THE CORRELATION BETWEEN VACCINIA RELATED KINASE mRNA EXPRESSION IN HUMAN BLOOD AND FOLLICULAR FLUID LYMPHOCYTES

Stephanie Gunderson, MD[1]; Pippa Simpson, PhD[3] ; Estil Strawn, MD[1,2] ; Kate D. Schoyer, MD[1,2]; Jayme Bosler, MD[1,2]

[1]Dept. of Obstetrics and Gynecology, [2]Division of Reproductive Endocrinology and Infertility, [3]Dept. of Quantitative Health Science and CRI Biostatistics Core

Background

Vaccinia related protein kinases (VRKs) are serine-threonine kinases necessary in cell division for both nuclear envelope breakdown and chromosome condensation as well as cell cycle regulation as immediate early response genes. VRK was originally found in human fetal liver samples by Nezu et al in 1997 and its expression levels have recently been correlated to infertility and poor embryo development in mice. To date, there have been no known studies identifying whether VRK depletion in humans leads to a subset of infertility.

Objective: To determine if VRK1 (a particular type of vaccinia related kinase studied in mice with infertility) expression in follicular fluid lymphocytes correlated with expression in the peripheral blood lymphocytes. Thus making peripheral blood levels of VRK1 expression a marker for expression of VRK1 at the ovary.

Design: Prospective case control study.

Methods

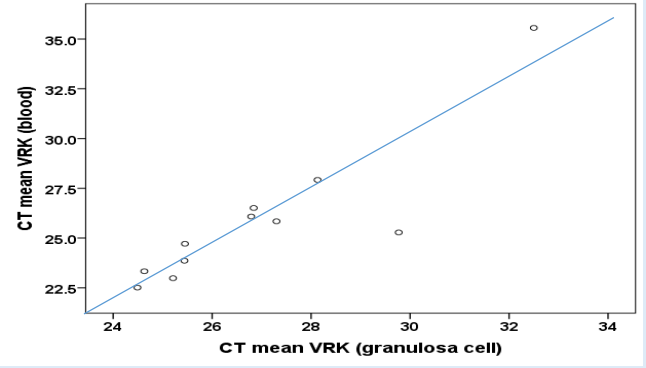
- ***Who:*** 11 women who presented to the reproductive endocrinology clinic to undergo in vitro fertilization (IVF) for diminished ovarian reserve, recurrent pregnancy loss, anovulation or unexplained infertility.
- ***What:*** Peripheral blood lymphocyte and follicular fluid samples were obtained from each patient.
- ***When:*** All samples were collected while patients were undergoing gonadotropic stimulation for IVF. Specifically, these samples were collected at the time of egg retrieval.
- Samples were processed using the steps below:
  - RNA was isolated using a *QIAamp RNA Blood Mini Kit* and converted into complementary DNA (cDNA).
  - Quality of RNA samples was confirmed using *Nanodrop Spectrophotometer* prior to proceeding to cDNA synthesis.
  - Real Time polymerase chain reaction (RT-PCR) was performed on all samples using a Bio-Rad iQ5 system.
  - VRK1 and a housekeeping gene, Glyceraldehyde 3-Phosphate Dehydrogenase (GAPDH), were amplified in triplicate for each patient sample using primers made from Integrated DNA Technologies quest software from the DNA sequence of the proteins.
  - Standard curve concentration time (CT) data from RT-PCR were analyzed.
  - GAPDH data was excluded due to unreliable amplification of this product within a given patient sample.

Statistical Analysis

A scatter plot of mean CT VRK1 data collected from RT-PCR was made and Pearson's correlation co-efficient was calculated.

Results

Mean CT VRK1 values of follicular fluid and blood obtained from the same patient were compared to determine if blood VRK1 mRNA levels were a good marker of follicular VRK1 mRNA levels.



There was a strong positive correlation between blood and follicular fluid VRK1 levels (r=0.891, p value=0.001).

Conclusions

Our data show a positive correlation between follicular fluid VRK1 and peripheral blood lymphocyte VRK1 expression levels. Given this statistically significant correlation we have concluded that peripheral blood analysis of VRK1 is a good marker for VRK1 expression at the ovary. This will allow for future studies to investigate the impact of VRK activity on fertility.

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