Molecular profiling of human oocytes after vitrification strongly suggests that they are biologically comparable with freshly isolated gametes

To assess the effects of vitrification on the biomolecular profile of oocytes, we analyzed through real-time reverse transcriptase–polymerase chain reaction eight genes encoding critically important proteins for embryo development and compared this partial transcriptome with that of freshly collected gametes isolated from the same women. The comparison of the molecular profiles demonstrated that our vitrification protocol does not alter the biomolecular quality of oocytes: in fact, between the two groups we found the absence of statistically significant variations. Accordingly, this cryopreservation technique might be helpful in preserving women's fertility. (Fertil Steril® 2010;94:2804–7. ©2010 by American Society for Reproductive Medicine.)

Key Words: Human oocytes, gene expression, molecular markers, vitrification, real time RT-PCR

Oocyte cryopreservation is a helpful fertility preservation technique for women at risk of losing their ovarian functions after disease, surgery, or chemotherapy (1). Moreover, avoiding embryo cryopreservation would solve religious, ethical, and legal problems, connected to the laws that actually regulate medically assisted reproduction in various countries. There are two major techniques for cryopreservation: slow freezing and vitrification (2, 3). Many published studies have compared frozen-thawed human oocytes, after either slow freezing or vitrification, with fresh collected ones, and they have analyzed their biologic behavior (e.g., survival, fertilization, early embryonic development), as well as more specific structural cellular features (e.g., meiotic spindle assembly, chromosome alignment) (4–11). However, there are no published data on the molecular profile of oocytes after cryopreservation.

To assess the effects of vitrification on the biologic quality of oocytes, we compared the expression profile of messenger RNAs in single vitrificated-thawed oocytes with that of fresh collected oocytes without cryopreservation. In this article, we report the expression analysis of eight different genes: three perform housekeeping functions, because they encode proteins involved in the basic cellular functions and are expressed constantly in all human cells (12) (hypoxanthine phosphoribosyltransferase [HPRT], glyceraldehyde-3-phosphate dehydrogenase [GAPDH], and peptidylprolyl isomerase A [cyclophilin A, CYCLOPHILIN]); the other five genes encode proteins essential for oocyte development and specific functions (bone morphogenetic protein 15 [BMP15], growth differentiation factor 9 [GDF9], folliculogenesis-specific basic helix-loop-helix [FIGLA], POU class 5 homeobox 1 [POU5f1-OCT4], and TATA box binding protein [TBP]–associated factor 4B [TAF4B]). GDF9 and BMP15 (also known as GDF9b) are two closely related oocyte-specific growth factors, members of the transforming growth factor–β (TGFβ) superfamily, that are expressed in oocytes throughout most of folliculogenesis. Both GDF9 and BMP15 are involved in specific functions of granulosa and cumulus cells (i.e., proliferation, cumulus expansion, apoptosis) (13–15). FIGLA, OCT4, and TAF4B are transcription factors preferentially expressed in germ cells. FIGLA (factor in the germline), expressed exclusively in germ cells, is a critical transcription factor during the early steps of

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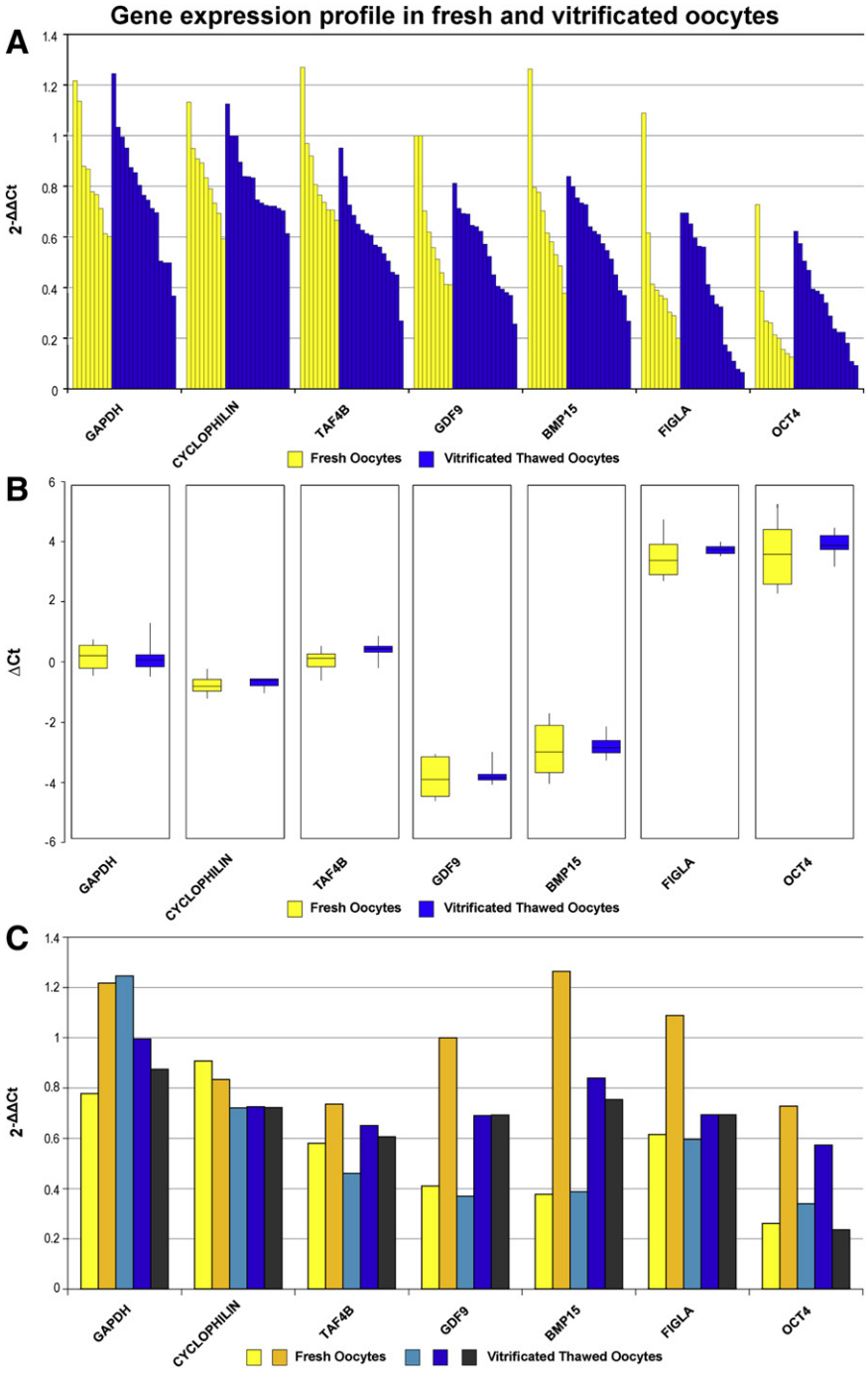
FIGURE 1

Gene expression profile in fresh and vitrificated oocytes. (**A**) The histogram shows the expression profiles of analyzed genes in 9 fresh (*yellow*) and 15 vitrificated oocytes (*blue*) estimated with use of the $2^{-\Delta\Delta Ct}$ method. We used hypoxanthine phosphoribosyltransferase as reference gene and the same fresh oocyte as calibrator. (**B**) Box and whisker plot of ΔCt means of the analyzed genes in fresh and thawed oocytes after vitrification. It provides a simple description of a distribution of values by depicting the 25th and 75th percentile values as the bottom and top of a box, respectively. The Y axis represents the ΔCt values. The median expression values of fresh and vitrificated thawed oocytes are marked by *horizontal lines* in the boxes. (**C**) Expression profile of analyzed genes in five oocytes collected from a woman during the same ovarian stimulation protocol. Normalization has been performed by $2^{-\Delta\Delta Ct}$ method with use of hypoxanthine phosphoribosyltransferase as reference gene and the same fresh oocyte as calibrator. Statistical analysis of data demonstrates that the expression profiles of the analyzed genes are not significantly different ($P<.01$).



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