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Metabolomics shows no impairment of the microenvironment of the cumulus-oocyte complex in the follicular fluid of women with isolated endometriosis.

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Short Title

Metabolomics of follicular fluid and endometriosis.

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Abstract

Research question: Is there any metabolomic evidence of impairment of the cumulus-oocyte complex (COC) microenvironment in the follicular fluid (FF) of women with endometriosis?

Design: We performed a prospective observational study from January to July 2018 at the Angers University Hospital, France. 79 women undergoing in vitro fertilization (IVF) with or without intracytoplasmic sperm injection (ICSI) were included: 39 for endometriosis related infertility and 40 controls with other causes of infertility. A targeted quantitative metabolomic and lipidomic analysis was performed.

Results: The patients' characteristics (age, body mass index, smoking status, hormonal profile and ovarian reserve markers) were comparable between the endometriosis and the control group. There was no significant difference in the cumulative FSH dose used for stimulation between the endometriosis and the control group (2732 *vs*. 2257 IU, p=0.09, respectively). There were no differences in the oocyte maturity rates (72.2% vs 77.7%, p=0.6), and in the fertilization rates in IVF and ICSI (49.4% vs 50.2%, p=0.9 and 76.4% vs 68.8%, p=0.53, respectively) between the endometriosis and control group. Among the 188 metabolites analysed, 141 were accurately measured. Univariate analysis did not reveal any significant modification of metabolite concentrations, and none of the multivariate models discriminated between the two groups of patients, even when the study was restricted to the most severe form of endometriosis.

Conclusions: We did not find any specific metabolomic signature of endometriosis in the follicular fluid of women undergoing IVF. These results suggest that there is no micro-environmental impairment of the COC in cases of isolated endometriosis among women with infertility.

Keywords: Follicular fluid, metabolomics, endometriosis, oocyte.

Introduction

Endometriosis is a common gynecological pathology affecting 5-10% of women of childbearing age (*Bulun et al.*, 2009), and about 25% of women seeking infertility treatment are likely to be affected by it (*Schenken et al.*, 1997; *Wellbery*, 1999). Endometriosis is difficult to diagnose since the symptoms, such as dysmenorrhea, and dyspareunia, are not specific. The current gold standard for confirming the diagnosis is laparoscopy (*Poncelet and Ducarme*, 2007), and it is formally established after a 7-year delay on average (*Nnoaham et al.*, 2011). The identification of biomarkers, especially in the blood or urine, would offer a less invasive technique than surgery and greatly facilitate the diagnosis of endometriosis (*May et al.*, 2010). The mechanisms by which endometriosis causes infertility are presumably numerous but are not yet clearly established (*Bulun et al.*, 2009). It could have a negative impact on folliculogenesis, ovulation, oocyte quality, tubal mobility and implantation, and could involve mechanical, molecular, genetic and environmental factors (*Macer et al.*, 2012). Data from the current available literature are insufficient to conclude whether there is a link between endometriosis and oocyte quality impairment (*Sanchez et al.*, 2017), with contradictory results coming from four different meta-analyses (*Barnhart et al.*, 2002; *Harb et al.*, 2013; *Yang et al.*, 2015; *Rossi et al.*, 2016). Moreover, studies confirming the negative impact of endometriosis on oocyte quality have several confounding factors that could affect the results, such as the stage of endometriosis, previous disease related medical/surgical treatments, and the possible concurrence of endometriosis and ovarian insufficiency, two of the most common causes of infertility.

The follicular fluid (FF) is produced by the transfer of blood plasma components across the blood follicular barrier, secretions of the theca and the granulosa cells, and molecules excreted

by the oocytes (*De la Barca et al.*, 2017). The composition of the FF reflects the exchanges occurring between the oocyte and its microenvironment during the acquisition of gametic competence (*Revelli et al.*, 2009). Qualitative and quantitative alterations in the composition of the FF may be related to oocyte quality and thus to embryonic quality (*Da Broi et al.*, 2018). Moreover, studies of the FF have allowed the identification of several biomarkers and altered metabolic pathways in several ovarian disorders such as diminished ovarian reserve (DOR) (*Fujii et al.*, 2010; *De la Barca et al.*, 2017) and polycystic ovarian syndrome (PCOS) (*Cordeiro et al.*, JARG 2015).

Endometriosis patients have been found to have significantly higher levels of interleukin 8,12, and adrenomedullin, an angiogenic mediator, as well as decreased fertilization rates and potentially decreased oocyte quality when compared with controls (*Singh et al.*, 2016). Lo Turco et al (*Lo Turco et al.*, 2013) used proteomic analysis of FF to show significant differences between women with endometriosis and women without, and between women with endometriosis who successfully conceived and those who did not. The proteins involved were apolipoprotein-AIV, transthyretin, complement factor I, vitronectin, kininogen-1 and FAK 1 (*Lo Turco et al.*, 2013). Only a few studies have focused on the metabolomic or lipidomic profiles in the FF of patients with endometriosis, and most of them included were limited by the low number of participants (*Cordeiro et al.*, 2015; *Marianna et al.*, 2017; *Sun et al.*, 2018; *Karaer et al.*, 2018). The aim of our study was to compare the metabolomic profiles of the FF in patients undergoing *in vitro* fertilization (IVF) for infertility secondary to endometriosis to those of patients without endometriosis, in order to analyze the potential impact of the disease on the oocyte microenvironment.

Materials and Methods

We performed a prospective observational study at the Angers University Hospital, France, from January to July 2018.

Study Population

We analysed the FF retrieved from 79 women undergoing IVF, 39 patients with endometriosis (endometriosis group) and 40 patients free from endometriosis (control group). The endometriosis group included women with minimal, mild or severe forms of endometriosis and for whom the diagnosis of endometriosis had been made either with ultrasound echography or Magnetic Resonance Imaging (MRI) or after abdominal or pelvic surgery. Of these 39 patients, 32 had severe endometriosis, defined as stage III or stage IV endometriosis, according to the classification of the American Society of Reproductive Medicine (*Schenken et al.*, 1997). All seven patients with Stage I and II disease had a history of abdominopelvic surgery that identified the peritoneal lesions and confirmed the diagnosis.

The control group included non-endometriosis women, under infertility management for unexplained infertility, male infertility or a tubal factor.

All the women included were treated with IVF (in vitro fertilization) or with ICSI (intracytoplasmic sperm injection) depending on the type of infertility. Our indications for IVF included unexplained infertility and tubal factor and for ICSI antecedents of failed fertilization or suboptimal fertilization during an earlier IVF attempt, i.e. a fertilization rate lower than 20%, and male infertility with severe sperm abnormalities.

Exclusion criteria for both groups were polycystic ovarian syndrome, a history of cancer, and premature ovarian failure.

In our center, the first line protocol for endometriosis is the long protocol, based on several studies showing a better pregnancy rate with this type of protocol (*Ma et al.*, 2008; *Kolanska et al.*, 2017). All patients had ovarian stimulation with either a long protocol or an antagonistic protocol, using either recombinant or urinary gonadotropins. When at least 3 follicles reached a size of 17 mm at ultrasound monitoring, ovulation was triggered with total 250 µg of recombinant HCG (Ovitrelle®). Ultrasound-guided, transvaginal oocyte retrieval was performed 36 h after the administration HCG. Patients were enrolled on the morning of oocyte retrieval.

Samples

Once the oocytes were isolated for fertilization and culture, FF samples were collected in our laboratory, pooled and immediately centrifuged for 10 minutes at 3000 g at +4°C before recovery of the supernatant, which was conserved at -80°C in 500 µL aliquots until the metabolomic analysis.

Targeted quantitative metabolomic analysis was carried out using the Biocrates® Absolute IDQ p180 kit (Biocrates Life sciences AG, Innsbruck, Austria). This kit uses mass spectrometry (QTRAP 5500, SCIEX, Villebon-sur-Yvette, France) to quantify up to 188 different endogenous molecules distributed as follows: free carnitine (C0), 39 acylcarnitines (C), the sum of hexoses (H1), 21 amino acids, 21 biogenic amines and 105 lipids. Lipids are distributed in the kit in four different classes: 14 lysophosphatidylcholines (lysoPC), 38 diacyl-phosphatidylcholines (PCaa), 38 acyl-alkyl-phosphatidylcholines (PCae) and 15 sphingomyelins (SM). Flow-injection analysis coupled with tandem mass spectrometry (FIA-MS/MS) was used for the analysis of carnitine, acylcarnitines, lipids and hexoses. Liquid chromatography (LC) was used for separating amino acids and biogenic amines before quantitation with mass spectrometry.

Each FF sample was thoroughly vortexed after thawing and centrifuged at 4°C for 5 minutes at 5000 g. Ten microliters of each sample were then added to the filter on the upper wells of the 96-well plate. Metabolites were extracted and derivatized for the quantitation of amino acids and biogenic amines. The extracts were finally diluted with MS running solvent before FIA and LC-MS/MS analysis. Three quality controls (QCs) composed of human plasma samples at three concentration levels: low (QC1), medium (QC2) and high (QC3), were used to evaluate the performance of the analytical assay. A seven-point serial dilution of calibrators was added to the kit's 96-well plate to generate calibration curves for the quantification of amino acids and biogenic amines.

Our primary endpoint was to analyse and compare the mean concentrations of several metabolites to try and identify specific metabolic biomarkers of endometriosis.

Statistical analysis

The raw data were examined before statistical analysis in order to exclude metabolites with concentration values that are more than 20% below the lower limit of quantitation (LLOQ) or above the upper limit of quantitation (ULOQ). Multivariate analysis was performed using principal component analysis (PCA) for the detection of sample grouping and outliers. Orthogonal partial least squares discriminant analysis (OPLS-DA) was then applied to maximize the variation between the endometriosis and control groups, and to determine the metabolites contributing to this variation. The quality of the OPLS-DA model was validated by two parameters, i.e. goodness of fit (R^2), and goodness of prediction indicated by the cumulated Q^2 value (Q^2cum). A threshold of 0.5 for Q^2cum was used to determine whether an OPLS-DA model could be considered to have a good (Q^2cum ≥ 0.5) or a poor (Q^2cum < 0.5) predictive capability. Multivariate data analysis was conducted using SIMCA-P v.14.0 (Umetrics, Umeå, Sweden).

Univariate analysis was performed with the bilateral Student's *t*-test. The Benjamini-Hochberg correction was applied in order to keep the risk of type I error lower than 5%.

Ethical approval

The collection of FF samples was approved of by the Ethics Committee of the University Hospital of Angers, France (Number DC-2014-2224 and AC-2016-2799), and all 79 participants gave their written informed consent.

Results

Baseline characteristics of patients and cycles

Patient's characteristics (age, body mass index, tobacco use, hormonal profile, ovarian reserve (OR) markers) are listed in **table 1**. They were comparable between the endometriosis and control groups. Out of 39 patients in the endometriosis group, 32 (82%) had severe endometriosis, all of them with endometrioma. On the other hand, seven patients (18%) in the endometriosis group had only peritoneal disease that was diagnosed by laparoscopy, without endometriomas, and were therefore classified as stage I-II. Characteristics were also comparable between patients with stage I-II and patients with severe endometriosis.

The cycles' characteristics are also described in Table I. The rate of oocyte maturity was defined as the ratio between the number of oocytes injected and the number of oocytes recovered. Fertilization rates in IVF or IVF-ICSI procedures were defined as the ratio of the number of fertilized oocytes, with two pronuclei observed at 18 h post-fertilization, to the number of oocytes inseminated or injected. There were no differences in the oocyte maturity rates (72.2% vs 77.7%, p=0.6), and in the fertilization rates in IVF and ICSI (49.4% vs 50.2%, p=0.9 and 76.4% vs 68.8%, p=0.53, respectively) between the endometriosis and control group.

We performed a sub-analysis comparing the 32 patients with severe endometriosis to the control group (**Table 2**). The patients' characteristics (age, body mass index, tobacco usage, hormonal profile, and OR markers) were also comparable between these two groups. We found no significant difference in the oocyte maturity rate (72.6% vs 77.7%, p = 0.64) and in the fertilization rates in IVF and ICSI (50% *vs.* 50.2%, p = 0.98, and 75.4% *vs* 68.8%, p = 0.59, respectively) between the severe endometriosis group and the control group (**Table 2**).

Metabolomic analysis

Among the 188 metabolites analysed, 141 were accurately measured, including hexoses, carnitine and four short chain acyl-carnitines, 21 amino acids, 16 biogenic amines and serotonin, histamine, putrescine, spemine, spermidine and taurine, 16 lysophosphatidylcholines, 70 phosphatidylcholines and 15 sphingomyelins. The FF concentrations (µmol/L) of the 11,139 measured metabolites in the 79 individuals are given in **supplementary table S1**.

After application of the Benjamini-Hochberg correction, no significant differences in mean metabolite concentrations were found between the endometriosis and the control group. PCA showed no grouping of data according to the presence of endometriosis or any other characteristic (**Figure 1**: parameters included in PC1 and PC2). We performed principal component analysis for all endometriosis patients and controls (**Figure 1A**), as well as for the subgroup of patients with severe endometriosis and controls (**Figure 1B**).

In order to analyze whether the FSH dose received had any impact on the metabolomic signature in patients with endometriosis, we divided them into two groups: those receiving a total dose of FSH per cycle ≥ 3000 IU (n=15) and those receiving < 3000 IU (n= 24). We did not find any predictive multivariate OPLS-DA to separate these two groups. Furthermore, when the FSH dose was set as the predictive dependent variable, no predictive multivariate (OPLS) model was found.

Finally, in order to assess the impact of age, we divided patients in both groups into 3 sub-groups: ≤30 years old, between 30 and 34 years old, and ≥35 years old, and we constructed OPLS-DA models for each subgroup in order to take age into account as a possible confusion

factor. However, we did not find any predictive OPLS-DA model to separate controls from patients with endometriosis, in all age subgroups.

Multivariate supervised modelling failed to find a predictive OPLS-DA model discriminating between the endometriosis and control groups with an even negative value for cumulated Q^2_{cum} (**Figure 2 A**), and between patients with severe endometriosis (n=32) and the control group (**Figure 2B**). Negative Q^2_{cum} values indicate very poor predictive capabilities, even when relatively well-separated groups appear in the scatter plot. This separation is due to overoptimistic models that find directions or latent variables where compared groups seem well discriminated. However, these latent variables do not contain systematic variations of the original predictive variables (i.e., metabolites) determining group discrimination, but (random) noise instead. Therefore, when a sample set, not used for model construction, but instead coming from the same population in which the model has been built, is submitted to this model, its predictive capabilities (i.e., right class allocation) are very poor and not very different from the prediction of a random model (i.e., flipping a coin to predict whether a patient belong to the endometriosis or to the control group). This is in contrast with our previous results using the same methodology in patients with DOR (n=28 vs n=29), where we found a highly OPLS-DA predictive model ($Q^2CUM = 0.76$) (*De la Barca et al.*, 2017).

Discussion

The current study aimed to analyze the impact of endometriosis on female fertility based on metabolomic analysis of the follicular fluid in patients undergoing IVF/ICSI. We tried to find specific biomarkers of the disease that would improve our understanding of the pathophysiologic mechanisms by which endometriosis negatively affects the oocyte microenvironment and fertility.

Several metabolomic signatures related to endometriosis have been recently identified in biological fluids. Indeed, altered levels of amino acids, purines, and phospholipids were found in the endometrium (*Li et al.*, 2018a; *Li et al.*, 2018), while altered levels of carnitine, acylcarnitines, phosphatidylcholines and sphingomyelins were found in the peritoneal fluid (*Vouk et al.*, 2016), and altered levels of acylcarnitines were found in the plasma (*Letsiou et al.*, 2017). Except for the purine metabolites, all these metabolites were analyzed by our analytical pipeline.

On the other hand, several metabolomic studies have identified metabolomic or lipidomic signatures in the FF of patients with endometriosis. Cordeiro et al (*Cordeiro et al.*, 2015), compared the FF lipidome of 10 patients with endometriosis to 10 controls, and found increased concentrations of sphingolipids and phosphatidylcholines in the endometriosis group. Marianna et al (*Marianna et al.*, 2017), compared the metabolome of the FF of 16 patients with endometriosis stage I-II (n = 8) or stage III-IV (n = 8) to 7 controls, and found significantly higher concentrations of phospholipids and lactates in the endometriosis group, and significantly lower concentrations of fatty acids, lysine, choline, glucose, aspartate, alanine, leucine, valine, proline, and phosphocholine. A recent metabolomic study that used nuclear magnetic resonance (NMR) spectroscopy in 12 endometriotic patients and 12 controls found elevated lactate, glucose, pyruvate and valine levels (*Karaer* et al., 2018).

In the current study, we assessed these same metabolites, but we did not find any discriminant signature in the FF of patients with endometriosis, regardless of the stage of the disease, despite using the same rigorously standardized targeted metabolomic methods that we had used in a previous study in patients with DOR, and which showed significant differences (*De la Barca et al.*, 2017). Thus, we did not identify any significantly modified metabolites with univariate analysis coupled with the Bonferroni-Hochberg correction (a necessary correction in the context of multiple comparisons to avoid type I errors). Furthermore, we did not find any OPLS-DA model discriminating between the endometriosis and the control group, even when the study was restricted to the most severe forms of endometriosis (stages III-IV).

Several factors could explain the discordance between our findings and the published literature – i.e. the lack of a specific metabolomics signature in the FF of patients with endometriosis. First of all, most of the published studies included relatively small numbers of patients, around 10-15 in the endometriosis and control group, whereas our study included 39 patients and 40 controls, thus improving its statistical power. On the other hand, most of these studies included women with a high risk of DOR: Marianna et al. (*Marianna et al.*, 2017) included women with an average age of 35 years and with low serum AMH levels (<2 ng/mL), whereas Karaer et al. (*Karaer et al.*, 2018) included women until 40 years of age. We have previously shown that women with DOR had a significantly different metabolomics signature in the FF when compared to controls with a normal OR (*De la Barca et al.*, 2017). Therefore, the DOR could have influenced the differences found that were attributed to endometriosis in the aforementioned studies.

The number of oocytes retrieved was significantly lower in the endometriosis group compared to the control group, but the rate of oocyte maturity and fertilization rate were comparable between the two groups. Several studies have already reported a lower number of oocytes retrieved in endometriosis patients (*Sanchez et al.*, 2017). This could be due to the

difficulties sometimes encountered during retrieval in cases of severe endometriosis (*Benaglia et al.*, 2018). Indeed, the access to the follicles may be difficult because of the presence of a large endometrioma, or because of the position of the ovary (retro-uterine or high in the pelvic cavity). Moreover, there is the risk of an infectious complication should the puncture needle pass through an endometrioma or a dilated tube (*Benaglia et al.*, 2018).

To date, there is no consensus on the direct impact of endometriosis on the capacity of the oocyte to fertilize and sustain the early embryo development A recent literature review (*Sanchez et al.*, 2017) analyzed the conflicting results of all published meta-analyses that evaluated the fertilization rate for patients with endometriosis (*Barnhart et al.*, 2002; *Harb et al.*, 2013; *Yang et al.*, 2015; *Rossi et al.*, 2016). The oldest study, dating back to 2002, compared the fertilization rate for patients with isolated endometriosis, i.e. with the absence of any other infertility factors, to that of a control group of patients with tubal factor infertility (*Barnhart et al.*, 2002). The authors found a significantly lower fertilization rate in the isolated endometriosis group compared to the control group (59.5% vs. 66.1%, $p < 0.001$) (*Barnhart et al.*, 2002). More recently, two other meta-analyses found no significant decrease in the fertilization rate or the embryo quality, even in patients with severe endometriosis or endometriomas (*Harb et al.*, 2013; *Yang et al.*, 2015). In our study, the OR reserve markers (AFC, AMH, FSH) were comparable between the endometriosis and control groups, even in the presence of endometriomas. These findings are in accordance with studies showing that endometriosis and endometriomas do not necessarily diminish the ovarian reserve by themselves, but it is the surgery of these lesions that does the damage (*Streuli et al,* 2012). Furthermore, we found no difference in the oocyte maturity and fertilization rates between the two groups. The relationship between oocyte quantity (OR) and quality is still the subject of debate: while some studies report a decrease in oocyte quality that parallels the decrease in OR (*Gleicher et al.*, 2009; *Gleicher et al.*, 2011), others report that there is no direct

correlation, with women with DOR having equivalent embryo quality and live birth rates to matched controls with normal OR (*Morin et al.,* 2018*).* The endometriosis patients in our study had normal OR, thus a probably normal oocyte quality. The fact that we found no specific metabolomic signature in their FFs could indicate that, in the absence of other confounding factors, endometriosis had no deleterious effect on the oocyte microenvironment.

One of the limitations of our study is the fact that ovarian stimulation with exogenous gonadotropins could have altered follicular metabolomics. Indeed, several in vitro models and animal studies have shown that ovarian stimulation with gonadotropins could modify the metabolomic and the lipid profile, in the serum as well as the follicular fluid (*Perovic et al.*, 2019; *Santos et al.*, 2017).

Conclusion

Several ovarian pathologies have been shown to alter the micro-environment of the cumulus-oocyte complex (COC), but the impact of endometriosis in women with infertility remains the subject of debate. In the current study, we did not find any specific metabolomic signature of endometriosis in the follicular fluid of women with infertility undergoing IVF/ICSI, thus confirming the lack of impairment of the COC microenvironment by endometriosis.

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Authorship:

PEB, JMCB, PR and PMP, the principal investigators, take primary responsibility for the paper. PEB, JMCB, PR and PMP contributed to the conception, design and coordination of the research. PEB, GL and PD recruited the patients. PEB, JMCB, PR and PMP contributed to the collection and analysis of data. PEB, JMCB, HEH, PR and PMP contributed to writing the manuscript. PEB, JMCB, HEH, GL, PD, PR and PMP contributed to the revision of the article, and the final version was approved by all the authors.

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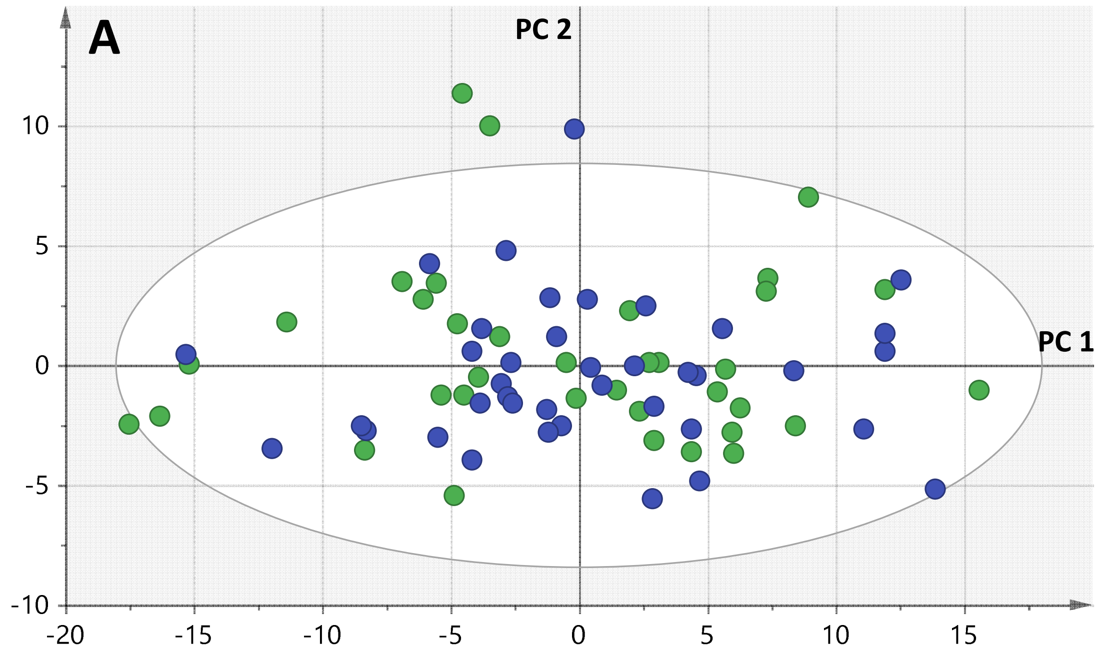
Figure 1. First principal plan scatter plot of the PCA built with 141 metabolites accurately measured in folicular fluid of endometriosis and control patients (**A**) or severe endometriosis and control patients (**B**). No grouping of data can be distinguished. Even when some points appear out of the confidence ellipse, they are not stronger outliers when considering not only the first two but all the principal components. Green circles represent endometriosis patients and blue circles control patients. Legend: PC 1, 2: First and second principal component.



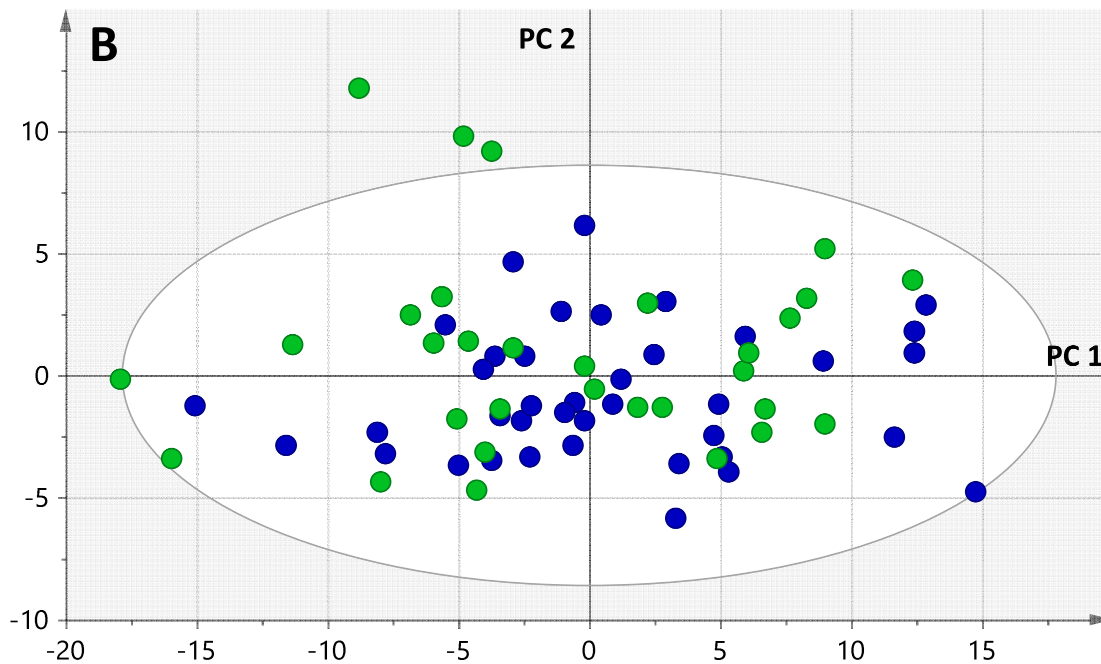
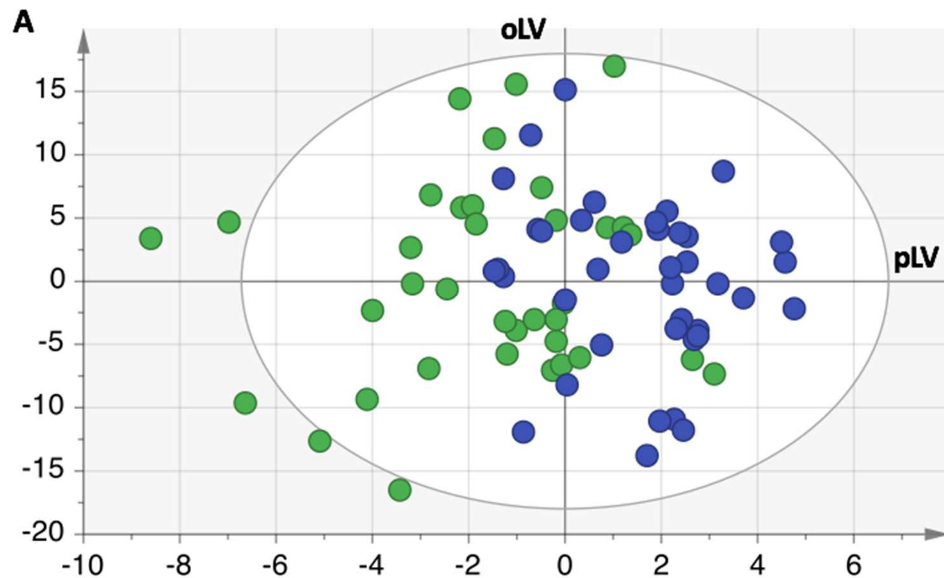


Figure 2A & 2B. OPLS-DA scatter plot for models comparing all endometriosis patient (**A**) or only patients with severe endometriosis (**B**) to the control group, using 141 accurately measured metabolites as predictive variables and endometriosis status as explained variable. When points are projected to the predictive latent variable (pLV), no group separation is achieved in either cases by the OPLS-DA models. This graphical representation is in total agreement with the poor predictive qualities of the supervised models. Green circles represent endometriosis patients and blue circles control patients. Legend: pLV: predictive latent variable; oLV: orthogonal latent variable.



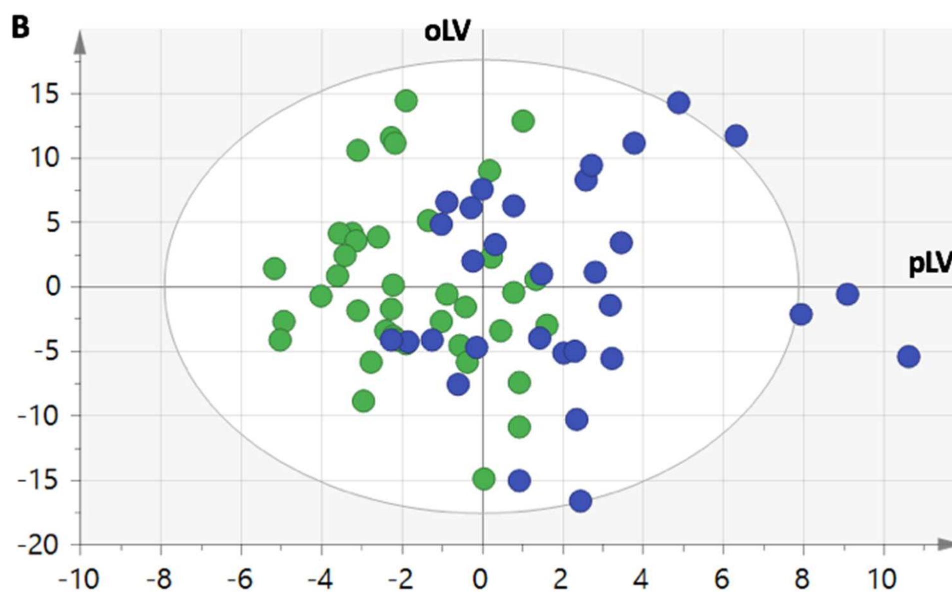


Table 1. Baseline characteristics of patients and cycles.

		Endometriosis group (n=39)	Control Group (n=40)	p-value
Age (years)		32.3 ± 4.1	31.6 ± 4.4	0.63
Body Mass Index (kg/m^2)		23.5 ± 4.2	24.1 ± 3.9	0.52
Smoking status				0.78
• Non-smoker		24 (61.5)	24 (60)	
• Smoker		3 (7.7)	5 (12.5)	
• Former smoker		8 (20.5)	10 (25)	
• Information missing		4 (10.3)	1 (2.5)	
Baseline E2 (pg/mL)		51.7 ± 45	37.6 ± 16.1	0.55
Baseline FSH (IU/L)		7.6 ± 3.2	8.5 ± 2.4	0.13
Baseline LH (IU/L)		4.7 ±1.9	5.5 ± 2.2	0.1
Baseline AMH (ng/mL)		2.6 ± 1.7	3.1 ±1.9	0.15
Antral follicle count		17.3 ± 7.9	17.9 ± 6.8	0.77
Total dose of FSH per cycle (UI)		2732 ±1250	2257 ± 705	0.09
Type of protocol	Antagonist	21 (54)	39 (97.5)	< 0.001
	Agonist	18 (46)	1 (2.5)	
Stimulation	FSH	26 (66.7)	33 (82.5)	0.13
	FSH + LH	13 (33.3)	7 (17.5)	
Treatment type	IVF	19 (48.7)	16 (40)	0.43
	ICSI	20 (51.3)	24 (60)	
Oocytes per retrieval		8.6 ± 6.8	13.3 ± 5.8	< 0.001
Rate of oocyte maturity in ICSI (%)[1]		72.2	77.7	0.6
Fertilization rate in IVF (%)[2]		49.4	50.2	0.9
Fertilization rate in ICSI (%)[3]		76.4	68.8	0.53
Ongoing pregnancy rate (%)[4]		13	20	0.4

Data are expressed as n (%), percentage or mean +/- standard deviation.
[1]Number of mature oocytes injected / number of oocytes recovered.
[2]Number of embryos obtained at stage 2 pronuclei / number of inseminated oocytes.
[3]Number of embryos obtained at stage 2 pronuclei / number of oocytes injected.
[4]Ongoing pregnancy rate was defined when the pregnancy had completed > 20 weeks of gestation.

Table 2. Comparison between patients with severe endometriosis and control group.

		Severe Endometriosis group (n=32)	Control Group (n=40)	p-value
Age (years)		32 .4 ± 3.9	31.6 ± 4.4	0.57
Body Mass Index (kg/m^2)		23.1 ± 4.1	24.1 ± 3.9	0.33
Smoking status				0.93
• Non-smoker		19 (59.3)	24 (60)	
• Smoker		7 (21.9)	5 (12.5)	
• Former smoker		3 (9.4)	10 (25)	
• Information missing		3 (9.4)	1 (2.5)	
Baseline E2 (pg/mL)		50.4 ± 47.9	37.6 ± 16.1	0.94
Baseline FSH (IU/L)		7.6 ± 3.4	8.5 ± 2.4	0.15
Baseline LH (IU/L)		4.5 ±1.6	5.5 ± 2.2	0.06
Baseline AMH (ng/mL)		2.8 ± 1.9	3.1 ±1.9	0.4
Antral follicle count		17.6 ± 7.8	17.9 ± 6.8	0.97
Total dose of FSH per cycle (UI)		2787 ±1136	2257 ± 705	0.053
Type of protocol	Antagonist	16 (50)	39 (97.5)	< 0.001
	Agonist	16 (50)	1 (2.5)	
Stimulation	FSH	22 (68.7)	33 (82.5)	0.13
	FSH + LH	10 (31.3)	7 (17.5)	
Treatment type	IVF	13 (40.6)	16 (40)	0.43
	ICSI	19 (59.4)	24 (60)	
Oocytes per retrieval		8.3 ± 7.3	13.3 ± 5.8	< 0.001
Rate of oocyte maturity in ICSI (%)[1]		72.6	77.7	0.64
Fertilization rate in IVF (%)[2]		50	50.2	0.98
Fertilization rate in ICSI (%)[3]		75.4	68.8	0.59
Ongoing pregnancy rate (%)[4]		15.6	20	0.63

Data are expressed as n (%), percentage or mean +/- standard deviation.
[1]Number of mature oocytes injected / number of oocytes recovered.
[2]Number of embryos obtained at stage 2 pronuclei / number of inseminated oocytes.
[3]Number of embryos obtained at stage 2 pronuclei / number of oocytes injected.
[4]Ongoing pregnancy rate was defined when the pregnancy had completed > 20 weeks of gestation.