Randomised Trial of Lipiodol Uterine Bathing Effect (LUBE) in Women with Endometriosis-Related Infertility

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ABSTRACT

BACKGROUND: We aimed to assess whether lipiodol alters endometrial gene expression through a uterine bathing effect that might enhance receptivity to embryo implantation.

METHODS: An open-label randomised controlled trial design in a single-centre tertiary infertility service. Twelve women with endometriosis (n = 11) or previous successful lipiodol procedure (n = 1) were randomised to receive immediate or delayed lipiodol hysterosalpingography, followed by endometrial biopsy. Endometrial samples were assessed for gene expression, using Affymetrix microarrays and validation studies using reverse transcriptase quantitative polymerase chain reaction analysis. Subsequent endometrial gene expression responses to treatment and clinical fertility outcomes were assessed.

RESULTS: Eleven of 12 women had successful endometrial sampling procedures. Nine women had successful pregnancies within the 9-month follow-up phase. Following lipiodol bathing we identified 20 down-regulated and 13 up-regulated genes with $p \leq 0.05$ and with magnitude of change ≥ 1.5 -fold in at least three of the four women, with osteopontin being the only gene down-regulated in all four women.

CONCLUSIONS: This study supports the concept of a uterine bathing effect of lipiodol altering endometrial biology and gene expression. Whether regulation of inflammation and immune response pathways by lipiodol might contribute to an increase in endometrial receptivity to embryo implantation merits further investigation.

Keywords: Endometriosis; Lipiodol; Microarray; Osteopontin; Randomised Controlled Trial; Uterine Bathing.

INTRODUCTION

Fertility benefit has been demonstrated from a hysterosalpingogram (HSG) with oil soluble contrast media (OSCM) such as lipiodol versus no intervention (Mohiyiddeen et al., 2015) and now versus water soluble contrast media (WSCM) in a Dutch multi-centre trial that has re-invigorated interest in the fertility benefit of lipiodol (Dreyer et al., 2017). We found the most marked benefit amongst women with endometriosis (Johnson et al., 2004). We have shown that lipiodol does not need to flush the fallopian tubes to attain this benefit (Johnson, 2014) and we have proposed a possible uterine bathing effect of lipiodol that might alter the endometrial environment thus enhancing implantation (Johnson et al., 2005).

Our aim was to test the hypothesis that lipiodol regulates endometrial gene expression through a uterine bathing effect that might enhance receptivity to embryo implantation.

MATERIALS AND METHODS

Ethics and consent

A single-centre open-label parallel group randomised controlled trial (RCT) was undertaken in women with a good prognosis for treatment success following lipiodol uterine bathing treatment (i.e., who either had endometriosis or a previous successful pregnancy following lipiodol). Approval for the trial was granted by the New Zealand Health and Disability National Ethics Committee (approval number AKY/04/06/144) prior to commencement and annually

thereafter; the ethical standards of the Helsinki Declaration of 1975, as revised in 1983, were met. This RCT was not registered a priori in a clinical trial registry as recruitment took place from 2005 to 2006 before this requirement was in place. The full protocol for the trial has previously been described (<https://researchspace.auckland.ac.nz/bitstream/handle/2292/5544/02whole.pdf?sequence=2>) (Johnson, 2007). All study participants gave full informed signed consent to participate in the research.

Study design

Women were randomised to receive lipiodol either 'immediately' (on the same cycle as randomisation) or 'after delay' of at least three completed menstrual cycles. Endometrial sampling procedures were undertaken, then fertility outcomes were then assessed. Women were asked to refrain from other fertility treatments from randomisation for the 9-month duration of follow-up. The design of the study was such that a comparison could be made between: (a) women randomised to immediate lipiodol versus those not randomised to immediate lipiodol, (b) women subsequently becoming pregnant versus those not subsequently becoming pregnant, (c) pre- and post-lipiodol amongst individual women (Fig. 1). No formal sample size calculation was undertaken, but we estimated that approximately 12 participants would be required to show important differences in endometrial gene activation between women receiving lipiodol versus those not receiving lipiodol.

Eligibility criteria were women aged 18 to 39 years inclusive, with infertility of duration 12 months or more, who either had endometriosis (confirmed previously through laparoscopic inspection by a gynaecologist, but if laparoscopic removal of endometriosis had been undertaken, this was more than 6 months previously) or who had previously become pregnant within 6 months of a lipiodol bathing procedure. Full investigation of infertility needed to have confirmed bilateral tubal patency either by dye studies at laparoscopy or hysterosalpingogram (HSG), early follicular phase follicle stimulating hormone (FSH) level of 10 IU/L or less; mid-luteal progesterone level of 25 mmol/L or more in a spontaneous cycle; the partner's semen analysis had to be normal by the existing World Health Organization (1992) criteria. Exclusion criteria included tubal damage, previous ectopic pregnancy, or iodine allergy.

Women were recruited from a tertiary level fertility clinic in Auckland, New Zealand (Fertility Plus, Auckland District Health Board). Women were randomised at the beginning of menses. Randomisation was performed using a computer-generated random number sequence (unknown to the executors of the assignment). The randomisation was in a block of 12 but this was unknown to all, other than a statistician who generated the allocation sequence and the principal investigator (NJ). Participants were enrolled by a research fellow (SJ), who also assigned participants to their treatment groups. Allocation concealment was securely maintained by storage in sealed, sequentially-numbered opaque envelopes until the interventions were assigned. Allocation was strictly maintained sequentially, all envelopes in the sequence being used. The allocated groups were intended to be analysed for clinical outcomes on an intention to treat (ITT) basis, as well as being analysed by the a priori determined methods described.

It was not possible to blind participants to treatment allocation since the treatment involved immediate HSG procedure and the control involved delayed HSG. There was also no blinding of the executor of the assignment, the clinician performing the lipiodol procedure, nor of the assessor of clinical outcomes at follow-up. However the assessor of endometrial gene expression analyses

was blinded to the origin of the endometrial samples in terms of treatment allocation (see below).

The protocol dictated that the immediate lipiodol group underwent a lipiodol procedure on the cycle of randomisation followed by an endometrial sampling at day 5 post-ovulation (day 6 after the peak of the LH surge, determined by daily serum LH estimations); the delayed lipiodol group had an endometrial sampling at day 5 post-ovulation on the cycle of randomisation then, after three completed menstrual cycles following randomisation, they underwent a lipiodol procedure followed by an endometrial sampling at day 5 after ovulation (Fig. 1). Lipiodol procedures were performed as previously described (Johnson et al., 2004) after menses, on or before day 12 of the cycle, by one of the investigators (NJ or WH), in a radiology facility under fluoroscopic screening at Auckland Radiology Group, using the Leech-Wilkinson cannula as the delivery system. The endometrial sampling procedures were performed by pipelle sampling — using a repeated sampling technique up to three passes until it was deemed that sufficient tissue had been retrieved — by one of the investigators (NJ or SJ). After collection, the endometrial samples were divided into two approximately equal parts. The first part was placed into RNeasy (Life Technologies, NY, USA) and was subsequently used to extract RNA needed for microarray and reverse transcriptase quantitative polymerase chain reaction (RT-qPCR) analysis. The second part was prepared for immunohistochemical analyses and these results will be presented elsewhere (Baidya et al., 2009).

The primary clinical outcome was clinical pregnancy (positive pregnancy test with positive intrauterine gestation sac on ultrasound), with last menstrual period (LMP) commencing within 9 months following randomisation, that led to live birth. Clinical data were collected from study participants by a telephone consultation with a member of the research team (SJ). Other outcomes were the up- or down-regulated gene expression based on microarray studies of endometrial tissue.

For microarray analysis of the endometrial tissue, total RNA from the endometrial biopsies were isolated using TRIzol (Life Technologies) and purified using RNA affinity mini columns (manufacturer's protocol: Qiagen, Hilden, Germany). RNA yields and purity were determined using the NanoDrop spectrophotometer (NanoDrop Technologies Inc., DE, USA) and RNA integrity was assessed using the Agilent 2100 Bioanalyser (Agilent Technologies Inc., CA, USA). Total RNA (5 µg) was labelled using the MessageAmp™ Premier RNA Amplification Kit (manufacturer's instructions; Life Technologies), and hybridised to Affymetrix Human Genome U133 Plus 2.0 gene chips (Affymetrix, Santa Clara, CA, USA) by the Centre of Proteomics and Genomics (The University of Auckland, NZ) according to manufacturer's instructions.

Bioinformatic analysis was carried out using the 'R' statistical framework (R Core Team, 2013). Raw microarray "cel" files were background corrected, summarised, normalised and log2-transformed using the Robust Multichip Averaging (RMA) algorithm (Irizarry et al., 2003), as implemented in the R "affy" package (Gautier et al., 2004). Independent Component Analysis (ICA), which is a machine learning method useful for separating gene expression changes associated with different experimental parameters, was performed using Matlab software as we have previously described (Saidi et al., 2004). Paired statistical analysis of differential abundance between groups was performed on the log2-transformed data using the "linear models for microarray data" (LIMMA) package (Smyth et al., 2005). Given the small number of replicates available, a consistency of change filtering step was also included, where genes were prioritised if their expression was 1.5-fold up- or down-regulated in the same direction

following lipiodol treatment in at least 3 of the 4 women's 'before' and 'after' endometrial samples. Lists of differentially expressed probe sets were tested for enrichment of particular functional categories using Ingenuity Pathways Analysis (IPA; Ingenuity Systems, <http://www.ingenuity.com>, Redwood, CA), GeneSetDB (Araki et al., 2012) and GATHER (Chang and Nevins, 2006) web applications, which incorporated functional information from the Gene Ontology (GO) database (Ashburner et al., 2000).

RT-qPCR was used to validate a subset of the microarray study findings. Eight RNAs were chosen for RT-qPCR validation. These RNAs were selected on the basis of passing the criteria for differential expression described above, as well as encoding proteins that are associated with cell growth, cell signalling and interaction, cell death, immune response, metabolic disease or reproductive system disease (biological plausibility), or being previously reported in the literature to be regulated in infertility-related endometrial pathologies (prior evidence of gene regulation). Total RNA was DNase treated (manufacturer's protocol; Deoxyribonuclease I Amplification Grade Kit, Life Technologies), reverse transcribed to cDNA (manufacturer's protocol; Superscript III First Strand Synthesis System for RT-PCR, Life Technologies) and diluted four-fold with sterile water. The efficiency of the reverse transcription was assessed by PCR amplification of an *ACTB* (forward 5'-3': CCTCGCCTTTGCCGATCC; reverse 5'-3': GGATCTTCATGAGGTAGTCAGTC) fragment from the cDNA using standard PCR cycling conditions (annealing temperature of 58°C). Using RT-qPCR (manufacturer's protocol; Platinum SYBR Green qPCR SuperMix-UDG with ROX, Life Technologies), cDNA samples were assayed in triplicate for the expression of the eight selected RNAs and for two housekeeping RNAs: *PPIA* (a member of the peptidyl-propyl isomerase family) and *RPLP0* (a component of the 60S subunit of ribosomes), and data were analysed using the $\Delta\Delta C_T$ method, normalising against the geometric mean of the two housekeeping RNAs (Schmittgen and Livak, 2008). See Supplementary Table 1 for primer sequences.

RESULTS

Participant flow and clinical outcomes

Twelve women were recruited and randomised between August 2005 and July 2006. Follow-up continued for nine months from randomisation to April 2007 and outcomes of all pregnancies were ascertained in March 2008. Figure 1 shows the flow of participants through the study. Eleven out of the 12 women had successful endometrial sampling procedures. Clinical outcomes are clarified in Table 1. Two women receiving lipiodol in the immediate treatment group had spontaneous pregnancies within 6 months (one woman became pregnant within 3 months post-lipiodol but miscarried; the other woman had a pregnancy within 6 months post-lipiodol that eventuated in live birth); a further woman became pregnant through in vitro fertilisation (IVF) treatment between 6 and 9 months post-lipiodol (and this woman was the only participant to undergo further fertility treatment during the follow-up phase of the study). In the delayed treatment group, two women became pregnant in the 3 months prior to planned lipiodol treatment (one woman miscarried then received lipiodol at the planned time 3 months post-randomisation; the other woman had an ongoing pregnancy that eventuated in live birth thus did not receive lipiodol); two further women became pregnant following lipiodol (both within 3 months, although it was not possible to obtain a pipelle endometrial sample from one of these women); the woman who miscarried prior to lipiodol again became pregnant within 3 months post-lipiodol but miscarried again. There were no reported adverse events from the lipiodol procedures or the endometrial sampling procedures.

Microarray analyses

To identify possible biological mechanisms by which lipiodol bathing may alter endometrial receptivity, microarray analysis was undertaken on endometrial tissue from four of the women in the delayed treatment group, comparing pre- and post-lipiodol treatment gene expression profiles (two other women randomised to

Fig. 1. Flow of participants through the trial.

Table 1. Clinical outcomes for study population.

Immediate lipiodol (IL) group				
Patient	Diagnosis	Pregnant at 3 months?	Pregnant at 6 months?	Samples available?
IL 1	Prev lipiodol success	No	No	Yes
IL 2	Endometriosis	No	Yes (IVF)	Yes
IL 3	Endometriosis	No	No	Yes
IL 4	Endometriosis	No	Yes (spontaneous)	Yes
IL 5	Endometriosis	Yes (spontaneous)		Yes
IL 6	Endometriosis	No	Yes (IVF)	Yes
Delayed lipiodol (DL) group				
Patient	Diagnosis	Pregnant at 3 months (pre-lipiodol)?	Pregnant at 6 months (post-lipiodol)?	Samples available?
DL 1	Endometriosis	Yes (spontaneous, but miscarried)	No	Yes, both
DL 2	Endometriosis	No	Yes (spontaneous)	Yes, both
DL 3	Endometriosis	No	Yes (by IUI, converted to IUI from IVF)	Yes, both
DL 4	Endometriosis	No	No	Yes, both
DL 5	Endometriosis	No	Yes	No
DL 6	Endometriosis	No	No	Yes, both

delayed treatment were unable to provide before and after samples owing to pregnancy in one case and unsuccessful sampling in the other case). Data processing by ICA identified strong patient-specific gene expression patterns that were robust from the first to the second endometrial sampling in those women who successfully underwent repeat endometrial sampling, as well as samples that had lower RNA yield than others (Fig. 2). However the ICA was not sensitive enough to visualise any systematic effects associated with lipiodol bathing or subsequent pregnancy (Fig. 2). Nevertheless, paired statistical analysis (using the LIMMA method with consistency of change filtering) identified 37 Affymetrix probe sets (corresponding to 33 distinct mRNAs) that appeared to be regulated in endometrial expression following lipiodol bathing. For four mRNAs there were multiple concordantly regulated probe sets (*ATAD2*, *SORD*, *NRP1* and *OAF*), adding confidence to the results for these mRNAs. Analyses using the GeneSetDB and GATHER web tools showed that the differentially expressed mRNAs were significantly enriched for mRNAs associated with inflammation (e.g. GATHER Bayes factor = 7, $p \leq 0.0001$ for gene set GO:0006954 ‘inflammatory response’). These included the inflammation-associated mRNAs: *AIF1*, *CCR1*, *CXCL10*, *SERPINA3* and *SPP1* (osteopontin). Osteopontin (*SPP1*) was the only gene transcript regulated in all four women. These mRNAs are described in Supplementary Table 2 and in Fig. 3.

RT-qPCR analyses for mRNA

As proof of principle a small subset of the differentially expressed RNAs were validated using RT-qPCR. After normalisation of the data against two housekeeping genes, RT-qPCR gave good amplification above the threshold with one distinct peak for each sample for four mRNAs (*CD86*, *HLA-DMB*, *SERPINA3* and *SPHK1*). The other four RNAs (*BCL2A1*, *CCR1*, *HPSE* and *SPP1*) failed to amplify above the threshold level after 40 cycles of PCR and this was confirmed in a repeat RT-qPCR for the four genes failing to amplify above the threshold, indicating their expression was insufficient for reliable quantification by RT-qPCR in these samples using the selected primers. Hence RT-qPCR validation of the microarray results was performed for only four mRNAs (*CD86*, *HLA-DMB*, *SERPINA3* and *SPHK1*). Of the four mRNAs selected, one of these (*CD86*) could not be assessed in three samples. The remainder demonstrated

Fig. 2. Hinton plot showing Independent Component Analysis (ICA) of microarray data. ICA identified gene expression patterns associated with clinical and technical parameters, but not with lipiodol flushing. Samples analysed by microarray are shown in columns, and gene expression patterns (components) are shown in rows. ‘Pre’ and ‘Post’ indicate timing of endometrial biopsy relative to lipiodol flushing. Letters A-D indicate individual patients. Yellow and red boxes indicate positive and negative component values, respectively, while the size of boxes indicates component magnitude. One component (component 1) appeared to be associated with a technical feature of the endometrial samples, since it was associated with RNA yield from the tissues. Three components appeared to be associated with individual patients irrespective of lipiodol treatment (components 10, 13 and 15 associated with patients A, B and D, respectively). No components associated with a lipiodol bathing effect were identified.

Fig. 3. Heatmap showing mRNAs identified to be differentially expressed in a paired LIMMA analysis comparing pre- and post-lipiodol treated endometrial samples. Tissue biopsies are shown in columns, with numbers indicating patients, and with 'Pr' and 'Po' indicating the biopsy was taken pre- or post lipiodol flushing, respectively. Microarray RNA expression data were transformed to standardised Z-scores then mapped to colours, with red indicating low and blue high RNA abundance (see colour key at bottom of diagram). Affymetrix probe sets IDs are shown in rows, with the official gene symbol corresponding to each probe set given as a suffix to the probe set ID. Probe sets are arranged so the probe set most significantly down-regulated after lipiodol bathing is at the top of the figure, while the probe set most significantly up-regulated after lipiodol bathing is at the bottom, with a white horizontal line separating down- and up-regulated probe sets. Red arrows indicate probe sets chosen for RT-qPCR validation.

concordant regulation (with the microarray data) by lipiodol in at least three of the four women (Fig. 4).

DISCUSSION

The main finding from this study was that microarray analysis indicated 20 genes were down-regulated (reflecting 22 probe sets, but in two instances there were two probes sets for the same gene) and 13 genes up-regulated (reflecting 15 probe sets, but in two instances there were again two probes sets for the same gene) with $p \leq 0.05$ and with magnitude of change ≥ 1.5 -fold in the endometrium of at least three out of four women exposed to lipiodol. Osteopontin was the only gene transcript that was regulated in the endometrium of all women exposed to lipiodol, osteopontin being down-regulated.

Of the three predominating theories for the mechanism of lipiodol fertility enhancement — that lipiodol is an effective

'flushing agent' of amorphous debris from fallopian tubes (Watson et al., 1994); that it alters peritoneal immuno-biology through an increase in regulatory T-cells apparently mediated by a change in the activation status of mature myeloid dendritic cells due to incorporation of lipiodol by these dendritic cells (Saidi et al., 2004); that it may act through a uterine bathing effect which improves endometrial receptivity (Johnson, 2005; Johnson, 2014; Izumi et al., 2017) — this study goes some way to supporting the latter uterine bathing theory. An immuno-biological effect of lipiodol is supported by the more dramatic short term effect on fertility among women with endometriosis (Johnson et al., 2004) and an endometrial effect is further supported by our murine study that showed an increase in endometrial activated dendritic cells in mice exposed to lipiodol (Johnson et al., 2005). The lipiodol uterine bathing theory is further supported in our previous study by eight spontaneous pregnancies

Fig. 4. Comparison of RT-qPCR and microarray endometrial expression data for four women pre- and post-lipiodol bathing. For each of the four mRNAs, fold changes pre- to post-lipiodol bathing in endometrial mRNA abundance are shown for the microarray data in blue and for the RT-qPCR data in red. RT-qPCR data could not be generated for CD86 for three of the four paired patient samples.

amongst 17 women in whom lipiodol did not pass through either fallopian tube (although bilateral tubal patency had previously been confirmed), a pregnancy rate of 47% amongst women whose fallopian tubes remained 'unflushed' in their lipiodol procedure (Court et al., 2014), which compares favourably with the 40% pregnancy rate amongst all women receiving lipiodol (Court et al., 2014). It has been debated whether the adverse impact of endometriosis on fertility results purely from established poorer egg quality or whether the endometrium of women with endometriosis is less receptive (Pellicer et al., 2001). The eutopic endometrium of women with endometriosis has been recognised to have different expression of bio-markers associated with the implantation window compared to women who do not have endometriosis (Wei et al., 2009). In particular, women with endometriosis have been shown to over-express endometrial osteopontin (Cho et al., 2009; Hapangama et al., 2012), a molecule whose binding with $\alpha\text{v}\beta 3$ integrins is important to promote endometrial implantation receptivity and whose expression to just the right level in endometrium appears to be exacting in its impact on implantation receptivity (Johnson et al., 2003). The fact that osteopontin was the only candidate gene consistently down-regulated by lipiodol in the endometrium of all four women in our study, along with the regulation of other inflammation-associated genes such as *AIF1*, *CCR1*, *CXCL10* and *SERPINA3*, suggests a putative mechanism involving down-modulation of endometrial inflammation for improving fertility soon after lipiodol uterine bathing, which merits additional study.

There were a number of weaknesses in this study. As with any small trial, the power to detect small differences was limited, and this was likely why no clear signals appeared beyond the robust

sample-to-sample signatures of individual women. Although the study was designed with the intention of comparing microarray results in women randomised to immediate lipiodol versus those not randomised to immediate lipiodol and women subsequently becoming pregnant versus those not subsequently becoming pregnant, the gene signatures of these women's endometrium were so characteristic of each individual that the differences between the participants' idiosyncratic expression profiles appeared to be much larger than the lipiodol-induced gene expression changes. Thus, although 12 participants entered the pilot randomised trial, the useful analysis could only be undertaken in one arm of the RCT — the delayed treatment group. So despite the intention to make comparisons across groups and to correlate the laboratory findings with clinical outcomes, ultimately the meaningful part of the study could be considered as a prospective own-control study in a small cohort of women recruited for one treatment arm of this RCT. And even for this comparison of pre- and post-lipiodol amongst individual women, the results were based only on four women, rather than on six women in this category as was originally hoped (we had anticipated that the ability to make this comparison would be a strength of our study), since one woman in the delayed lipiodol group had a pregnancy in the first 3 months that resulted in a live birth so that only a pre-lipiodol sample and no post-lipiodol sample was available, and another woman had unsuccessful endometrial sampling procedures in whom it was not possible to obtain endometrial tissue by pipelle sampling. Further, it was disappointing that osteopontin differential expression could not be validated using RT-qPCR, however this was due to technical reasons, rather than being due to any issue with the osteopontin RT-qPCR results being

non-concordant with the array results. It is likely that osteopontin had too low an expression to be measurable and it is recognised that RT-qPCR may sometimes be less sensitive than microarrays. Nonetheless our inability to verify the osteopontin microarray data means that a substantial cautionary note needs to be applied to our conclusions, which can, for all the reasons above, only be 'suggestive' at best.

CONCLUSION

Our study provides suggestive data to support the concept of a uterine bathing effect of lipiodol. Whether the down-regulation of endometrial inflammatory regulators, including osteopontin, might contribute to an increase in endometrial receptivity to embryo implantation merits further investigation.

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CONFLICT OF INTEREST

Neil Johnson reports a consultancy for Guerbet, the company that manufactures lipiodol, from 2016 to date.

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Supplementary Table 1. RT-qPCR primer sequences.

Gene symbol	Gene description	Primer orientation	5' to 3' sequence
<i>BCL2A1</i>	BCL2-related protein A1	Forward	TTTGTGTCGGAGTTCATAATGA
		Reverse	ATTTTCCCAGCCTCCGTTTT
<i>CD86</i>	CD86 molecule	Forward	CATTCCCTGATGTTACGAGCAA
		Reverse	GCCGCGTCTTGTCAGTTTC
<i>HLADMB</i>	Major histocompatibility complex class II, DM beta	Forward	TGGTTTCTCCATCCAATTCTTTG
		Reverse	GGTCCCCCAAGTTGCTAAGTT
<i>PPIA</i>	Peptidylprolyl isomerase A	Forward	GGGTTCTGCTTTACAGAATT
		Reverse	GGACCCGTATGCTTAGGATGA
<i>RPLP0</i>	Ribosomal protein, large, P0	Forward	ATGGGCAAGAACCACCATGATG
		Reverse	CCTCCTTGGTGAACACAAAGC
<i>SERPINA3</i>	Serpine peptidase inhibitor, clade A (alpha-1 antitrypsin), member 3	Forward	CAGGCCCTTCTGATGATCA
		Reverse	GGATTGGTGACTTTGCTCATGA
<i>SPP1</i>	Secreted phosphoprotein 1	Forward	AGATATGCTGGTTGTAGACCCCAA
		Reverse	TGCACTATCTAATTCATGAGAAATACGA

Supplementary Table 2. List of mRNAs in endometrium found to be differentially expressed following lipiodol treatment.

Along with mean fold change across the four patients and paired LIMMA p value, this list includes mRNAs that pass both of the following criteria: (i) Paired LIMMA p value of less than 0.05 and (ii) more than 1.5 fold differential expression between the pre- and post-lipiodol treatment biopsies in at least 3 of the 4 patients.

Probe Set ID	Gene ID	Description	Expression fold change in endometrium	p Value	Patients in which RNA regulated $\geq +1.5$	Patients in which RNA regulated ≤ -1.5
233713_at	OAF	OAF homolog	1.58	0.00094	3	0
206100_at	CPM	carboxypeptidase M	1.82	0.00178	3	0
240286_at	GPM6B	glycoprotein M6B	1.59	0.00235	3	0
239519_at	NRP1	neuropilin 1	1.8	0.00249	3	0
232472_at	FNDC3B	fibronectin type III domain containing 3B	1.43	0.01000	3	0
225740_x_at	MDM4	Mdm4 p53 binding protein homolog	1.59	0.01023	3	0
208138_at	GAST	gastrin	2.08	0.01339	3	0
1563478_at	CTA-221G9.4	KIAA1671 protein	1.47	0.01608	3	0
203571_s_at	C10orf16	chromosome 10 open reading frame 116	1.7	0.02238	3	0
226333_at	IL6R	interleukin 6 receptor	1.61	0.02724	3	0
212298_at	NRP1	neuropilin 1	1.5	0.02991	3	0
235274_at	NA	unknown	1.91	0.03073	3	0
234269_at	NA	unknown	1.44	0.04497	3	0
1558613_at	OAF	OAF homolog	1.56	0.04683	3	0
1552532_a_at	ATP6V1C2	ATPase, H+ transporting, lysosomal 42kDa, V1 subunit C2	1.44	0.04729	3	0
209875_s_at	SPP1	secreted phosphoprotein 1 (osteopontin)	-1.98	0.00076	0	4
205099_s_at	CCR1	chemokine (C-C motif) receptor 1	-1.6	0.00286	0	3
220133_at	ODAM	odontogenic, ameloblast associated	-1.86	0.00295	0	3
202376_at	SERPINA3	serpin peptidase inhibitor, clade A member 3	-2.32	0.00488	0	3
243330_at	LRRTM1	leucine rich repeat transmembrane neuronal 1	-1.6	0.00715	0	3
209173_at	AGR2	anterior gradient homolog 2	-2.05	0.00896	0	3
204629_at	PARVB	parvin, beta	-1.41	0.01514	0	3
229759_s_at	VEPH1	ventricular zone expressed PH domain homolog 1	-1.76	0.01548	0	3
202741_at	PRKACB	protein kinase, cAMP-dependent, catalytic, beta	-1.49	0.01762	0	3
204533_at	CXCL10	chemokine (C-X-C motif) ligand 10	-1.94	0.01850	0	3
215051_x_at	AIF1	allograft inflammatory factor 1	-1.5	0.01966	0	3
212862_at	CDS2	CDP-diacylglycerol synthase (phosphatidate cytidylyltransferase) 2	-1.41	0.02305	0	3
218782_s_at	ATAD2	ATPase family, AAA domain containing 2	-1.98	0.02766	0	3
209301_at	CA2	carbonic anhydrase II	-2.49	0.02770	0	3
235266_at	ATAD2	ATPase family, AAA domain containing 2	-1.97	0.02796	0	3
242814_at	SERPINB9	serpin peptidase inhibitor, clade B (ovalbumin), member 9	-1.9	0.03120	0	3
201562_s_at	SORD	sorbitol dehydrogenase	-2.44	0.03170	0	3
205552_s_at	OAS1	2',5'-oligoadenylate synthetase 1, 40/46kDa	-1.61	0.03450	0	3
212531_at	LCN2	lipocalin 2	-2.38	0.03955	0	3
201563_at	SORD	sorbitol dehydrogenase	-2.35	0.04132	0	3
202554_s_at	GSTM3	glutathione S-transferase M3 (brain)	-1.51	0.04249	0	3
205681_at	BCL2A1	BCL2-related protein A1	-3.73	0.04407	0	3