Endometrial gene expression in the window of implantation is altered in obese women especially in association with polycystic ovary syndrome

José Bellver, M.D.,[a,b] *José Antonio Martínez-Conejero, Ph.D.,*[b,c] *Elena Labarta, M.D.,*[a] *Pilar Alamá, M.D.,*[a] *Marco Antonio Barreto Melo, M.D.,*[a] *José Remohí, M.D.,*[a,b] *Antonio Pellicer, M.D.,*[a,b] *and José Antonio Horcajadas, Ph.D.*[b,c]

[a] Instituto Valenciano de Infertilidad; [b] Fundación IVI-Instituto Universitario IVI-University of Valencia, Calle Guadassuar; and [c] iGenomix, Calle Catedrático Agustín Escardino, Valencia, Spain

Objective: To determine whether luteal phase endometrial transcriptome is altered in obese women during the window of implantation (WOI), considering the presence of infertility, fat distribution and association with polycystic ovary syndrome (PCOS).
Design: Prospective study.
Setting: University-affiliated infertility clinic, between May 2007 and March 2009.
Patient(s): One control group of women with normal weight (n = 4), and four study groups of obese women (n = 6 each one) according to the association with infertility, PCOS, and ovarian stimulation.
Intervention(s): The endometrium was biopsied 7 days after LH surge or hCG administration in 28 women.
Main Outcome Measure(s): Endometrial gene expression during the WOI.
Result(s): One hundred and fifty-one genes were dysregulated in obese groups compared with controls. This dysregulation was more pronounced when infertility was associated. The biologic processes of these genes belonged mainly to development and regulation of different biological functions such as transcription and biosynthesis. The molecular functions overrepresented were transcription and peptide receptor activity. The endometrium of obese women with PCOS showed dysregulated genes related to biologic processes such as development, morphogenesis, and the immune system, as well as different molecular functions such as protein binding, binding, growth factor activity, and carboxylic acid transmembrane transporter activity. Some of these genes have been previously related to implantation and unexplained infertility.
Conclusion(s): Obese women present a different endometrial gene expression than controls during the WOI, which is more pronounced when infertility or polycystic ovary syndrome are associated. (Fertil Steril® 2011;95:2335–41. ©2011 by American Society for Reproductive Medicine.)

Key Words: Obesity, infertility, polycystic ovary syndrome, gene expression, microarray, window of implantation

Infertility affects an estimated 12% of women of reproductive age (1). Obesity is acquiring epidemic proportions in developed countries (2, 3). In obese women, higher rates of infertility and subfecundity (increased time-to-pregnancy) have been described (4–7). Recently, adolescent obesity has been associated with a threefold increased risk of lifetime nulliparity and a fourfold increased risk of lifetime nulligravidity (8). Considering these data, many obese women will be able to conceive without difficulty. The increased risk of infertility has been mainly related to ovulatory disorders (4, 9, 10). However, recent studies have also detected a progressive reduction of spontaneous conception with the increase of body mass index (BMI) in women with regular menses who are ovulatory (11–13), indicating other possible altered factors such as the oocyte itself, the embryo, or the uterine environment (12). In ART, despite no consensus having been reached, recent systematic reviews (14) and retrospective studies with large sample sizes (15, 16) have highlighted a significant reduction in implantation, pregnancy, and live birth rates in obese women undergoing IVF. Two of the most recent papers included 6,500 cycles of IVF in a single-center setting (15), and 45,163 ART embryo transfers from 345 U.S. clinics (16), respectively.

An increased risk of obstetric complications has been also described in obese women after either spontaneous or assisted conceptions (14, 17–19). The combination of lower implantation and pregnancy rates, higher miscarriage rates, and increased maternal and fetal complications reduces the probability of a healthy liveborn (20). Most second- and third-trimester complications are due to the expression of the metabolic syndrome of obesity together with an underlying maternal subclinical inflammation and vascular

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Reprint requests: José Bellver, M.D., Instituto Valenciano de Infertilidad, Plaza de la Policía Local, 3, 46015, Valencia, Spain (E-mail: jbellver@ivi.es).

dysfunction (21). However, what occurs between conception and the end of the first trimester seems to be the result of an abnormal dialogue between embryo and endometrium (22).

Some reports have described a compromised number and quality of oocytes and embryos in obese patients undergoing IVF (23, 24), whereas others have shown no such deleterious effects (25, 26).In the largest single-center study regarding this topic (15), despite a significant reduction in implantation, pregnancy and live birth rates as BMI increased, no differences were observed in embryo quality, pointing to the endometrium as the main player in the impaired reproductive outcome of obese patients or to the contribution of unknown embryonic factors.

The best human model for studying the endometrium is ovum donation, because good quality oocytes from nonobese women are used. Recently, 2,656 first-cycle ovum recipients were assessed according to their BMI (22). The ongoing pregnancy rate per cycle initiated was significantly lower among obese women than among lean and healthy controls, highlighting the role of the endometrium or its environment in poor reproductive performance. Two other recent clinical studies using ovum donation in surrogate mothers (27) and frozen embryos (28) have reached similar conclusions. A recent multicenter retrospective study did not find poorer results in obese oocyte recipients, but confounding factors were not controlled, including the BMI of oocyte donors, which was unknown (16).

Based on this clinical evidence, our aim was to determine whether the human luteal phase endometrial transcriptome is altered in obese women at a molecular level during the peri-implantation period. For this purpose, we analyzed the endometrium during the window of implantation (WOI) by gene expression (GE) microarray analysis according to the presence of obesity and other correlated factors of poor reproductive outcome, such as central distribution of fat and association with polycystic ovary syndrome (PCOS). In addition, we assessed natural and stimulated cycles to rule out the previously described deleterious effect of controlled ovarian stimulation (COS) on the endometrium (29).

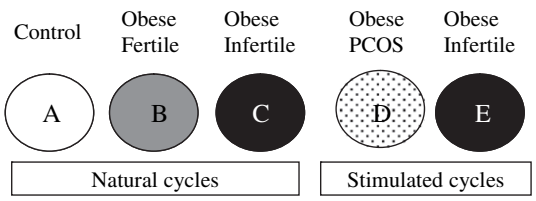
MATERIALS AND METHODS

Study Design

Five groups of young women (18–35 years old), with normal uterus, both ovaries, and no endometriosis were included between May 2007 and March 2009 (Fig. 1). Four endometrial samples were taken with Pipelle catheters (Genetics, Namont-Achel, Belgium) in a natural cycle (LH+7) in the normal weight (BMI, 20–24.9 kg/m^2) group (A), and six samples in each one of the four obese (BMI, >30 kg/m^2) groups—in two of them (B and C) in a natural cycle and in the other two (D and E) in a stimulated cycle with 100 IU/day rFSH (Puregon, Organon, Barcelona, Spain; hCG+7), as described elsewhere (30). Women in groups A, B, C, and E were ovulatory (menses every 25–35 days) with normal day 3 basal hormones, and in group D exhibied PCOS according to the Rotterdam criteria (31). Groups A and B were fertile (previous spontaneous pregnancies at term), and groups C, D, and E presented normal hysterosalpingography, no male factor, and infertility ≥2 years. In groups C and E, the infertility was of unknown origin. In group D, PCOS was considered the origin of infertility. All women with PCOS presented menses every 35 days or greater with oligoanovulation and a polycystic ovary pattern on ultrasound examination. Four of them associated biochemical or clinical signs of hyperandrogenism, or both.

FIGURE 1

Experimental design.



Bellver. Obesity, polycystic ovary, and endometrium. Fertil Steril 2011.

Regarding the number of patients included in each group, in microarray studies the needed sample size calculated by the proposed permutation method will ensure detecting at least the desired sensitivity with 95% probability. The method is shown to perform well for a real example dataset using a small pilot dataset with four to six samples (32).

The study was approved by the Institutional Research Board and Ethics committee and registered in Clinicaltrials.gov (NCT00505986). Subjects were not trying to conceive during the considered cycle and were recruited prospectively after a detailed explanation of the study and inform consent. No drugs were taken during the study except for recombinant follicle stimulating hormone and hCG in groups D and E.

RNA Isolation and Microarray Hybridization

Endometrial samples were snap-frozen in liquid nitrogen. Total RNA was extracted and analyzed as described previously (33). Sample preparation and hybridization of the RNA were adapted from the Agilent technical manual (one color).

Data Processing and Analysis

Spot intensities (medians) without background subtraction were transformed to the $\log_2$ scale. Data were normalized by quantile normalization. The replicates by gene symbol were merged, and the data were filtered to delete unknown sequences or probes without gene description. The R-statistical software system (www.bioconductor.org) was used for these purposes and for downstream analysis (34).

GE profile was determined by means of nonparametric tests, which perform comparisons 2 by 2, and two criteria were used to define genes with altered mRNA abundance among the different sample sets: an absolute fold change ≥2.0 and a corresponding fold-change $P<0.05$. To detect activations or inactivations in biologic functions or pathways, we used the Database for Annotation, Visualization, and Integrated Discovery (DAVID) (35).

Clustering and Principal Component Analysis

Expression data were normalized by Z-score. Hierarchical clustering was performed with MeV4.2.02 software (http://www.tm4.org) (36) with a complete-linkage hierarchical clustering algorithm. The Euclidean distance was chosen as the similarity measure.

The table data of genes and endometrial samples were transposed, and principal component analysis (PCA) was run to reduce the number of variables to two or three principal components, which represent the majority of the variability in the dataset. A three-dimensional scatterplot was produced to visualize the differences in sample sets based on each sample's gene expression profile.

Microarray Validation by Real-Time PCR

RT-PCR was performed for genes *GPx3*, *PAEP*, *HABP2*, and *SORD* as described previously (30). Oligonucleotides are listed in Supplemental Table 1.

RESULTS

Patient Characteristics

Study groups and patient characteristics are described in Table 1. Twenty of the 24 patients (83.3%) included in the obese groups (B–E) exhibited central obesity, defined as waist-to-hip ratio >0.80.

Differential Gene Expression

To identify differentially expressed genes, two different methods were used: parametric (significance analysis of microarrays