Metabolic profiling of follicular fluid and plasma from natural cycle in vitro fertilization patients—a pilot study

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Objective: To investigate changes in follicular fluid (FF) and plasma composition during the follicular and periovulatory phases of the menstrual cycle in patients undergoing assisted conception, using proton nuclear magnetic resonance (^{1}H-NMR) spectroscopy–based metabolite profiling.
Design: A pilot prospective laboratory study.
Setting: Assisted conception clinic in a university hospital.
Patient(s): Ten women undergoing natural-cycle (NC) in vitro fertilization (IVF) or intracytoplasmic sperm injection (ICSI) with either male factor subfertility or unexplained subfertility.
Intervention(s): FF and plasma were collected during the midfollicular phase or at the LH-surge and at the time of oocyte collection.
Main Outcome Measure(s): ^{1}H-NMR spectroscopy was performed on the fluids and the metabolic profiles compared across the phases with the use of principal components analysis (PCA).
Result(s): LH-surge FF resembled periovulatory FF more than midfollicular FF, with higher levels of lactate and pyruvate and lower glucose. Periovulatory plasma contained higher levels of glucose and acetate and lower glycoprotein, trimethylamine, and glycine compared with midfollicular and LH-surge plasma.
Conclusion(s): NMR-based metabolite profiling of FF and plasma has potential for identifying changes across menstrual stages, studying the impact of exogenous hCG administration, and in the pursuit of biomarkers to predict fertility treatment outcome. (Fertil Steril® 2012;98:1449–57. ©2012 by American Society for Reproductive Medicine.)
Key Words: Blood plasma, follicular fluid, in vitro fertilization (IVF), metabolomics, nuclear magnetic resonance (NMR)

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Current challenges in assisted reproduction technologies (ART) include optimizing the time of oocyte collection for in vitro fertilization (IVF) and selecting oocytes with the greatest potential for embryo development. Understanding more about changes in the environment of the oocyte during the menstrual cycle, both locally and systemically, in assisted conception patients is an essential first step in this process. Gaining a better understanding of the follicular dynamics associated with oocyte maturation also is desirable. Cyclic metabolic fluctuations in parallel with endocrine changes during a menstrual cycle have been recognized in the investigation of premenstrual syndrome since the 1920s (1–3). Cyclic fluctuations in lipid metabolism have been determined in normally cycling women (4, 5), and metabolite concentration changes in rat urine have been correlated with different stages of the estrous cycle (6). More recently, Wallace et al. (7) analyzed the composition of plasma and urine from women during four phases of their menstrual cycles and identified a separation in plasma between the menstrual and luteal phases.

Follicular fluid (FF) perhaps has the most potential to reveal information about the impact of menstrual cycle metabolic fluctuations on the oocyte. FF is a plasma transudate that fills the follicle antrum and whose composition

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is in part determined by local follicular metabolic processes. The follicle wall acts as a coarse molecular sieve, allowing small metabolites to pass through while restricting the access of molecules >100 kDa. FF supports oocyte maturation and thus its composition may have a direct influence on the oocyte, in both its ability to mature and its quality (8). Although FF composition in relation to follicle size and the estrous cycle has been studied, this has principally been carried out in animal models on a select number of metabolites. Levels of carbohydrates, such as glucose, lactate, and pyruvate have been examined in human (9, 10), murine (11, 12), ovine (13), bubaline (13), and bovine (14) FF. The latter study also investigated changes in FF amino acid levels with the estrous cycle. Others have focused on other FF components, including steroids, enzymes, proteins, and ions in sheep (15), buffalos (16), cattle (17, 18), and gray seals (19). Some of these studies included the examination of small panels of metabolites, e.g., Nandi et al. (15) measured glucose, cholesterol, triglycerides, lactate, urea, and creatinine in ovine FF; Schweigert and Schams (19) profiled triglycerides and cholesterol in seal FF; Leroy et al. (18) measured concentrations of glucose, 3-hydroxybutyrate (3HB), lactate, urea, triglycerides, cholesterol, and fatty acids; and Eissa (16) also profiled glucose in buffalo FF.

Nuclear magnetic resonance (NMR) spectroscopy is ideal for the analysis of biofluids, because it allows the simultaneous identification of all the low-molecular-weight metabolites in the intact FF sample with little sample preparation. Using this approach, Gérard et al. (20) measured the concentrations of several metabolites in equine FF and compared them at very early follicular, late dominant, and preovulatory stages. Sarty et al. (21) worked with bovine FF from four selected stages of the estrous cycle. The latter study and the plasma study by Wallace et al. (7) both used a metabolite profiling approach called metabolomics or metabonomics (22, 23). Metabolomics is the transcendent of genomics, transcriptomics, and proteomics and is concerned with the quantitative measurement of all the metabolites in the "system" (the "metabolome") (Nicholson, et al. 1999). In a recent review, Baskind et al. (24) concluded that metabolite analysis, specifically by NMR spectroscopy, has great potential for monitoring menstrual cycle–associated metabolic processes, with the possibility of identifying candidate biomarkers that may allow the assessment of reproductive function and predict fertility treatment outcomes.

The aim of the present study was to investigate the metabolic profiles of FF and blood plasma as a function of follicular phase during the natural menstrual cycle. Fluids were collected from ten women undergoing natural-cycle (NC) IVF or intracytoplasmic sperm injection (ICSI) in two consecutive cycles. In the first cycle, fluids were taken during the follicular phase or at the time of the LH-surge and no oocyte retrieval was performed. In the subsequent cycle, fluids were taken during the periovulatory phase as part the normal oocyte retrieval in the treatment. Proton (^{1}H) NMR spectroscopy was used to analyze the fluids, and principal components analysis (PCA) was used to interpret the multivariate spectral data. These techniques allowed a nontargeted comparison of fluid metabolites in the midfollicular and periovulatory phases.

MATERIALS AND METHODS

Sample Collection

From November 2008 to March 2009, ten couples were selected according to strict inclusion criteria from those undergoing natural cycle NC-IVF/ICSI at the Assisted Conception Unit, St James's University Hospital, Leeds, United Kingdom; see Table 1 for patient demographics. The inclusion criteria included body mass index 19–30 kg/m^2, age 25–35 years, and nondiabetic. The NC cohort was selected to remove the impact of gonadotropin stimulation effects on metabolite profiles. The second cycle of treatment was accompanied by administration of hCG to trigger ovulation to reduce cycle cancellation as a result of failed or unidentified LH-surge

TABLE 1

Patient demographics, cycle details, hormone levels, and treatment outcome.

				Menstrual cycle phase at time of aspiration		Blood hormone level[a]							
						Cycle 1			Cycle 2				
Patient	Age, y	BMI, kg/m^2	Etiology	Cycle 1	Cycle 2	FSH	LH	E_2	FSH	LH	E_2	IVF/ICSI	IVF/ICSI outcome
1	30	22	MFI	Midfollicular	Periovulatory	3.4	5.7	878	10.5	22	248	ICSI	No pregnancy
2	34	28	MFI	Midfollicular	Periovulatory	5	8.5	584	15.8	84.5	1213	ICSI	Immature oocyte
3	33	23	U	LH-surge	Periovulatory	8.8	31.7	1508	14.9	79	738	IVF	No pregnancy
4	29	27	MFI	Midfollicular	Periovulatory	3.8	7.5	749	4.4	14.7	392	ICSI	Biochemical pregnancy
5	30	20	MFI	LH-surge	Periovulatory	11.7	71.9	1604	20.8	129.2	1708	ICSI	First-trimester miscarriage
6	33	21	MFI	LH-surge	Periovulatory	7.2	20.9	1890	12.2	17.6	1450	ICSI	No pregnancy
7	29	23	MFI	Midfollicular	Periovulatory	5.4	9.7	1120	8.8	25.6	389	ICSI	No pregnancy
8	27	24	MFI	Midfollicular	—	5.6	9.2	171	4.9	12.6	798	—	Ovulated before UDOR
9	33	21	U	Midfollicular	Periovulatory	4.5	5.4	1764	18.4	35.4	619	IVF	Live birth
10	30	26	U	Midfollicular	Periovulatory	6.7	4.7	281	12.4	13.4	322	IVF	Immature oocyte
Mean	30.8 ± 2.3	23.5 ± 2.7											

Note: ICSI = intracytoplasmic sperm injection; IVF = in vitro fertilization; MFI = male factor infertility; U = unexplained; UDOR = ultrasound-directed oocyte recovery.
[a] FSH and LH are in units of IU/L and E_2 is in units of pmol/L.

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