Comparison of endometrial prostanoid profiles in three infertile subgroups: the missing part of receptivity?

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Objective: To study the prostanoid profile of the endometria of patients with recurrent implantation failure (RIF), unexplained infertility (UIF), and recurrent miscarriages (RM), and to compare them with the endometria of healthy fertile controls.
Design: Prospective cohort study.
Setting: University hospital.
Patient(s): Fifteen patients with RIF, 18 patients with UIF, 16 patients with RM, and 23 fertile controls were recruited.
Intervention(s): Endometrial samples were taken during the window of implantation. After tissue homogenization and extraction, analysis with ultra-performance liquid chromatography diode array detector electrospray ionisation tandem mass spectrometry was performed.
Main Outcome Measures: Concentrations of prostaglandin (PG) D1, PGE1, PGF1α, 6-ketoPGF1α, PGD2, PGE2, PGF2α, 15-deoxy-Δ12,14-PGJ2, PGD3, PGE3, PGF3α, thromboxane B2, 13,14-dihydro-PGE1, 13,14-dihydro-PGF1α, 13,14-dihydro-PGF2α, 13,14-dihydro-15-keto-PGE1, 13,14-dihydro-15-keto-PGE2, and 13,14-dihydro-15-keto-PGF2α were assessed.
Result(s): Comparison of the endometria of patients with UIF and the controls showed no statistically significant differences. When the endometria of patients with RIF were compared with the controls, thromboxane B2 (TXB2) was found significantly higher (843.1 pg/mg vs. 133.5 pg/mg). When the endometria of patients with RM were compared with controls, 13,14-dihydro-15-keto PGF2α and TXB2 were found significantly higher (3907.30 pg/mg vs. 17.80 pg/mg and 858.7 pg/mg vs. 133.5 pg/mg respectively).
Conclusion(s): We identified increased endometrial presence of TXB2 in patients with RM and RIF, and 13,14-dihydro-15-keto PGF2α in patients with RM. Although common ground is observed for RM and RIF, prostanoids, on the other hand, might make their own contribution to endometrial receptivity as important as genes and proteins. Attempts to normalize the prostaglandin profile of the endometrium via enzymatic activity can open new therapeutic options. (Fertil Steril® 2020;113:670-8. ©2019 by American Society for Reproductive Medicine.)
El resumen está disponible en Español al final del artículo.

Key Words: Endometrium, receptivity, prostanoids, implantation

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The implantation process remains an enigma. Today, we accept that three basic factors are needed for a successful implantation: a healthy blastocyst strong enough to initiate the process, a well-prepared endometrium for the coming embryo, and adequate communication between these two elements (1). A well-prepared endometrium means a receptive endometrium during the window of implantation (WOI). Endometrial receptivity has been studied in terms of genomics, and as a result, some tests have been developed and are now commercially available.

Recently, lipids, especially prostaglandins (PGs), were found to play an important role during embryo implantation (2). PGE2 and PGF2α were reported to be the most important prostaglandins in animal implantation (3). The consistency observed between animal studies suggested that lipids might also influence human implantation. In 2010, an attempt to understand the lipidomic profile of the myometrium between pregnant and non-pregnant women was made by Durn et al. (4) who identified 19 prostanoids. The same year, Achache et al. (5) showed that prostaglandin synthesis in the endometrium of patients with repeated implantation failure was disrupted. Later, endometrial fluid became the material studied for the identification of the lipids (3, 6, 7); and nine specific lipids were identified, with PGE2 and PGF2α being the most relevant lipids in implantation. Thus, endometrial receptivity might be associated with genes, proteins, and lipids.

One of the main goals in infertility is to find biomarkers that reveal endometrial receptivity. By combining Durn et al. (4) and Achache et al.'s (5) studies, we hypothesized that lipids identified from the myometrium may also affect the underlying endometrium and its receptivity. We looked for these lipids in the endometrium during the window of implantation in patients with repeated implantation failure and extended the study by including two others infertility subgroups (recurrent miscarriages and unexplained infertility) and healthy fertile controls.

MATERIALS AND METHODS

Patient selection and sample collection

In this prospective cohort study, endometrial samples were collected from 18 patients with unexplained infertility (UIF), 16 patients with recurrent miscarriages (RM), 15 patients with repeated implantation failure (RIF), and 23 fertile controls during the WOI at the gynecology and infertility clinics of Istanbul University Istanbul Faculty of Medicine in 2014 through 2015. Institutional Review Board approval was obtained from the Ethics Committee of Istanbul University School of Medicine (Istanbul, Turkey), and informed consent was given by all participants. There is no conflict of interest to disclose from the authors.

UIF was defined as infertility of more than 24 months despite a normal hormone panel at Day 3, normal uterine cavity, bilateral tubal patency observed in a hysterosalpingogram, and normal spermiogram parameters. RIF was accepted as failure of pregnancy in ≥ 3 consecutive in vitro fertilization cycles with ≥ 1 transfer(s) of good quality Day-3 embryos in each cycle. RM was characterized as at least 2 miscarriages at 20 weeks or earlier in a couple with an ordinary test panel, which included a hormone profile at Day 3, hysterosalpingogram, spermiogram, karyotype of both partners, lupus anticoagulant evaluation, cardiolipin, and β2-glycoprotein antibodies (immunoglobulin [Ig]-M and IgG). For all groups, the exclusion criteria were age over 35 years, active pelvic infections, undiagnosed vaginal bleeding, uterine anomalies, endometriosis or karyotype anomalies in one or both partners, and active use of medications that interfere with prostaglandin synthesis such as non-steroidal anti-inflammatory drugs (NSAIDs). Recruited from our gynecologic clinic for well woman examinations, fertile control patients were women aged younger than 35 years, with at least one live birth and no associated medical and gynecologic comorbidities.

Patients were regularly followed up with transvaginal ultrasound for the development of a dominant follicle. Once the ovulation of the dominant follicle was confirmed with ultrasound, the patients were scheduled for sampling. After confirmation of ovulation based on blood progesterone levels, endometrial samples were taken between days 19-21, which are believed to reflect the window of implantation. Endometrial biopsies obtained using a Pipelle (Laboratoire CCD) were immediately snap frozen and transferred in liquid nitrogen to a -80°C refrigerator. Once all samples were obtained, they were again transferred in liquid nitrogen to the Biology Laboratory in Istanbul University.

In the biology laboratory, to isolate the compounds of interest, partial purification was achieved through extraction using C18 solid-phase extraction columns. The columns were then washed with 2.5 mL high Performance liquid chromatography-grade water and 1.5 mL 40% methanol. Elutions of 1.5 mL of 60%, 75%, 85%, and 100% methanol were collected in individual autosampler vials. The vials were stored at -80°C until required for mass spectrometry (MS) analysis. Ultra-performance liquid chromatography diode array detector electrospray ionisation tandem mass spectrometry (UPLC-DAD-ESI-MSMS) analysis was performed following the protocol described by Walker et al. (8).

Chemicals and Reagents

Prostaglandin D1 (PGD1), prostaglandin E1 (PGE1), prostaglandin F1α (PGF1α), 6-ketoprostaglandin F1α (6-keto-PGF1α), prostaglandin D2 (PGD2), prostaglandin E2 (PGE2), prostaglandin F2α (PGF2α), 15-deoxy-Δ12,14 prostaglandin J2 (15-deoxy-Δ12,14 2-PGJ2), prostaglandin D3 (PGD3), prostaglandin E3 (PGE3), prostaglandin F3α (PGF3α), thromboxane B2 (TXB2), 13,14-dihydro PGE1, 13,14-dihydro PGF1α, 13,14-dihydro PGF2α, 13,14-dihydro-15-keto PGE1, 13,14-dihydro-15-keto PGE2 and 13,14-dihydro-15-keto PGF2α were purchased from Cayman Chemicals. The acetonitrile, glacial acetic acid, and ethanol were purchased from Sigma Aldrich. TXB3 was not purchased because of funding and company limitations. We looked for 18 of the 19 reported prostanoids.

UPLC-DAD-ESI-MS/MS analysis

UPLC-DAD-ESI-MS/MS analysis was performed using a Waters Acquity UPLC system coupled to a diode array detector (DAD) and Xevo TQD (double-quadrupole analyzer) mass spectrometer (Waters), which was equipped with a Z-spray

TABLE 1

Demographic features of patients with repeated implantation failure, unexplained infertility, recurrent miscarriages, and controls.

Variable	Control (n = 23)	RIF (n = 15)	UIF (n = 18)	RM (n = 16)
Age (y)	32.52 ± 3 .80	33.77 ± 2.33	34.15 ± 3.80	34.43 ± 1.53
BMI (kg/m2)	24.15 ± 1.56	25.36 ± 1.19	23.14 ± 1.84	24.27 ± 1.32
Day 3 E2 (pg/mL)	51.85 ± 15.36	38.41 ± 45.10	47.43 ± 22.96	42.54 ± 35.34
Day 3 FSH (mIU/mL)	6.75 ± 1.97	7.12 ± 2.18	6.47 ± 2.67	7.02 ± 1.05
Day 3 TSH (mIU/mL)	2.46 ± 1.22	2.18 ± 1.04	2.03 ± 1.31	2.32 ± 0.84
Mean day of biopsy	19.78 ± 0.97	19.68 ± 0.84	20.00 ± 0.78	20.14 ± 0.79

Note: Data presented as mean ± standard deviation, unless stated otherwise. BMI = body mass index; E2 = estradiol; FSH = follicle-stimulating hormone; RIF = repeated implantation failure; RM = recurrent miscarriages; TSH = thyroid stimulating hormone; UIF = unexplained infertility. *P* values were not significant.

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ESI source operating in negative mode/positive mode. Data acquisition was accomplished using MassLynx v 4.1 software (Waters). The instrument was operated in the negative mode.

The analyses were performed using an Acquity BEH C18 analytical column (100 × 2.1 mm, 1.7 μm). To analyze the prostanoids, the mobile phases consisted of two solvents, i.e., 90:10:0.02 (v/v/v) bidistilled water: acetonitrile: glacial acetic acid (solvent A) and 10:90:0.02 (v/v/v) bidistilled water: acetonitrile: glacial acetic acid (solvent B). The standards of prostanoids were analyzed using gradient elution: (Flow rate = 0.2 mL/min; column temperature: 30°C): 0 mins 70% A–30% B; 4th min 70% A–30% B; 7th min 60% A–40% B (slope 1.0); 9th min 40% A–60% B (slope 1.0); 13th min 10% A–90% B (slope 1.0); 16th min 70% A–30% B (slope 1.0). All injected solutions were stored at 8°C in the autosampler. The injection volume was set to 5 μL in partial loop with needle overfill (PLINO) mode. The ionization working conditions were set for the Xevo TQD mass spectrometer as follows: capillary voltage, 3.9 kV; collision energy, 3 V; cone voltage, 40 V; cone gas flow rate, 35 L/h; desolvation temperature, 400°C; desolvation gas flow rate, 650 L/h. Cone voltage and collision energy were optimized for each compound using the IntelliStart software (Waters).

Stock standard solutions of eighteen prostanoids were prepared in ethanol at a concentration of 100 mg/L (ppm) each. A linear concentration range was studied using mixed standard solutions ranging from 1 to 1000 μg/L (ppb) by dilution of the stock solutions in ethanol–water (1:1, v/v). All stock and working solutions were stored at −20°C before analysis.

Statically analysis

To compare the concentrations of prostanoids between each disease group (UIF, RIF, RM) with fertile controls, Wilcoxon rank sum tests were performed. We believe this method was the most appropriate because we had multicollinear variables. Multiple Bonferroni testing correction was needed because we performed Wilcoxon rank sum tests comparing concentrations of multiple lipids.

RESULTS

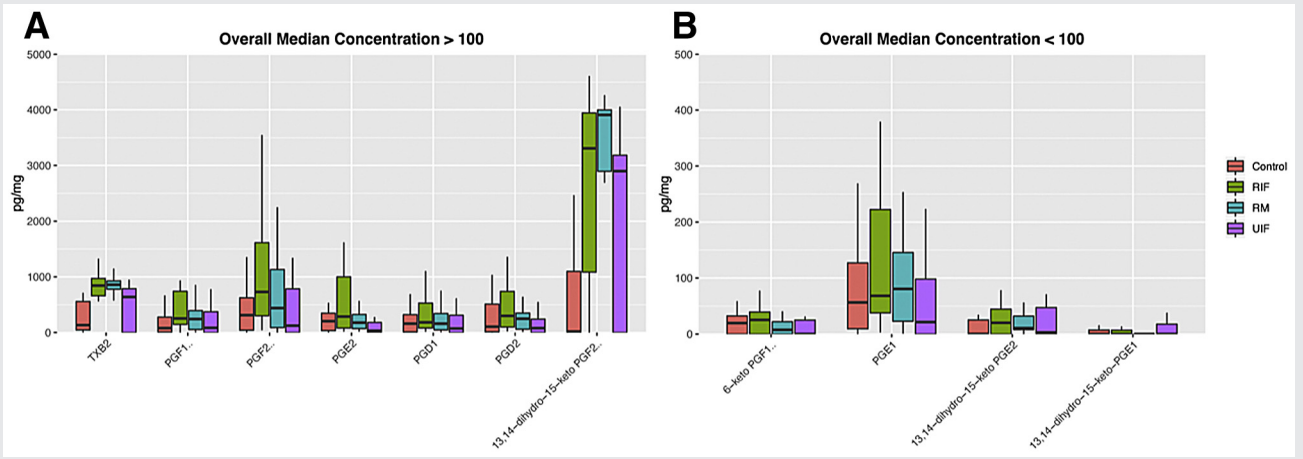
Eighteen prostanoids were identified using mass-to-charge (m/z) transitions of precursor and product ions in the multiple reaction monitoring mode (Supplemental Table 1). Using the above working mode, the prostanoids were identified by matching retention times, ultraviolet–visible spectroscopy, and mass spectral data with those of the standards concerned. Calibration curves were established for the compounds of interest as peak area versus concentration (deuterated standards were not used for quantification in this study; reference certified materials were used).

The extracted ion chromatograms of individual prostanoids are shown in Supplemental Figure 1. Calibration curves for concentration (10 different concentrations) versus response ratios were plotted for each analyte and slope and (a) intercept (b) and correlation coefficients (r) were evaluated. The limit of detection and limit of quantification were calculated at the critical signal-to-noise ratios of 3 and 10, respectively (Supplemental Table 2).

The demographic features of each group are shown in Table 1. Seven of 18 prostanoids were not detected in endometrial samples. The non-detected prostanoids were 13,14-dihydro PGE1, 13,14-dihydro-15-keto PGF1α, 13,14-dihydro-PGF2α, 15-deoxy-Δ12,14 2-PGJ2, PGD3, PGE3 and PGDF3α. For the 11 detected prostanoids, the distributions of concentrations of prostanoids for each group are illustrated as boxplots in Figure 1. It can be observed that the concentrations of detected prostanoids varied greatly. The median concentrations of all prostanoids across all samples ranged from 0 (i.e. non-detected for 13,14-dihydro-15-keto-PGE1) to 2480.15 pg/mg (for 13,14-dihydro-15-keto PGF2α).

The results of the pairwise comparisons of prostanoid concentrations between each disease groups (UIF, RM and RIF) versus the fertile controls are presented in Table 2. When prostanoid concentrations in the endometrium samples of patients wih RM were compared with fertile controls, TXB2 and 13,14-dihydro-15-keto PGF2α were significantly higher: 858.70 pg/mg vs. 133,50 pg/mg and 3907.30 pg/mg vs. 17.80 pg/mg, respectively (adjusted *P* values $<$.001). Similarly, TXB2 was higher in the endometrium samples of patients with RIF in comparison with the fertile controls (adjusted *P* $<$.001): 843.10 pg/mg vs. 133.50 pg/mg. When the TXB2 concentration was further compared between the samples of patients with RM and RIF, there was no statistically significant difference ($P >$.05).

FIGURE 1



Distributions of concentrations for the 11 detected prostanoids. For each prostanoid, boxplots of concentration values for fertile control (Control), repeated implantation failure (RIF), recurrent miscarriages (RM), and unexplained infertility (UIF) are shown.

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DISCUSSION

This study is the first to assess previously reported infertility-related prostanoids in the endometria of three infertility subgroups during the WOI. We observed two prostanoids of significant importance: high TXB2 levels in RM and RIF, and high levels of 13,14-dihydro-15-keto PGF2α (PGFM) in RM.

The first publication suggesting the association of prostaglandins with fertility was in 1969 by Nutting and Cammarata (9). This study was performed on rats. With this study, the question about the potential relationship between prostanoid and implantation/infertility was raised and it was in 1981 when the first paper assessing prostanoids in infertile women was released: TXB2 was found increased in the peritoneal fluid of infertile women with endometriosis (10).

A literature search revealed numerous studies on prostaglandins and their role in implantation. The first studies highlighted prostanoid production from the blastocyst side (11–15). Later, the endometrium became the focus of investigation. Recently, attention was paid to NSAIDs: during pregnancy, the use of NSAIDs, by inhibiting prostaglandin synthesis, was associated with an increased risk of miscarriage (16). In infertile women, it can be suggested to limit the use of NSAIDs because they decrease PGE2, the latter being positively involved in fertility (17). In our study, we found two lipid prostanoids at increased concentrations in the endometrium compared with the fertile controls: TXB2 in patients with RIF and RM, and 13,14-dihydro-15-keto PGF2α in patients with RM. Although it is impossible to differentiate whether these prostaglandins are the cause or result of infertility, they most likely have a strong influence on the endometria of patients with RIF and RM. Their potential roles are discussed below.

Thromboxane B2

Thromboxane A2 (TXA2) is synthesized from PGH2 via thromboxane synthase. Within 30 seconds, TXA2 is converted to its metabolite thromboxane B2 (TXB2). The latter, whose half-life is 7 minutes, is further metabolized into 11-dehydro-TXB2 (18). Therefore, instead of TXA2, TXB2 and 11-dehydro-TXB2 are measured in biologic samples. The main functions of thromboxanes are platelet activation, recruitment of platelets into the injury site, smooth muscle proliferation, and vasoconstriction (19).

Swanson et al. (20) showed that thromboxane synthase was expressed in human endometrial glands, stroma, myometrial smooth muscle, and uterine blood vessels, along with TXA2 receptors. This means that thromboxane is transported via blood vessels and synthesized locally in the uterus. Multiple studies reported its association with normal menstruation, dysmenorrhea, uterine smooth contractions, endometrial cancer, and preeclampsia (20, 21).

In terms of infertility, thromboxane was first investigated in endometriosis; its level was found increased in the peritoneal fluid of patients with endometriosis (10, 22, 23). In 1991, Tulppala et al. (24) reported that a thromboxane dominance and prostacyclin deficiency was observed in the urine of pregnant women with RM experiencing miscarriages when compared with pregnant women with no history of abortion. According to the same authors, when not pregnant, patients with RM demonstrated similar thromboxane levels in urine compared with fertile women (25). In our study, we found increased TXB2 levels in the endometria of non-pregnant patients with RM. These outcomes may not be contradictory because we assessed endometrial biopsies and Tulppala urine samples. We might even hypothesize that the thromboxane dominance observed in the endometrial environment starts being reflected in the urine when a patient with RM becomes pregnant. Pregnancy might induce a shift from a local to a systemic effect.

A balance between thromboxane and prostacyclin is important for tissue perfusion (26). In patients with RM, a defect in perfusion may lead to thrombosis in the vasculature, especially in the placenta. Antiphospholipid antibodies are

TABLE 2

Results of the Wilcoxon Rank Sum tests comparing the disease groups unexplained infertility, recurrent miscarriages, and repeated implantation failure versus the fertile control group (control).

	C (n = 23)	UIF (n = 18)		RM (n = 16)		RIF (n = 15)	
Prostanoid	Median (pg/mg)	Median (pg/mg)	vs. C Adj-*P*	Median (pg/mg)	vs. C Adj-*P*	Median (pg/mg)	vs. C Adj-*P*
6-keto $PGF_{1\alpha}$	19.5	0	1	7.55	1	25.3	1
TXB_2	133.5	637.4	1	858.7	<0.001	843.1	<0.001
$PGF_{1\alpha}$	78.7	84	1	243.65	1	253	0.41
$PGF_{2\alpha}$	312.2	123.85	1	439.45	1	728.6	0.5
PGE_2	203.9	34	1	177.75	1	286.2	1
PGE_1	56.4	21.45	1	80.5	1	70.8	0.86
PGD_1	160.1	73	1	160.5	1	183.8	1
PGD_2	105	79.15	1	248.15	1	299.3	1
13,14-dihydro-15-keto $PGF_{2\alpha}$	17.8	2897.05	1	3907.3	<0.001	3308	0.07
13,14-dihydro-15-keto PGE_2	0	2.9	1	10.65	1	20.1	1
13,14-dihydro-15-keto-PGE_1	0	0	1	0	1	0	1

Note: Adj-*P* = adjusted *P* value; C = control; RIF = repeated implantation failure; RM = recurrent miscarriages; UIF = unexplained infertility. Median (pg/mg) is median concentration value of the prostanoid in the given group.

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found in 20% of patients with RM; one of the mechanisms suggested for the thrombosis associated with these antibodies is the increased production of thromboxane by platelets (27). Therefore aspirin, by inducing a shift from thromboxane to prostacyclin, might be suggested during the pregnancy of patients with RM.

Although increased TXB in the endometrium leads to adverse pregnancy outcomes, the embryonic side of the fetomaternal crosstalk in implantation has a different feature: TXB is a molecule consistently found in studies assessing the prostaglandin production of blastocysts/embyros (11, 12, 14). One study reported lower TXB levels in the villous tissue of patients with early pregnancy losses when compared with women who underwent legal induced abortion (28).

The association between thromboxane and RIF is not strongly supported by previous literature. Battaglia et al. (29) demonstrated that patients who become pregnant after in vitro fertilization treatment had lower endometrium thromboxane concentrations. This study supports our results because endometrial levels of thromboxane were found to be higher in patients with RIF.

Taking the literature and our results into consideration, the embryo should synthesize and secrete thromboxane. However, on the opposite side, if the endometrium has a high thromboxane level, the implantation area might experience a perfusion defect meaning that the embryo cannot develop properly. In other words, TXB2 levels exceeding a 'threshold' become detrimental for implantation and development of pregnancy.

13,14-dihydro-15-keto PGF2α

13,14-dihydro-15-keto PGF2α (PGFM), the first and major stable metabolite of PGF2α, is a successful marker of in vivo PGF2α production (30–32). The measurement of PGFM reflects PGF2α because it is rapidly metabolized to its metabolite; therefore, we will base our discussion on studies with PGF2α and PGFM.

The functions of PGF2α on the reproductive tract are luteolysis (33), proliferation of endometrial epithelial cells (34), mediating vasoconstriction in endometrial spiral arterioles, and initiating myometrial contraction thus causing uterine ischemia (35–39), control of Na^+ and K^+ transport on surface endometrial epithelial cells (40), and induction of endometrial connexins (41). Its synthesis is controlled by estrogens (stimulating) and progestins (inhibiting) (42, 43). Therefore, its level varies during the cycle: elevated levels are observed during the implantation period (highest) and before menstruation (7, 44). While causing endometrial hypoxia, PGF2α also helps endometrial repair by increasing VEGF (45) and adrenomedullin, which boosts vascular and lymphatic endothelial proliferation and branching (46). On a pregnant endometrium, PGF2α has additional effects: it increases matrix metalloproteinase activity and decreases its inhibitor activity, increases oxytocin and its receptor expression, and decreases the response to progesterone. These effects are important because they include 'decidual activation' and are involved in parturition (47). Therefore, PGF2α is known to cause abortion, along with PGE2.

The homeostasis between the fetal unit and the maternal immune system is maintained through cytokines. When this very delicate and accurate balance is negatively affected, an aberrant immunoregulatory mechanism may break this homeostasis and cause pregnancy failure (48). PGF2α controls this complex 'cytokine activity' via its receptor (49–51). Thus, the delicate balance of PGF2α in the endometrium has important reflections. Vilella et al. (52) showed that the inhibition of PGF2α, along with PGE2, prevented embryo attachment. The use of NSAIDs during pregnancy, by inhibiting cyclooxygenase and subsequent prostaglandin synthesis, may be associated with miscarriages (52). Patients with RIF have defective prostaglandin synthesis in their endometria (5). On the other hand, pregnancy is an anti-inflammatory state. PGFM and PGF2α were found to be significantly lower in the first trimester-decidua of patients

choosing elective pregnancy termination when compared with secretory endometria of non-pregnant women (53). Bajekal and Li suggested that an increased production of PGF2α resulted in a dysfunctional uterine contraction that subsequently led to infertility issues such as abnormal sperm migration, defective transport of fertilized eggs, and impaired nidation (54, 55). Intramural myomas, which are reported to have higher PGF2α levels in the nodule and the corresponding endometrium, cause lower pregnancy and implantation rates even when they do not distort the uterine cavity (55).

PGF2α has been studied in patients experiencing miscarriages. Spontaneous abortion was shown to be associated with elevated levels of prostaglandins in amniotic fluid (56). One study reported that increased levels of PGFα were observed in the decidua of incomplete abortion compared with missed abortion and normal pregnancy (57). The authors concluded that PGF2α caused contraction, which led to the expulsion of the uterine content. Another study confirmed these findings by reporting higher PGF2α levels in the decidua of spontaneous abortion with vaginal bleeding compared with spontaneous abortion without vaginal bleeding and normal pregnancy (58). These findings were reinforced by Miura who hypothesized that, with the help of vascular channels, the increased PGF2α was transported to the subendometrial myometrium (junctional zone) and endometrium, which are believed to be the areas initiating uterine contraction and peristaltic movement (59).

The embryo itself also has a strong influence on PGF2α. A study performed on pigs showed the estrogen derived from the conceptus directed PGF2α from the uterine vasculature to the uterine lumen (60). In the uterine lumen, PGF2α was observed to promote the proliferation of trophoblasts and their adhesion to extracellular matrix protein (61). Indeed, the conceptus upregulates PGF2α through its receptor (62). We may conclude that the conceptus lowers its levels in the endometrium to a point that the detrimental effects of PGF2α are no longer effective. If the conceptus cannot induce this shift or the endometrium is refractory to its signal, 'too much' PGF2α triggers the 'pregnancy loss' cascade.

PGF2α is a dangerous key player; it controls the crosstalk between the endometrium and blastocyst; therefore, it is essential. However, too much PGFα causes disharmony, resulting in pregnancy loss.

Before concluding, we would like to assess the strengths and limitations of our study. Samples were taken during the WOI; accordingly, great care was taken to obtain enough endometrial tissue such that analysis could be performed properly. Samples were washed so that blood, coagulum, and mucus were removed, only clean tissues were assessed. Transfer of tissues was performed by the same person (I.D.K.).

We have several limitations. For patients included in the RM group, karyotypes were performed on all patients but not on all products of conception. Therefore, some miscarriages lacked karyotyping in order to rule out an embryo factor. Another limitation is that pathologic examinations were not performed on endometrial samples. The schedule of sampling was performed according to ovulation confirmed on ultrasound. We assumed that days 19-21 would reflect the window of implantation for all patients. Pathologic examinations of endometria would have given more strength to our results. To confirm ovulation, we checked progesterone levels before sampling; any level above 10 ng/mL was accepted as positive for ovulation. The potential relationship between progesterone and each group/each prostanoid is seen as beyond this paper's aim; therefore, we did not record any progesterone levels. With a Pipelle, 85%-90% of the uterine cavity is sampled; however, the area of implantation might still have been missed. Sufficient endometria are retrieved using a Pipelle for microarray analysis; however, the amount of tissue is less and more superficial compared with dilatation and curettage.

CONCLUSION

Negative pregnancy outcomes from natural or stimulated cycles remain psychological and economic burdens for society. Therefore, endometrial receptivity holds crucial importance and needs to be revealed as soon as possible. Recent studies suggested that lipids might play role in implantation.

By looking for 18 previously reported prostanoids, we identified the increased endometrial presence of TXB2 in patients with RM and RIF, as well as PGFM/PGF2α in patients with RM. The increased concentration of TBX2 in patients with RIF and RM indicates that there might be a common component in the pathogenesis of these conditions. Although the association of TXB2 with RM has been reported previously, PGF2α may become a new tool for deeper investigation in the multifactorial etiology of RM. Another interesting feature of our results is that these two molecules are essential in the establishment and development of pregnancy; however, their levels should be within limits: increased levels start the cascade that leads to adverse pregnancy outcomes.

By revealing these important components of infertility, we believe that this study provides further insight into the common and distinct aspects of various causes of infertility. Differences in endometrial prostanoid-producing enzymes could be a new area for investigation: 'normalizing' the endometrial prostanoid profile might open new treatment options.

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REFERENCES

1. Simon C, Martin JC, Pellicer A. Paracrine regulators of implantation. Baillieres Best Pract Res Clin Obstet Gynaecol 2000;14:815–26.
2. Sordelli MS, Beltrame JS, Cella M, Gervasi MG, Perez Martinez S, Burdet J, et al. Interaction between lysophosphatidic acid, prostaglandins and the endocannabinoid system during the window of implantation in the rat uterus. PLoS One 2012;7:e46059.
3. Vilella F, Ramirez LB, Simon C. Lipidomics as an emerging tool to predict endometrial receptivity. Fertil Steril 2013;99:1100–6.

4. Durn JH, Marshall KM, Farrar D, O'Donovan P, Scally AJ, Woodward DF, et al. Lipidomic analysis reveals prostanoid profiles in human term pregnant myometrium. Prostaglandins Leukot Essent Fatty Acids 2010;82:21–6.
5. Achache H, Tsafrir A, Prus D, Reich R, Revel A. Defective endometrial prostaglandin synthesis identified in patients with repeated implantation failure undergoing in vitro fertilization. Fertil Steril 2010;94:1271–8.
6. Berlanga O, Bradshaw HB, Vilella-Mitjana F, Garrido-Gomez T, Simon C. How endometrial secretomics can help in predicting implantation. Placenta 2011;32:271–5.
7. Vilella F, Ramirez L, Berlanga O, Martinez S, Alama P, Meseguer M, et al. PGE2 and PGF2alpha concentrations in human endometrial fluid as biomarkers for embryonic implantation. J Clin Endocrinol Metab 2013;98:4123–32.
8. Walker JM, Huang SM, Strangman NM, Tsou K, Sanudo-Pena MC. Pain modulation by release of the endogenous cannabinoid anandamide. Proceedings of the National Academy of Sciences of the United States of America 1999;96:12198–203.
9. Nutting EF, Cammarata PS. Effects of prostaglandins on fertility in female rats. Nature 1969;222:287–8.
10. Drake TS, O'Brien WF, Ramwell PW, Metz SA. Peritoneal fluid thromboxane B2 and 6-keto-prostaglandin F1 alpha in endometriosis. Am J Obstet Gynecol 1981;140:401–4.
11. Racowsky C, Biggers JD. Are blastocyst prostaglandins produced endogenously? Biol Reprod 1983;29:379–88.
12. Geissler FT, Kuzan FB, Faustman EM, Henderson WR Jr. Lipid mediator production by post-implantation rat embryos in vitro. Prostaglandins 1989;38:145–55.
13. Neulen J, Williams RF, Schaefer W, Flecken U, Hodgen GD. Arachidonic acid metabolism and prostaglandin production by primate preimplantation blastocysts. Prostaglandins 1991;42:121–5.
14. Shutt DA, Lopata A. The secretion of hormones during the culture of human preimplantation embryos with corona cells. Fertil Steril 1981;35:413–6.
15. Holmes PV, Sjogren A, Hamberger L. Prostaglandin-E2 released by preimplantation human conceptuses. J Reprod Immunol 1990;17:79–86.
16. Li DK, Ferber JR, Odouli R, Quesenberry C. Use of non-steroidal anti-inflammatory drugs during pregnancy and the risk of miscarriage. Am J Obstet Gynecol 2018.
17. Niringiyumukiza JD, Cai H, Xiang W. Prostaglandin E2 involvement in mammalian female fertility: ovulation, fertilization, embryo development and early implantation. Reprod Biol Endocrinol 2018;16:43.
18. Patrono C, Ciabattoni G, Pugliese F, Pierucci A, Fitzgerald GA. Estimated rate of thromboxane secretion into the circulation of normal humans. J Clin Invest 1986;77:590–4.
19. Hirata T, Narumiya S. Prostanoids as regulators of innate and adaptive immunity. Adv Immunol 2012;116:143–74.
20. Swanson ML, Lei ZM, Swanson PH, Rao CV, Narumiya S, Hirata M. The expression of thromboxane A2 synthase and thromboxane A2 receptor gene in human uterus. Biol Reprod 1992;47:105–17.
21. Rowe J, Campbell S, Gallery ED. Effects of hypoxia on regulation of prostanoid production in decidual endothelial cells in normal and preeclamptic pregnancy. J Soc Gynecol Investig 2000;7:118–24.
22. Koike H, Egawa H, Ohtsuka T, Yamaguchi M, Ikenoue T, Mori N. Eicosanoids production in endometriosis. Prostaglandins Leukot Essent Fatty Acids 1992;45:313–7.
23. Pungetti D, Lenzi M, Travisani D, Cantiero D, Maurizio G, Sarti G, et al. Prostanoids in peritoneal fluid of infertile women with pelvic endometriosis and PID. Acta Eur Fertil 1987;18:189–92.
24. Tulppala M, Viinikka L, Ylikorkala O. Thromboxane dominance and prostacyclin deficiency in habitual abortion. Lancet 1991;337:879–81.
25. Tulppala M, Viinikka L, Ylikorkala O. Nonpregnant women with a history of habitual abortion have normal and luteal function independent production of prostacyclin and thromboxane A2. Fertil Steril 1992;57:1216–9.
26. Bussolino F, Benedetto C, Massobrio M, Camussi G. Maternal vascular prostacyclin activity in pre-eclampsia. Lancet 1980;2:702.
27. Kutteh WH. Antiphospholipid antibodies and reproduction. J Reprod Immunol 1997;35:151–71.
28. Li K, Zhang X, Chen G, Pei L, Xiao H, Jiang J, et al. Association of fatty acids and lipids metabolism in placenta with early spontaneous pregnancy loss in Chinese women. Food Funct 2018;9:1179–86.
29. Battaglia C, Artini PG, Giulini S, Salvatori M, Maxia N, Petraglia F, et al. Colour doppler changes and thromboxane production after ovarian stimulation with gonadotrophin-releasing hormone agonist. Hum Reprod 1997;12:2477–82.
30. Samuelsson B, Goldyne M, Granström E. Prostaglandins and thromboxanes. Annu Rev Biochem 1978;47:997–1029.
31. Meyer HHD, Eisele K, Osaso J. A biotin-streptavidin amplified enzyme immunoassay for 13,14-dihydro-15-keto-PGF2α. Prostaglandins 1989;38:375–83.
32. Del Vecchio RP, Maxey KM, Lewis GS. A quantitative solid-phase enzymeimmunoassay for 13,14-dihydro-15-keto-prostaglandin F2α in plasma. Prostaglandins 1992;43:321–30.
33. McCracken JA, Custer EE, Lamsa JC. Luteolysis: a neuroendocrine-mediated event. Physiol Rev 1999;79:263–323.
34. Milne SA, Jabbour HN. Prostaglandin (PG) F(2alpha) receptor expression and signaling in human endometrium: role of PGF(2alpha) in epithelial cell proliferation. J Clin Endocrinol Metab 2003;88:1825–32.
35. Benedetto C. Eicosanoids in primary dysmenorrhea, endometriosis and menstrual migraine. Gynecol Endocrinol 1989;3:71–94.
36. Zahradnik HP, Breckwoldt M. Contribution to the pathogenesis of dysmenorrhea. Arch Gynecol 1984;236:99–108.
37. Pickles VR, Hall WJ, Best FA, Smith GN. Prostaglandins in Endometrium and Menstrual Fluid from Normal and Dysmenorrhoeic Subjects. J Obstet Gynaecol Br Commonw 1965;72:185–92.
38. Maybin JA, Critchley HO, Jabbour HN. Inflammatory pathways in endometrial disorders. Mol Cell Endocrinol 2011;335:42–51.
39. Martin JN Jr, Bygdeman M, Eneroth P. The influence of locally administered prostaglandin E2 and F2alpha on uterine motility in the intact non-pregnant human uterus. Acta Obstet Gynecol Scand 1978;57:141–7.
40. Vetter AE, O'Grady SM. Mechanisms of electrolyte transport across the endometrium. I. Regulation by PGF2 alpha and cAMP. Am J Physiol 1996;270:C663–72.
41. Grummer R, Winterhager E. Blastocyst-mediated induction of endometrial connexins: an inflammatory response? J Reprod Immunol 2011;90:9–13.
42. Abel MH, Baird DT. The effect of 17 beta-estradiol and progesterone on prostaglandin production by human endometrium maintained in organ culture. Endocrinology 1980;106:1599–606.
43. Schatz F, Markiewicz L, Gurpide E. Hormonal effects of PGF2 alpha output by cultures of epithelial and stromal cells in human endometrium. J Steroid Biochem 1986;24:297–301.
44. Catalano RD, Wilson MR, Boddy SC, Jabbour HN. Comprehensive expression analysis of prostanoid enzymes and receptors in the human endometrium across the menstrual cycle. Mol Hum Reprod 2011;17:182–92.
45. Maybin JA, Hirani N, Brown P, Jabbour HN, Critchley HO. The regulation of vascular endothelial growth factor by hypoxia and prostaglandin F(2)alpha during human endometrial repair. J Clin Endocrinol Metab 2011;96:2475–83.
46. Maybin JA, Battersby S, Hirani N, Nikitenko LL, Critchley HO, Jabbour HN. The expression and regulation of adrenomedullin in the human endometrium: a candidate for endometrial repair. Endocrinology 2011;152:2845–56.
47. Makino S, Zaragoza DB, Mitchell BF, Robertson S, Olson DM. Prostaglandin F2alpha and its receptor as activators of human decidua. Semin Reprod Med 2007;25:60–8.
48. Saini V, Arora S, Yadav A, Bhattacharjee J. Cytokines in recurrent pregnancy loss. Clin Chim Acta 2011;412:702–8.
49. Wallace AE, Sales KJ, Catalano RD, Anderson RA, Williams AR, Wilson MR, et al. Prostaglandin F2alpha-F-prostanoid receptor signaling promotes neutrophil chemotaxis via chemokine (C-X-C motif) ligand 1 in endometrial adenocarcinoma. Cancer Res 2009;69:5726–33.

50. Sales KJ, Grant V, Cook IH, Maldonado-Perez D, Anderson RA, Williams AR, et al. Interleukin-11 in endometrial adenocarcinoma is regulated by prostaglandin F2alpha-F-prostanoid receptor interaction via the calcium-calcineurin-nuclear factor of activated T cells pathway and negatively regulated by the regulator of calcineurin-1. Am J Pathol 2010;176:435–45.
51. Noguchi K, Endo H, Kondo H, Ishikawa I. Prostaglandin F2alpha upregulates interleukin-6 production in human gingival fibroblasts. J Periodontal Res 2001;36:80–7.
52. Li DK, Ferber JR, Odouli R, Quesenberry C. Use of nonsteroidal antiinflammatory drugs during pregnancy and the risk of miscarriage. Am J Obstet Gynecol 2018;219:275.e1–8.
53. Norwitz ER, Wilson T. Secretory component: a potential regulator of endometrial-decidual prostaglandin production in early human pregnancy. Am J Obstet Gynecol 2000;183:108–17.
54. Bajekal N, Li TC. Fibroids, infertility and pregnancy wastage. Hum Reprod Update 2000;6:614–20.
55. Miura S, Khan KN, Kitajima M, Hiraki K, Moriyama S, Masuzaki H, et al. Differential infiltration of macrophages and prostaglandin production by different uterine leiomyomas. Hum Reprod 2006;21:2545–54.
56. Karim SM, Hillier K. Prostaglandins and spontaneous abortion. J Obstet Gynaecol Br Commonw 1970;77:837–9.
57. Sugino N, Karube-Harada A, Kashida S, Takiguchi S, Kato H. Reactive oxygen species stimulate prostaglandin F2 alpha production in human endometrial stromal cells in vitro. Hum Reprod 2001;16:1797–801.
58. Sugino N, Nakata M, Kashida S, Karube A, Takiguchi S, Kato H. Decreased superoxide dismutase expression and increased concentrations of lipid peroxide and prostaglandin F(2alpha) in the decidua of failed pregnancy. Mol Hum Reprod 2000;6:642–7.
59. Kido A, Togashi K, Nakai A, Kataoka ML, Koyama T, Fujii S. Oral contraceptives and uterine peristalsis: evaluation with MRI. J Magn Reson Imaging 2005;22:265–70.
60. Ka H, Seo H, Choi Y, Yoo I, Han J. Endometrial response to conceptus-derived estrogen and interleukin-1beta at the time of implantation in pigs. J Anim Sci Biotechnol 2018;9:44.
61. Kaczynski P, Baryla M, Goryszewska E, Bauersachs S, WacLawik A. Prostaglandin F2alpha promotes embryo implantation and development in the pig. Reproduction 2018;156:405–19.
62. Kaczynski P, Waclawik A. Effect of conceptus on expression of prostaglandin F2alpha receptor in the porcine endometrium. Theriogenology 2013;79:784–90.

Comparación de los perfiles de prostanoides endometriales en tres subgrupos infértiles: ¿La parte perdida de la receptividad?

Objetivo: Estudiar el perfil de prostanoides del endometrio de pacientes con fallo de implantación recurrente (RIF), infertilidad de causa desconocida (UIF) y abortos recurrentes (RM), y compararlos con el endometrio de controles sanas y fértiles.

Diseño: Estudio prospectivo de cohorte.

Ubicación: Hospital universitario.

Paciente(s): Se reclutaron quince pacientes con RIF, 18 pacientes con UIF, 16 pacientes con RM y 23 controles fértiles.

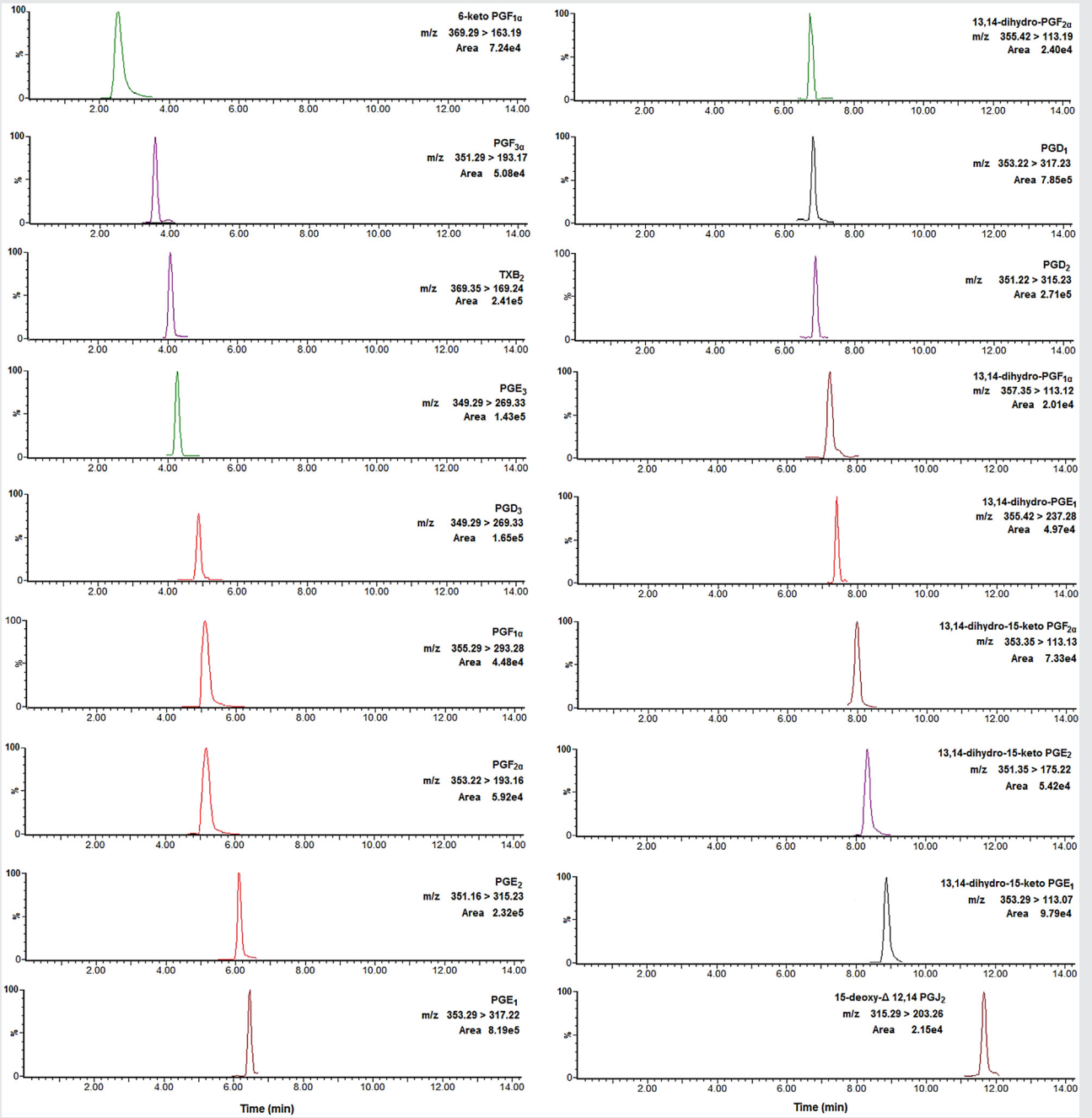
Intervención(es): Se tomaron muestras endometriales durante la ventana de implantación. Después de la homogenización celular y extracción, se realizó un análisis mediante cromatografía líquida de ultra rendimiento y espectrometría de masas en doble tandem con detector de matriz de diodos e ionización por electropulverización.

Principales medidas de resultado: Se evaluaron las concentraciones de prostaglandina (PG) D1, PGE1, PGF1α, 6-ketoPGF1α, PGD2, PGE2, PGF2α, 15-desoxy-Delta12, 14-PGJ2, PGD3, PGE3, PGF3α, tromboxano B2, 13,14-dihidro-PGE1, 13,14-dihidro-PGF1α, 13,14-dihidro-PGF2α, 13,14-dihidro-15-ceto-PGE1, 13,14-dihidro-15-ceto-PGE2, y 13,14-dihidro-15-ceto-PGF2α.

Resultado(s): La comparación del endometrio de pacientes con UIF con controles no mostró diferencias significativas. Al comparar el endometrio de las pacientes con RIF con los controles, el tromboxano B2 (TXB2) se encontró significativamente más elevado (843.1 pg/mg vs. 133.5 pg/mg). Cuando se comparó el endometrio de los pacientes con RM con el de los controles, se encontró que el 13,14-dihidro-15-ceto PGF2α y el TXB2 eran significativamente más altos.

Conclusión(es): Identificamos un incremento en la presencia endometrial de TBX2 en pacientes con RM y RIF, y de 13,14-dihydro-15-ceto PGF2α en pacientes con RM. Aunque se observa un terreno común para RM y RIF, los prostanoides, por otro lado, podrían hacer su propia contribución a la receptividad endometrial de manera tan importante como la de los genes y las proteínas. Los intentos de normalizar el perfil de prostaglandinas del endometrio a través de la actividad enzimática pueden abrir nuevas opciones terapéuticas.

SUPPLEMENTAL FIGURE 1



Extracted ion chromatograms of eighteen prostanoids

Demiral Keleş. Prostanoid profiles of infertile endometria. Fertil Steril 2019.