FOLLICULAR FLUID LEVELS OF POLYBROMINATED DIPHENYL ETHERS (PBDES), GENE EXPRESSION IN HUMAN MURAL AND CUMULUS GRANULOSA CELLS AND FERTILITY

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Introduction

There is increasing evidence that a number of the brominated flame retardants (BFRs), including the polybrominated diphenyl ethers (PBDEs), act as endocrine disruptors, affecting androgenic, estrogenic and thyroid hormone activities (1) and thus, reproductive health (2,3). The dietary exposure of adult female rats to an environmentally relevant BFR mixture significantly alters steroidogenesis and folliculogenesis (4). In the ovary, steroids are synthesized by granulosa cells, the ovarian follicular cells that line the fluid-filled antrum in antral follicles. In previous studies we demonstrated that in a human granulosa cell line, KGN cells, a mixture of PBDEs reflecting the congeners detected in human follicular fluid significantly affects steroidogenesis and induces oxidative stress (5).

In human studies, the detection of high levels of PBDEs in follicular fluid or in serum has been associated with a longer time to conceive (6), failure of embryo implantation (7), a decreased fertilization rate and a reduced proportion of high-quality embryos (8) after *in vitro* fertilization (IVF). Together, these observations has led us to hypothesize that high levels of PBDEs in follicular fluid may increase the risk of an unsuccessful pregnancy outcome. In this study, our objective was to investigate the link between follicular levels of PBDEs, granulosa cell gene expression, and fertility in women engaged in IVF with an intracytoplasmic sperm injection (ICSI) procedure.

Materials and methods

Fertile (male infertility) (n=21) and idiopathic infertile (n=19) women undergoing ICSI were recruited. Follicular fluid and cumulus and mural granulosa cells were isolated. Concentrations of PBDEs in follicular fluid were measured by gas chromatography-mass spectrometry and classified as high or low based on quartiles of detected PBDE congener concentrations.

The effects of PBDEs on the transcriptomes of granulosa cells were assessed in women from four experimental groups: 1) fertile women with low follicular fluid PBDE concentrations; 2) fertile women with high follicular fluid PBDE concentration; 3) infertile women with low follicular fluid PBDE concentrations; and 4) infertile women with high follicular fluid PBDE

concentrations. RNA was isolated from cumulus and mural granulosa cells and global gene expression was analyzed using SurePrint G3 Human Gene Expression v3 8x60K Microarray Kits from Agilent Technologies. qPCR was done to validate the expression of specific genes.

Results and discussion

Of the 23 PBDE congeners tested, 10 were detected in follicular fluid. The most abundant PBDE congeners were tetra-BDE-47, hexa-BDE-153, penta-BDE-99 and penta-BDE-100. The $\sum_{10}$ PBDEs were not significantly different between fertile (32.41 ± 5.66 pg/g) and infertile (27.86 ± 6.05 pg/g) women.

Transcriptomic profiles in both cumulus and mural cells were different when the cells were obtained from fertile rather than infertile women, suggesting that fertility status is associated with differential gene expression in cumulus and mural granulosa cells. Infertility-related changes in global gene expression in both granulosa cell types were modulated by follicular PBDE levels. In cumulus cells, 64 of the 112 sequences associated with infertility were identified specifically in cells isolated from women with high versus low PBDE levels in their follicular fluid. The most affected gene, *GDF3*, was significantly upregulated in cumulus cells collected from infertile women with high PBDE levels while fertility status had no effect on *GDF3* gene expression in women with low PBDE levels. In mural cells, the number of genes associated with infertility (386 genes) was strikingly reduced to 17 in cells obtained from women with high follicular PBDE levels when compared to women with low follicular fluid PBDE levels (350 genes). The most significantly affected genes, *FSHR* and *IHH*, were downregulated significantly in mural granulosa cells collected from infertile women with low PBDE levels; the expression of these genes were not affected by fertility status in women with high PBDE levels. In addition, genes involved in cell cycle regulation and repair of DNA damage signaling pathways, such as *CCNB2* and *CDK1*, were downregulated in mural granulosa cells obtained from infertile women with low PBDE levels and not affected by fertility status in women with high PBDE levels.

In summary, our findings reveal that PBDEs are detectable at variable levels in follicular fluid samples collected from fertile (ICSI indicated for male factor) and infertile (idiopathic infertility) women. Furthermore, infertility is clearly associated with modifications in cumulus and mural granulosa cell gene expression and these are modulated by follicular fluid PBDE levels. Overall, these data suggest that PBDEs influence the development of preovulatory follicles and the production of mature oocytes by modulating gene expression in follicular somatic cells.

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