Data Article

In vitro treatment of human granulosa cells with irisin and leptin: Quantitative RT-PCR array data (female infertility panel)

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A B S T R A C T

Reproduction is closely related to energy metabolism: physical activity and adiposity (either insufficient weight or obesity) can affect female fertility. Irisin is a myo- and adipokine produced by skeletal muscles during exercise or shivering as well as in smaller amounts by subcutaneous visceral adipocytes [1]. Leptin is a neuroendocrine adipokine regulating satiety and energy expenditure. Circulating levels of both, irisin and leptin, correlate with adiposity status and physical activity [2–6]. This article presents data from quantitative PCR array of the in vitro effects of irisin and leptin on cultured human ovarian granulosa cells. Granulosa cells were purified from follicular fluid samples obtained from women undergoing in vitro fertilization (IVF) procedure and were subjected to treatment with irisin (500 ng/mL) or leptin (100 ng/mL) for 24 h. The array included 84 genes involved in female fertility.

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Specifications Table

| Subject                                   | Health and medical sciences                                      |
|------------------------------------------|------------------------------------------------------------------|
| Specific subject area                    | Female reproductive endocrinology                                 |
| Type of data                             | Table                                                            |
| How the data were acquired               | Quantitative RT-PCR array using RT² Profiler PCR Array – Human Female Infertility kit (Qiagen) and QuantStudio 3 Real-Time PCR system (Thermo Fisher Scientific). Data were analyzed using GeneGlobe Data Analysis Center (Qiagen). |
| Data format                              | Raw and Analyzed                                                 |
| Description of data collection           | Human ovarian granulosa cells were purified from follicular fluid samples obtained during IVF procedure. Cells were cultured in vitro and were subjected to treatment with irisin (500 ng/mL) or leptin (100 ng/mL) for 24 h. The effect of irisin and leptin on modulation of 84 genes involved in female infertility was evaluated by using qRT-PCR array. |
| Data source location                     | • Institution: Friedman Diabetes Institute, Northwell Health<br>• City/Town/Region: New York, NY<br>• Country: USA |
| Data accessibility                       | Repository name: Mendeley Data<br>Data identification number: 10.17632/gr3dg36nzx.1<br>Direct URL to data: https://data.mendeley.com/datasets/gr3dg36nzx/1 |

Value of the Data

- Provided data are useful for understanding the relationship between energy metabolism and reproduction.
- Presented data may be of interest to researchers and physicians working in the field of reproduction.
- This dataset can be used as a basis for further investigations of the molecular mechanisms of irisin and leptin action in the ovary.

1. Data Description

This article presents data obtained from qRT-PCR array measuring the expression of various genes related to female infertility. Human ovarian granulosa cells purified from follicular fluid samples were grown in vitro and treated with irisin or leptin for 24 h. Array included 84 genes listed in Tables 1 and 2. Raw data from the analysis (Ct values) have been published in a data repository [7]. Analyzed data demonstrating fold-regulation and fold-change of irisin or leptin treatment vs. control are presented in Tables 3 and 4, Figs. 1 and 2.
Table 1  
List of genes included in the PCR array.

| Gene Symbol | Gene full name | UniGene ID | GeneBank ID |
|-------------|----------------|------------|-------------|
| AKT1 | V-Akt murine thymoma viral oncogene homolog 1 | HS.525622 | NM_005163 |
| ANXA2 | Annexin A2 | HS.511605 | NM_004039 |
| APOD | Apolipoprotein D | HS.522555 | NM_001647 |
| AR | Androgen receptor | HS.496240 | NM_000044 |
| AREG | Amphiregulin | HS.270833 | NM_001657 |
| BAX | BCL2-associated X protein | HS.624291 | NM_004324 |
| BCL2 | B-cell CLL/lymphoma 2 | HS.150749 | NM_000633 |
| C2 | Complement component 2 | HS.408903 | NM_000063 |
| C3 | Complement component 3 | HS.529053 | NM_000064 |
| CALCA | Calcitonin-related polypeptide, alpha | HS.37058 | NM_001741 |
| CASP3 | Caspase 3 | HS.141125 | NM_004346 |
| CCL5 | Chemokine (C-C motif) ligand 5 | HS.514821 | NM_002985 |
| CCNB1 | Cyclin B1 | HS.23960 | NM_001966 |
| CD55 | CD55 molecule | HS.126517 | NM_000574 |
| CDH1 | Cadherin 1 | HS.461086 | NM_004360 |
| CFD | Complement factor D | HS.155597 | NM_001928 |
| CLDN4 | Claudin 4 | HS.729359 | NM_001305 |
| COMP | Cartilage oligomeric matrix protein | HS.1584 | NM_000095 |
| CRABP2 | Cellular retinoic acid binding protein 2 | HS.405662 | NM_001878 |
| CSF1 | Colony stimulating factor 1 | HS.591402 | NM_000757 |
| CTNNB1 | Catenin, beta 1 | HS.476018 | NM_001904 |
| CXCL12 | Chemokine (C-X-C motif) ligand 12 | HS.522891 | NM_000609 |
| DKK1 | Dickkopf homolog 1 | HS.40499 | NM_012424 |
| EGF | Epidermal growth factor | HS.419815 | NM_001963 |
| EGFR | Epidermal growth factor receptor | HS.488293 | NM_005228 |
| ESR1 | Estrogen receptor 1 | HS.208124 | NM_000125 |
| ESR2 | Estrogen receptor 2 | HS.729020 | NM_001437 |
| F3 | Coagulation factor III | HS.62192 | NM_001993 |
| FBN1 | Fibrillin 1 | HS.591133 | NM_000138 |
| FN1 | Fibronecin 1 | HS.203717 | NM_002026 |
| GADD45A | Growth arrest and DNA-damage-inducible, alpha | HS.80409 | NM_001924 |
| GAST | Gastrin | HS.2681 | NM_000805 |
| GDF15 | Growth differentiation factor 15 | HS.616962 | NM_004864 |
| GPX3 | Glutathione peroxidase 3 | HS.386793 | NM_002084 |
| HBEGF | Heparin-binding EGF-like growth factor | HS.799 | NM_001945 |
| HOXA10 | Homeobox A10 | HS.110637 | NM_018951 |
| HOXA11 | Homeobox A11 | HS.249171 | NM_005523 |
| ICAM1 | Intercellular adhesion molecule 1 | HS.643447 | NM_000201 |
| IGFBP1 | Insulin-like growth factor binding protein 1 | HS.642938 | NM_000596 |
| IL11 | Interleukin 11 | HS.467304 | NM_000641 |
| IL15 | Interleukin 15 | HS.654378 | NM_000585 |
| IL1A | Interleukin 1, alpha | HS.1722 | NM_000575 |
| IL1B | Interleukin 1, beta | HS.126256 | NM_000576 |
| IL1R1 | Interleukin 1 receptor, type 1 | HS.701982 | NM_000877 |
| IL6 | Interleukin 6 | HS.654458 | NM_000600 |
| ITGAV | Integrin, alpha V | HS.436873 | NM_002210 |
| ITGB3 | Integrin, beta 3 | HS.218040 | NM_000212 |
| KDR | Kinase insert domain receptor | HS.479756 | NM_002253 |
| LAMC2 | Laminin, gamma 2 | HS.591484 | NM_000562 |
| LEP | Leptin | HS.194236 | NM_000230 |
| LIF | Leukemia inhibitory factor | HS.2250 | NM_002309 |
| LIFR | Leukemia inhibitory factor receptor, alpha | HS.133421 | NM_002310 |
| MAOA | Monoamine oxidase A | HS.183109 | NM_000240 |
| MID1 | Midline 1 | HS.27695 | NM_000381 |
| MKI67 | Antigen identified by monoclonal antibody Ki-67 | HS.689823 | NM_002417 |
| MMP2 | Matrix metalloproteinase 2 | HS.513617 | NM_004530 |
| MMP7 | Matrix metalloproteinase 7 | HS.2256 | NM_002423 |

(continued on next page)
### Table 1 (continued)

| Gene Symbol | Gene full name                                      | UniGene ID  | GeneBank ID |
|-------------|-----------------------------------------------------|-------------|-------------|
| MMP9        | Matrix metalloproteinase 9                         | Hs.297413   | NM_004994   |
| MSX1        | Msh homeobox 1                                     | Hs.424414   | NM_002448   |
| MUC1        | Mucin 1                                             | Hs.89603    | NM_0018016  |
| OLRF1       | Olfactomedin 1                                     | Hs.522484   | NM_006334   |
| PAEP        | Progestagen-associated endometrial protein         | Hs.532325   | NM_002571   |
| PCNA        | Proliferating cell nuclear antigen                 | Hs.728886   | NM_182.649  |
| PGF         | Placental growth factor                            | Hs.252820   | NM_002632   |
| PGR         | Progesterone receptor                              | Hs.32405    | NM_000926   |
| PRL         | Prolactin                                          | Hs.1905     | NM_000948   |
| PTGS1       | Prostaglandin-endoperoxide synthase 1              | Hs.201978   | NM_000962   |
| PTGS2       | Prostaglandin-endoperoxide synthase 2              | Hs.196384   | NM_000963   |
| SELK        | Selectin L                                         | Hs.728756   | NM_000655   |
| SFRP4       | Secreted frizzled-related protein 4                | Hs.658169   | NM_003014   |
| SOD1        | Superoxide dismutase 1                             | Hs.443914   | NM_004545   |
| SPP1        | Secreted phosphoprotein 1                          | Hs.313      | NM_000582   |
| STAT3       | Signal transducer and activator of transcription 3 | Hs.463059   | NM_003150   |
| STTN1       | Statmin 1                                           | Hs.209983   | NM_005563   |
| TGFB1       | Transforming growth factor, beta 1                 | Hs.645227   | NM_000660   |
| TIMP1       | TIMP metalloproteinase inhibitor 1                 | Hs.522632   | NM_003254   |
| TNF         | Tumor necrosis factor                              | Hs.241570   | NM_000594   |
| TFNRI0B     | Tumor necrosis factor receptor superfamily, member 10b | Hs.521456 | NM_003842 |
| TP53        | Tumor protein p53                                   | Hs.654481   | NM_000546   |
| VCAM1       | Vascular cell adhesion molecule 1                  | Hs.109225   | NM_001078   |
| VEGFA       | Vascular endothelial growth factor A               | Hs.73793    | NM_003376   |
| WNT2        | Wingless-type MMTV integration site family, member 2 | Hs.567356 | NM_003391 |
| ACTB        | Actin, beta                                         | Hs.520640   | NM_001101   |
| B2M         | Beta-2-microglobulin                               | Hs.534255   | NM_004048   |
| GADD45A     | Glyceraldehyde-3-phosphate dehydrogenase           | Hs.592355   | NM_002046   |
| HPR8T1      | Hypoxanthine phosphoribosyltransferase 1           | Hs.412707   | NM_000194   |
| RPLP0       | Ribosomal protein, large, P0                       | Hs.546285   | NM_001002   |

### Table 2

PCR array genes organized by function.

| Function                          | Gene                                    |
|-----------------------------------|-----------------------------------------|
| Disregulated during infertility   | CFD, CLDN4, COMP, CRABP2, DKK1, ESR2, GADD45A, GAST, GDF15, GPX3, IGFBP1, IL15, MAOA, MSX1, OLRF1, PAEP, SFRP4, SPP1 |
| Receptive endometrium             | AREG, CALCA, CSF1, EGF, HOXA10, HOXA11, LEP, LIF, LIFR, MUC1, PAEP, PGR, PTGS1, PTGS2, SELK |
| Signal transduction               | IL1A, IL1B, IL1R1                       |
| IL-1 signaling                    | CDH1, CTNNB1, DKK1, MMP7, PTGS2, SFRP4, TP53, WNT2 |
| Wnt signaling                     | PTGS1, PTGS2                           |
| Prostaglandin signaling           | AKT1, EGFR, GDF15, STAT3, TGFB1         |
| Other signal transduction genes   | CALCA, CLN5, CLDN4, CTNNB1, CXCL12, FB11, FN1, ICAM1, ITGA4, ITGAV, ITGB3, LAMC2, MMP2, MMP7, MMP9, SELK, SPP1, VCAM1 |
| Leukocyte migration               | CCNB1, GADD45A, MKI67, PCNA, STTN1, TGFB1, TP53 |
| Cell cycle                        | C2, C3, CD55, CFD, F3                    |
| Coagulation                       | BAX, BCL2, CASP3, COMP, GADD45A, IL1B, MSX1, TNF, TNFRSF10B, TP53 |
| Apoptosis                         | ACTB, BAX, BCL2, CASP3, COMP, GADD45A, IL1B, MSX1, TNF, TNFRSF10B, TP53 |
| Cytokines                         | ANXA2, APOD, AR, ESR1, HBEGF, IGFI, KDR, MID1, PGR, PTGS1, SOD1, TIMP1, VEGFA |
Table 3
Effect of irisin on the expression of genes related to female infertility.

| Gene Symbol | Fold Regulation | Fold Change | p-Value | Significance |
|-------------|----------------|-------------|---------|--------------|
| ANXA2       | -1.23          | 0.82        | 0.189   | ns           |
| APOD        | -1.35          | 0.74        | 0.4628  | ns           |
| AR          | 1.03           | 1.03        | 0.8525  | ns           |
| AREG        | 1.45           | 1.45        | 0.5115  | ns           |
| BAX         | 1.02           | 1.02        | 0.806   | ns           |
| BCL2        | -1.17          | 0.86        | 0.5586  | ns           |
| C2          | -1.18          | 0.85        | 0.8827  | ns           |
| C3          | -1.52          | 0.66        | 0.2588  | ns           |
| CALCA       | -1.07          | 0.94        | 0.8268  | ns           |
| CAP3        | -1.18          | 0.85        | 0.434   | ns           |
| CCL5        | -4.68          | 0.21        | 0.0774  | ns           |
| CNB1        | 1.01           | 1.01        | 0.7758  | ns           |
| CD55        | 1.06           | 1.06        | 0.5712  | ns           |
| CDK1        | -1.94          | 0.52        | 0.2382  | ns           |
| CFD         | 1.11           | 1.11        | 0.5235  | ns           |
| CDN4        | -1.17          | 0.85        | 0.5629  | ns           |
| COMP        | 1.26           | 1.26        | 0.6391  | ns           |
| CRABP2      | -1.73          | 0.58        | 0.276   | ns           |
| CSF1        | -3.57          | 0.28        | 0.000022| ****         |
| CTNNB1      | 1.06           | 1.06        | 0.5502  | ns           |
| CXCL12      | 1.06           | 1.06        | 0.6446  | ns           |
| DKK1        | -3.49          | 0.29        | 0.0037  | **           |
| EGF         | -1.11          | 0.9         | 0.5601  | ns           |
| EGFR        | -1.42          | 0.71        | 0.0562  | ns           |
| ESR1        | -1.25          | 0.8         | 0.485   | ns           |
| ESR2        | 1.31           | 1.31        | 0.9232  | ns           |
| F3          | -1.1           | 0.91        | 0.6093  | ns           |
| FBN1        | 1.04           | 1.04        | 0.8893  | ns           |
| FN1         | 1.55           | 1.55        | 0.1007  | ns           |
| GADD45A     | -1.08          | 0.92        | 0.9922  | ns           |
| GST         | -1.32          | 0.76        | 0.6517  | ns           |
| GDF15       | -4.22          | 0.24        | 0.0327  | *            |
| GPX3        | 1.83           | 1.8         | 0.1291  | ns           |
| HBEFG       | -1.27          | 0.79        | 0.0964  | ns           |
| HOXA10      | 1.81           | 1.81        | 0.0918  | ns           |
| HOXA11      | -1.1           | 0.91        | 0.7422  | ns           |
| ICAM1       | -2.01          | 0.48        | 0.0404  | *            |
| IGF1        | 1.24           | 1.24        | 0.5136  | ns           |
| IGBP1       | -2.44          | 0.41        | 0.301   | ns           |
| IL11        | 2.08           | 2.08        | 0.1221  | ns           |
| IL15        | -1.23          | 0.82        | 0.2788  | ns           |
| IL1A        | -1.29          | 0.78        | 0.4451  | ns           |
| IL1B        | -1.66          | 0.6         | 0.2715  | ns           |
| IL1R1       | 1.29           | 1.29        | 0.6692  | ns           |
| IL6         | -3.14          | 0.32        | 0.195   | ns           |
| ITGA4       | -1.46          | 0.69        | 0.6359  | ns           |
| ITGA5       | -1.41          | 0.71        | 0.2241  | ns           |
| ITGB3       | 6.48           | 6.4         | 0.451   | ns           |
| KDR         | -6.99          | 0.14        | 0.0115  | *            |
| LAMC2       | -1.69          | 0.59        | 0.3188  | ns           |
| LEP         | -1.12          | 0.9         | 0.5819  | ns           |
| LIF         | -1.59          | 0.63        | 0.0571  | ns           |
| LIFR        | 1.33           | 1.33        | 0.4115  | ns           |
| MAOA        | -1.73          | 0.58        | 0.5141  | ns           |
| MID1        | 1.1            | 1.1         | 0.467   | ns           |
| MKI67       | 1.6            | 1.6         | 0.2392  | ns           |
| MMP2        | -1.65          | 0.61        | 0.8731  | ns           |
| MMP7        | -19.16         | 0.05        | 0.0729  | ns           |
| MMP9        | -2.37          | 0.42        | 0.2073  | ns           |
| MSX1        | -1.22          | 0.82        | 0.5109  | ns           |
| MUC1        | 1.85           | 1.85        | 0.0189  | *            |
| OLFM1       | -1.25          | 0.8         | 0.3683  | ns           |

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Table 3 (continued)

| Gene Symbol | Fold Regulation | Fold Change | p-Value | Significance |
|-------------|-----------------|-------------|---------|--------------|
| PAEP        | -2.34           | 0.43        | 0.1269  | ns           |
| PCNA        | 1.08            | 1.08        | 0.3423  | ns           |
| PGF         | -1.87           | 0.53        | 0.1015  | ns           |
| PGR         | 1.27            | 1.27        | 0.3233  | ns           |
| PRL         | 1.73            | 1.73        | 0.375   | ns           |
| PTGS1       | -2.12           | 0.47        | 0.9528  | ns           |
| PTGS2       | -1.66           | 0.6         | 0.2325  | ns           |
| SEL         | 2.24            | 2.24        | 0.0045  | *            |
| SFRP4       | 2.53            | 2.53        | 0.014   | *            |
| SOD1        | -1.26           | 0.79        | 0.0084  | **           |
| SPP1        | -12.62          | 0.08        | 0.0185  | *            |
| STAT3       | 1.08            | 1.08        | 0.4506  | ns           |
| STMN1       | 1.57            | 1.57        | 0.0363  | *            |
| TGFβ1       | 1.36            | 1.36        | 0.177   | ns           |
| TIMP1       | 1.91            | 1.91        | 0.0363  | *            |
| TNFRSF10B   | -1.65           | 0.61        | 0.0033  | **           |
| TP53        | 1.01            | 1.01        | 0.9896  | ns           |
| VCA M1      | -1.68           | 0.59        | 0.2364  | ns           |
| VEGFA       | -1.47           | 0.68        | 0.1865  | ns           |
| WNT2        | 1.86            | 1.86        | 0.0508  | ns           |

Data are calculated as both, fold regulation and fold change of irisin treatment vs. control. Statistically significant data are presented in bold. ns, non-significant data (p > 0.05); *, p ≤ 0.05; **, p ≤ 0.01; ***, p ≤ 0.001; ****, p ≤ 0.0001.

Table 4

Effect of leptin on the expression of genes related to female infertility.

| Gene Symbol | Fold Regulation | Fold Change | p-Value | Significance |
|-------------|-----------------|-------------|---------|--------------|
| ANXA2       | 1.01            | 1.01        | 0.9824  | ns           |
| APOD        | 1.55            | 1.55        | 0.8023  | ns           |
| AR          | 1.09            | 1.09        | 0.5206  | ns           |
| AREG        | 2.55            | 2.55        | 0.1545  | ns           |
| BAX         | -1.08           | 0.92        | 0.6137  | ns           |
| BCL2        | 1.09            | 1.09        | 0.8415  | ns           |
| C2          | -1.22           | 0.82        | 0.8023  | ns           |
| C3          | -2.14           | 0.47        | 0.2792  | ns           |
| CALCA       | 1.3             | 1.3         | 0.9629  | ns           |
| CASP3       | -1.09           | 0.92        | 0.6023  | ns           |
| CCL5        | -1.78           | 0.56        | 0.8546  | ns           |
| CCNB1       | 1.04            | 1.04        | 0.8628  | ns           |
| CD55        | 1.12            | 1.12        | 0.2053  | ns           |
| CDH1        | 1.43            | 1.43        | 0.5925  | ns           |
| CFD         | 1.09            | 1.09        | 0.5935  | ns           |
| CLDN4       | -1.4            | 0.71        | 0.2672  | ns           |
| COMP        | -1.08           | 0.93        | 0.9617  | ns           |
| CRABP2      | -1.17           | 0.86        | 0.49    | ns           |
| CSF1        | -1.81           | 0.55        | 0.1526  | ns           |
| CTNBNB1     | 1.15            | 1.15        | 0.3522  | ns           |
| CXCL12      | 1.02            | 1.02        | 0.9724  | ns           |
| DKK1        | -1.55           | 0.65        | 0.1118  | ns           |
| EGF         | 1.26            | 1.26        | 0.5105  | ns           |
| EGFR        | -1.21           | 0.83        | 0.33    | ns           |
| ESR1        | -1.11           | 0.9         | 0.5697  | ns           |
| ESR2        | 1.39            | 1.39        | 0.7212  | ns           |
| F3          | 1.15            | 1.15        | 0.5501  | ns           |
| FBN1        | 1.22            | 1.22        | 0.2301  | ns           |
| FN1         | 1.6             | 1.6         | 0.0569  | ns           |
| GADD45A     | 1.15            | 1.15        | 0.1484  | ns           |

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Table 4 (continued)

| Gene Symbol | Fold Regulation | Fold Change | p-Value  | Significance |
|-------------|-----------------|-------------|----------|--------------|
| GAST        | 1.03            | 1.03        | 0.8392   | ns           |
| GDF15       | -2.52           | 0.4         | 0.2892   | ns           |
| GPX3        | 1.46            | 1.46        | 0.106    | ns           |
| HBEGF       | 1.51            | 1.51        | 0.0813   | ns           |
| HOXA10      | 1.76            | 1.76        | 0.3294   | ns           |
| HOXA11      | -1.04           | 0.96        | 0.8184   | ns           |
| ICAM1       | -1.89           | 0.53        | 0.1567   | ns           |
| IGF1        | 1.07            | 1.07        | 0.8605   | ns           |
| IGFBP1      | 1.31            | 1.31        | 0.2512   | ns           |
| IL11        | 1.44            | 1.44        | 0.3379   | ns           |
| IL15        | -1.2            | 0.83        | 0.3587   | ns           |
| IL1A        | -1.23           | 0.81        | 0.6621   | ns           |
| IL1B        | -2.23           | 0.45        | 0.1299   | ns           |
| IL1R1       | 1.59            | 1.59        | 0.2971   | ns           |
| IL6         | -1.97           | 0.51        | 0.2302   | ns           |
| ITGA4       | -1.32           | 0.76        | 0.0962   | ns           |
| ITGAV       | -1.07           | 0.93        | 0.3291   | ns           |
| ITGB3       | 4.51            | 4.51        | 0.9383   | ns           |
| KDR         | -2.02           | 0.5         | 0.3483   | ns           |
| LAMC2       | -3.23           | 0.31        | 0.0598   | ns           |
| LEP         | -1.46           | 0.69        | 0.1327   | ns           |
| LIF         | -1.1            | 0.91        | 0.655    | ns           |
| LIFR        | 1.49            | 1.49        | 0.187    | ns           |
| MAOA        | -1.1            | 0.91        | 0.5909   | ns           |
| MID1        | -1.15           | 0.87        | 0.3192   | ns           |
| MKI67       | 1.47            | 1.47        | 0.196    | ns           |
| MMP2        | -1.88           | 0.53        | 0.3988   | ns           |
| MMP7        | -7.72           | 0.13        | 0.251    | ns           |
| MMP9        | -1.56           | 0.64        | 0.553    | ns           |
| MSX1        | -1.08           | 0.92        | 0.6698   | ns           |
| MUC1        | 1.16            | 1.16        | 0.1898   | ns           |
| OLFM1       | -1.45           | 0.69        | 0.1941   | ns           |
| PAEP        | -1.49           | 0.67        | 0.553    | ns           |
| PCNA        | 1.02            | 1.02        | 0.7715   | ns           |
| PGE2        | -1.23           | 0.81        | 0.8469   | ns           |
| PGR         | 1.6             | 1.6         | 0.0639   | ns           |
| PRL         | 1.39            | 1.39        | 0.7569   | ns           |
| PTGCS1      | -1.94           | 0.52        | 0.2358   | ns           |
| PTGCS2      | -2.12           | 0.47        | 0.1376   | ns           |
| SELL        | 1.31            | 1.31        | 0.4546   | ns           |
| SFRP4       | 1.21            | 1.21        | 0.6447   | ns           |
| SOD1        | -1.01           | 0.99        | 0.9654   | ns           |
| SPP1        | -5.29           | 0.19        | 0.0955   | ns           |
| STAT3       | 1.02            | 1.02        | 0.8683   | ns           |
| STMN1       | 1.26            | 1.26        | 0.1182   | ns           |
| TGFBI       | 1.21            | 1.21        | 0.4503   | ns           |
| TIMP1       | 1.44            | 1.44        | 0.052    | ns           |
| TNF         | 1.82            | 1.82        | 0.3258   | ns           |
| TNFRSF10B   | -1.18           | 0.85        | 0.3564   | ns           |
| TP53        | -1.01           | 0.99        | 0.8372   | ns           |
| VCAM1       | -1.18           | 0.85        | 0.762    | ns           |
| VEGFA       | -1.14           | 0.87        | 0.563    | ns           |
| WNT2        | 1.04            | 1.04        | 0.6854   | ns           |

Data are calculated as both, fold regulation and fold change of leptin treatment vs. control.
Fig. 1. Volcano plot visualization of the effect of irisin (A) and leptin (B) on the expression of genes related to female infertility. Volcano plot graph presenting genes upregulated or downregulated more than two-fold. Genes upregulated more than two-fold (in orange) by the effect of irisin (A) are: IL11, SELL, SFRP4 and ITGB3, while genes downregulated more than two-fold (in blue) by the effect of irisin (A) are: ICAM1, PTGS1, PAEP, MMP9, IGFBP1, IL6, DKK1, CSF1, GDF15, CCL5, KDR, SPP1 and MMP7. Genes upregulated more than two-fold (in orange) by the effect of leptin (B) are AREG and ITGB3, while genes downregulated more than two-fold (in blue) by the effect of leptin (B) are: KDR, C3, PTGS2, IL1B, GDF15, LAMC2, SPP1 and MMP7.
Fig. 2. Scatter plot visualization of the effect of irisin (A) and leptin (B) on the expression of genes related to female infertility. Scatter plot graph presenting genes upregulated or downregulated more than two-fold. Genes upregulated more than two-fold (in red) by the effect of irisin (A) are: IL11, SELL, SFRP4 and ITGB3, while genes downregulated more than two-fold (in green) by the effect of irisin (A) are: ICAM1, PTGS1, PAEP, MMP9, IGFBP1, IL6, DKK1, CSF1, GDF15, CCL5, KDR, SPP1 and MMP7. Genes upregulated more than two-fold (in red) by the effect of leptin (B) are AREG and ITGB3, while genes downregulated more than two-fold (in green) by the effect of leptin (B) are: KDR, C3, PTGS2, IL1B, GDF15, LAMC2, SPP1 and MMP7.
2. Experimental Design, Materials and Methods

2.1. Cells

Human ovarian granulosa cells were purified from follicular fluid samples from patients undergoing in vitro fertilization (IVF) procedure by using Percoll PLUS reagent (GE Healthcare, Cat. # 17-5445-02). Initially, follicular fluid was centrifuged at 1000 g for 5 min. Pellet containing the cells was resuspended in 20 mL of PBS and layered onto 15 mL of 50% Percoll PLUS reagent followed by centrifugation at 400 g for 30 min. Granulosa cells were collected from the intermediate layer, washed two times with PBS and seeded in cell culture dishes with DMEM/F12 (50:50) medium (Corning, Cat. # 10-092-CM) supplemented with 10% FBS (VWR, Cat. # 89510-186) and antibiotic/antimycotic mixture (MP Biomedicals, Cat. # 1674049). For experiments, 0.3 × 10^6 cells were plated in 6-well plates and one day later the cell culture medium was replaced with serum-free medium supplemented with 500 ng/ml of irisin (Enzo, Cat. # ADI-908-307-0010) or 100 ng/ml of leptin (BioVision, Cat. # 4366-02) for 24 h.

2.1.1. RNA extraction and qRT-PCR array

After completing the experiment, cells were washed two times with PBS (Corning, Cat. # 46-013-CM) and total RNA was extracted using TRIzol reagent (Ambion, 15596018). RNA was quantified using NanoDrop One spectrophotometer (Thermo Fisher Scientific) and samples were normalized to 1 μg total RNA, then converted to cDNA using qScript cDNA SuperMix (Quantabio, Cat. # 95048). PCR array was performed using RT^2 Profiler PCR Array – Human Female Infertility (Qiagen, Cat. # 330231, GeneGlobe ID PAHS-164Z) and QuantStudio 3 Real-Time PCR System (Thermo Fisher Scientific) following manufacturer’s protocol.

2.1.2. Data calculation and statistical analysis

Obtained threshold cycle (Ct) values from the PCR array were analyzed using GeneGlobe Data Analysis Center (https://geneglobe.qiagen.com/us/analyze). Gene expression values were normalized using arithmetic mean of the Ct values and the following housekeeping genes: ACTB, B2M, GAPDH, HPRT1, POLP0. Data were accepted as statistically significant if p ≤ 0.05.

Ethics Statements

This research was approved by the Institutional Review Board of Northwell Health (IRB # 20-0449). Informed consent was obtained from all individuals involved in the study.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

CRediT Author Statement

Radoslav Stojchevski: Methodology, Validation, Formal analysis, Investigation, Writing – original draft, Writing – review & editing, Visualization; Leonid Poretsky: Conceptualization, Writing – review & editing, Supervision, Project administration, Funding acquisition; Dimiter Avtanski: Conceptualization, Methodology, Validation, Formal analysis, Resources, Writing – original draft, Writing – review & editing, Visualization, Supervision, Funding acquisition.
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