# Quantitative label-free proteomic analysis of human follicle fluid to identify novel candidate protein biomarker for endometriosis-associated infertility

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## Abstract

**Background:**Endometriosis (EM) leads to a decline in fertility, which is characterized by a decrease in the number and quality of follicles, and thus has a negative impact on in vitro fertilization (IVF) outcomes. However, the mechanism of how EM affects oocytes and leads to infertility remains unclear. As a potentially available sample directly related to oocyte growth, follicular fluid (FF) has important research value. Evaluating the association of FF content and EM-associated infertility through proteomics may helpful to explore the possible pathogenesis of EM-associated infertility.

**Methods:**In the present experimental study, from August 2019 to June 2020, FF samples were obtained as control group (CON-G; n = 10) from women with no one female factor of infertility and were undergoing IVF due to other reasons, 20 women with EM-associated infertility undergoing IVF with no other female factors were distributed into the EM group according to the time for IVF: (i) EM-group 1 (EM-G1, Stage I to Stage III, n = 10); (ii) EM-group 2 (EM-G2, Stage I to Stage III, n = 10). label-free quantitative proteomics (LFQP) technology and parallel reaction monitoring (PRM) approach were combined to aid in identifying and validating FF protein biomarkers for EM-associated infertility. In PRM analysis, another 20 subjects were enrolled as EM-associated infertility group (EM,Stage I to Stage III, n = 10) and controls (CON, n = 10) within the same time and inclusion criteria are the same as previously described. Finally, a potential protein biomarker panel of FF differential expressed proteins to EM-associated infertility was also evaluated by t-test and receiver operating characteristic (ROC) curve and binary Logistic regression models.

**Results:**7 significant differential expressed proteins which closely related to EM-associated infertility were found by LFQP technology, among which immunoglobulin lambda variable 7-46 (IGLV7-46), Immunoglobulin heavy constant gamma 2 (IGHG2), glia-derived nexin (GDN) and Inter-alpha-trypsin inhibitor heavy chain H3 (ITIH3) were significantly up-regulated (p < 0.05), while corticosteroid-binding globulin (CBG), angiotensinogen (AGT) and Fetuin-B (FETUB) were significantly down regulated (p < 0.05). Additionally, GDN and AGT was identified as a potential protein biomarker by further PRM analysis for EM-associated infertility according to ROC curve analysis and t-test (p < 0.05), the area under the curve (AUC) for GDN and AGT was 0.78 and 0.69 with optimum sensitivity of 50%, 70% and specificity of 100%, 90%, respectively. According to binary logistic regression and evaluated ROC analysis, the AUC for the combination of GDN and AGT was 0.80.

**Conclusions:**To the best of our knowledge, this is the first time that elevated GDN protein levels have been found in the FF of patients with EM-associated infertility. Combining LFQP technology and PRM method we found the abnormal of GDN and AGT in FF may be the potential cause of EM-associated infertility which may help to better understand the physiological and pathological mechanism of EM-associated infertility. Further experimental studies are required to confirm their mechanism in EM-associated infertility. The results of this study are also consistent with the previous conclusion that EM is a chronic inflammatory disease.

**Significance:**To the best of our knowledge, this is the first time that elevated GDN protein levels have been found in the follicular fluid of patients with EM-associated infertility. Combining LFQP technology and PRM methods we found the abnormal of GDN and AGT protein in FF may be the potential cause of EM-associated infertility which may help to better understand the physiological and pathological mechanism of EM-associated infertility. Clinically, it has been recognized that EM is related to infertility, but the mechanism remains unclear. Our study combines label-free quantitative proteomics technology and parallel reaction monitoring methods to identify and verify the FF protein biomarkers of EM-associated infertility, which provides a good research method for follow-up research.

**Keywords:**Endometriosis; Follicular fluid; In vitro fertilization; Label-free quantitative proteomics; Parallel reaction monitoring.