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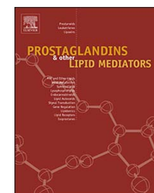

Original Research Article

Comprehensive profiling of prostaglandins in human ovarian follicular fluid using mass spectrometry

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ABSTRACT

Prostaglandins are formed by enzymatic and non-enzymatic mechanisms. They have been detected in human ovarian follicular fluid (HFF), a medium rich in growth factors and nutrients important for oocyte growth and fertility. However, the comprehensive identification of HFF prostaglandins has not been addressed. Here we use hybrid triple quadrupole time-of-flight and triple quadrupole mass spectrometers to comprehensively analyze prostaglandins in HFF. We identified PGE1, PGE2, PGF2α, and other prostaglandins synthesized via prostaglandin-endoperoxide synthase (i.e. Cox) cascades. We also identified specific PGF2α isomers (F2-isoprostanes) and PGF3α analogs whose structures are inconsistent with Cox-dependent formation. A prospective cohort pilot study of infertility patient subtypes revealed two potential associations. F2-isoprostanes are decreased in the diminished ovarian reserve subtype and elevated PGF2α may be associated with decreased live birth. Other than PGF2α, only body mass index > 25 kg/m^2 correlated with poor *in vitro* fertilization outcome. Our studies suggest that HFF contains prostaglandins formed from at least two mechanisms, which may correlate with distinct clinical parameters.

1. Introduction

Prostaglandins (PGs) are signaling molecules derived from dietary fats with clinically relevant roles in reproductive biology [1–4]. PGE2, for instance, promotes ovulation downstream of the luteinizing hormone surge [5,6]. Excess consumption of nonsteroidal anti-inflammatory drugs, which inhibit prostaglandin-endoperoxide synthase (a.k.a. cyclooxygenase or Cox), is associated with reversible female infertility, likely due to failed ovulation [7,8]. On the other hand, proinflammatory cytokines increase PGF2α associated with corpus luteum development and immune cell recruitment [9]. The mature human follicle contains mural and cumulus granulosa cells surrounding a single oocyte. During follicle development, an antrum forms that is filled with fluid containing PGs, steroids, peptide growth factors, and metabolites [10]. Human ovarian follicular fluid (HFF) is collected along with cumulus-oocyte complexes from mature follicles in patients undergoing *in vitro* fertilization (IVF), providing a window into the physiological signaling processes occurring in fertile and infertile women. Although PGs have been analyzed in HFF, most studies used radio- and enzyme-immunoassays, which lack the specificity to distinguish among the complexity of PG types and isomers known to exist today [11–13].

PGs are synthesized from the 20-carbon polyunsaturated fatty acids (PUFAs) dihommo-gamma-linolenic acid (DGLA), arachidonic acid (AA), and eicosapentaenoic acid (EPA) [1,14,15]. A key structural feature is the cyclopentane ring, which contains side groups that define classes. For instance, the F-series class member PGF2α is synthesized from AA and contains hydroxyl groups at the carbon-9 (C9) and C11 positions in the cyclopentane ring [16]. Classical PG synthesis is initiated by Cox enzymes, which convert AA into the bicyclic endoperoxide PGG2 and then PGH2 [17,18]. PGD, PGE, and PGF synthases convert PGH2 into bioactive forms [16,19,20]. PGs are also formed non-enzymatically by free radical-induced peroxidation [21,22]. In this mechanism, reactive oxygen species (ROS) produced during oxidative stress act on C20 PUFAs in phospholipids. PGs generated by Cox or ROS can be distinguished through structural information [23–25]. Cox pathways generate free PGs with specific stereochemistry [17]. In contrast, ROS produce a broad spectrum of PG classes and isomers that are esterified to phospholipids (Fig. 1). As auto-oxidation reactions lack specificity, free radical-induced peroxidation generates 64 esterified

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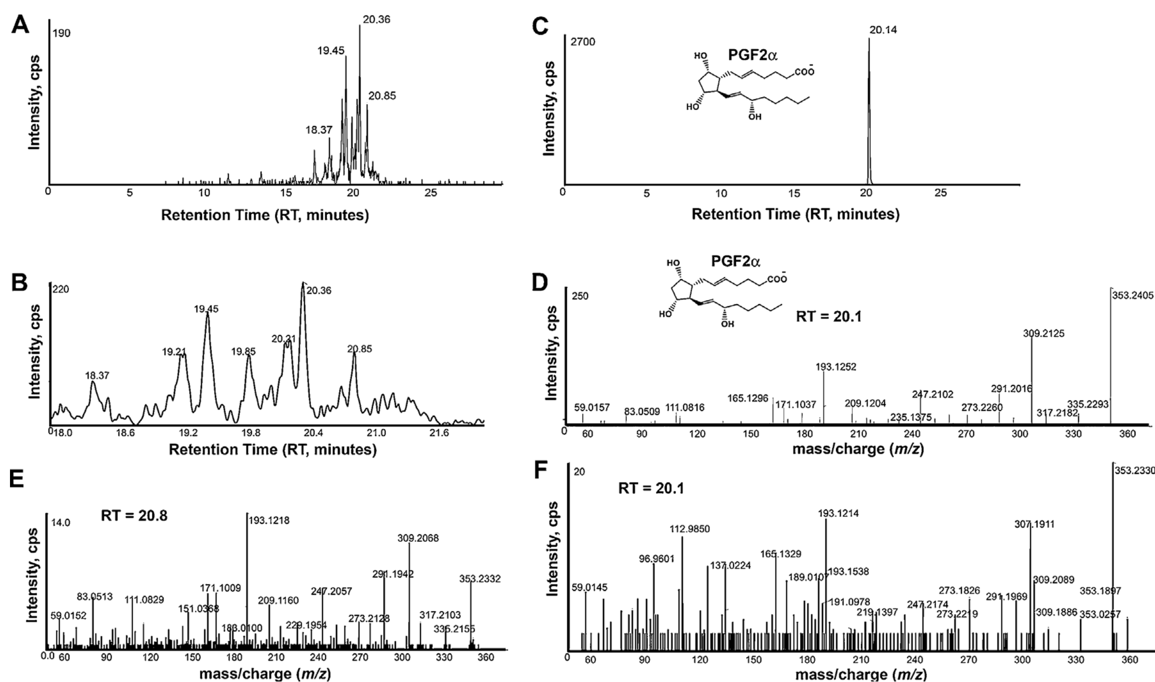

Fig. 1. PGF2α isomers in HFF. An extracted ion chromatogram (XIC) generated from the product ion m/z 193.122 that is found in 15-F2-isoprostanes (A). A magnified chromatogram focusing on RT 18–22 min is shown below (B). An XIC for product ion m/z 193.122 shows a single peak at RT 20.14 min from the PGF2α standard (10 ng/ml) (C). MS/MS product ion spectrum of the PGF2α standard (D). MS/MS product ion spectrum of m/z 353.2323 at RT 20.8 min (E). MS/MS product ion spectrum of m/z 353.232 at RT 20.2 min (F). This spectrum contains overlapping isobaric precursor ions, possibly a steroidal sulfate(s), as evidenced by m/z 96.960 ($HSO4^-$). Panels A, B, E, and F are from a single control patient. An XIC for a second control patient is shown in Supplementary Fig. 1B. Cps, counts per second.

PGF2α isomers alone (termed F2-isoprostanes), comprising four regioisomeric families each with 16 isomeric members. These families are called 5-F2-isoprostanes, 8-F2-isoprostanes, 12-F2-isoprostanes, and 15-F2-isoprostanes, based on position of the cyclopentane ring in the carbon chain [21,25]. Sensitive and specific analytical methods are necessary to resolve these PG species.

There is recent evidence for a third PG metabolism pathway. The nematode *C. elegans* produces specific F1-isoprostanes, F2-isoprostanes, and F3-isoprostanes independent of Cox enzymes [26–28]. These F-series PGs are formed from DGLA, AA, and EPA precursors, respectively [27], and have an important function to attract migrating sperm to oocytes within the oviduct [27–29]. While the metabolism pathway is not well understood, insulin and TGF-β signaling pathways regulate ovarian F-series PG metabolism [26,28,30]. Genetic ablation of the two *Cox* genes in mice eliminates PGI2 and other classical PGs. However, specific F2-isoprostanes are still observed in wild-type and Cox null mice that are similar to those in *C. elegans* [26]. A distinguishing feature thus far is specificity for F-series versus D-series, E-series, and I-series PGs [27]. The extent to which the metabolic process and products generated overlaps with free radical-induced peroxidation is not clear. An important distinction is that *C. elegans* PG metabolism is strictly regulated and has a function unrelated to oxidative stress.

In this study, we used a system comprising nanoscale liquid chromatography coupled to a triple-TOF 5600 instrument, a hybrid Q-time-of-flight tandem mass spectrometer (qTOF), to comprehensively analyze PGs in HFF. To correlate selected PG concentrations across patient HFF samples, we used a conventional liquid chromatography tandem mass spectrometry (LC–MS/MS) system on a triple quadruplole mass spectrometer operated in multiple reaction monitoring (MRM) mode. The most abundant PGs across all samples are PGE2 and PGF2α. In addition, PGE1, PGF1α, and specific F2-isoprostanes and PGF3α analogs were detected. Compared to control HFF from oocyte donor and male infertility patients, HFF from patients with diminished ovarian reserve (DOR) contained significantly reduced concentration of F2-isoprostanes co-eluting with the *8-iso*-PGF2α standard (also known as iPF2α-III or 15-F2t-IsoP). These results document diverse PG types in HFF and suggest that multiple F-series PGs are important for female fertility.

2. Materials and methods

2.1. Chemicals

PGF2α-d9, PGD2-d9, PGE2-d4, PGF1α-d9, PGF2α, *8-iso*-PGF2α, *ent*-PGF2α, *ent-8*-iso-PGF2α *ent-8*-iso-15(S)-PGF2α, *15(R)*-PGF2α, *8-iso-15(R)*-PGF2α, *11β*-PGF2α, *8-iso-9β*-PGF2α, PGF1α, PGF3α, PGD2, PGE2, PGE1, PGD1, *6-keto*-PGF1α, PGA1, and PGA2 standards were obtained from Cayman Chemical (Ann Arbor, MI). All HPLC solvents and reagents were purchased from Fisher Scientific Co. (Norcross, GA) and were of HPLC grade.

2.2. Patients and HFF collection

All patients gave consent for HFF donation for research purposes as part of a University of Alabama at Birmingham Institutional Review Board approved protocol. This work has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki). Subjects were premenopausal women presenting with subfertility or as oocyte donors. No patients undergoing oocyte retrieval were excluded from this study. Pertinent patient characteristics, including laboratory values, IVF cycle laboratory values, and outcome measures were recorded. Specific variables analyzed included subject age, history of live birth, body mass index, anti-mullerian hormone level (AMH), blastocyst conversion, and IVF outcomes (no pregnancy, biochemical pregnancy, spontaneous abortion, or ongoing pregnancy). Subjects were categorized by subfertility diagnosis that led to *IVF*. A control group was comprised of oocyte donors or patient couples presenting with male factor only infertility as a diagnosis.

Transvaginal cyst puncture was performed after controlled ovarian hyperstimulation with gonadotropins (recombinant follicle stimulating

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