The aim of this study was to determine for the first time, the equine follicular fluid protein composition by SDS-PAGE as the dominant follicle develops and maturates, and to compare follicular fluid and serum protein profiles in order to identify the proteins which may have an essential role in follicle development and maturation. For this purpose, fluids from ovarian follicles of three increasing diameters: early dominant, late dominant and preovulatory (34h after an injection of LH) were recovered by ultrasound guided follicle aspiration. Follicular fluids and serum proteins were separated by 1D-PAGE after enrichment and 2D-PAGE, and analyzed by mass spectrometry.
A total of 459 protein spots were visualized by 2D-PAGE in crude equine follicular fluid. No difference was observed between follicular fluids at the three developmental stages studied. Thirty proteins were observed as differentially expressed between serum and follicular fluid. The enrichement method increased the resolution of 2D-PAGE and many more proteins were visualized on 2D-PAGE (n= 992).
Our results of protein identification confirm the presence of several high abundance serum proteins in equine follicular fluid, and demonstrated that electrophoresis methods may be useful approaches to identify novel protein candidates that may have a special role in follicle development and maturation.

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