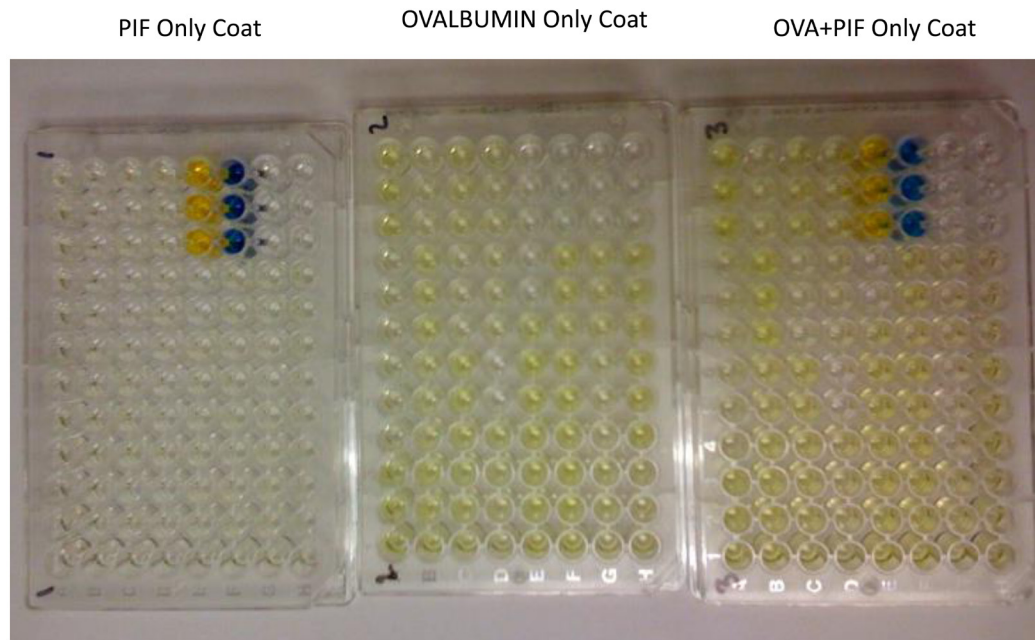# PreImplantation factor (PIF) protects cultured embryos against oxidative stress: relevance for recurrent pregnancy loss (RPL) therapy

## Supplementary Material

PIF Only Coat          OVALBUMIN Only Coat          OVA+PIF Only Coat



**Supplementary Figure 1: ELISA based PIF antibody detection: data shows that the antibody detected is likely against egg component and not against PIF**

OVA+PIF Only Coat              OVALBUMIN Only Coat



**Supplementary Figure 2: ELISA based PIF antibody detection:** data shows that the antibody detected is likely against egg component and not against PIF

PIF Only Coat                   OVALBUMIN Only Coat



**Supplementary Figure 3: ELISA based PIF antibody detection:** confirmation that only the ovalbumin presence and not PIF leads to the antibody detection.

**Supplementary Table 1: PIF PREVENTS EMBRYO DEMISE CAUSED BY RPL SERA**
**<3kDa  Fraction Causes  Embryo Delay & >3kDa Fraction Causes Embryo Demise**

| PIF+ | 34 Total | | PIF- | 34 Total |
|---|---|---|---|---|
| P29 Blast | 85.3% | | 15 Blast | 44.1% |
| 2 Eblast | 5.9% | | 4 Eblast | 11.8% |
| 1 Morula | 2.9% | | 8 Morula | 23.5% |
| 0 6-8 Cell | 0.0% | | 3 6-8 Cell | 8.8% |
| 2 2-4 Cell | 5.9% | | 3 2-4 Cell | 8.8% |
| 0 Atretic | 0.0% | | 1 Atretic | 2.9% |

## < 3 kDa sera fraction

| PIF+ | 33 Total | | PIF- | 33 Total |
|---|---|---|---|---|
| 16 Blast | 48.5% | | 12 Blast | 36.4% |
| 5 Eblast | 15.2% | | 2 Eblast | 6.1% |
| 6 Morula | 18.2% | | 4 Morula | 12.1% |
| 0 6-8 Cell | 0.0% | | 0 6-8 Cell | 0.0% |
| 1 2-4 Cell | 3.0% | | 0 2-4 Cell | 0.0% |
| 5 Atretic | 15.2% | | 15 Atretic | 45.5% |

## > 3 kDa sera fraction

**Supplementary Table 2: PIF PREVENTS EMBRYO DEMISE CAUSED BY OXIDATIVE STRESS Blocks PDI / Thioredoxin Inhibitor Effect**

| Group | | Cleaved | | Blastocyst | | |
|---|---|---|---|---|---|---|
| | | N | % | N | % (of total) | % (of cleaved) |
| Control | 248 | 176 | 70.0[a] | 71 | 28.6[a] | 40.3[a] |
| **PIF** | **155** | **104** | **67.1[ab]** | **46** | **29.7[a]** | **44.2[a]** |
| PDI-inhibitor | 247 | 145 | 58.7[b] | 15 | 6.1[b] | 10.3[b] |
| **PDI inh+PIF** | **236** | **148** | **60.1[ab]** | **33** | **14.0[c]** | **22.3[c]** |

PIF effect on cultured bovine zygotes cleavage and reaching the blastocyst stage.
 $(P < 0.05)$. N = No. of Embryos
Total zygote cleavage and embryo development to blastocyst stage for each treatment group.
 (Replicates are combined.) Values in the same column with different superscripts are significantly different $(p < 0.05)$.

**Supplementary Table 3.** Ligand-receptor interaction analysis in docking models of PIF and 16F16 to either oxidized PDI or reduced PDI form [1].

| | oxi PDI - PIF | oxi PDI - 16F16 | red PDI - PIF | red PDI - 16F16 |
|---|---|---|---|---|
| APBS Binding energy [kJ/mol] | -4.07 | 5.95 | **-6.95** | 0.40 |
| Electrostatic Interaction energies [kcal/mol] | 9.43 | -13.42 | **-18.47** | 3.00 |
| Electrostatic Interaction energies (distance dependent) [kcal/mol] | -5.46 | -2.77 | -5.46 | -0.45 |
| Electrostatic energy [kcal/mol] | -23249.38 | -22311.24 | **-23798.96** | -22779.24 |
| Total charge | -19 | -20 | -20 | -21 |
| APBS Solvation energy [kcal/mol] | -9866.09 | -9690.88 | **-10326.38** | -10230.40 |
| MEP interaction energy (electrostatic interaction) | 9.4380 | -13.4179 | **-18.4713** | 3.0079 |

[1] Ligand-receptor interaction was evaluated by modeling biomolecular solvation through solving the Poisson-Boltzmann equation and related equations, to calculate solvation energies and electrostatic properties of the complex. The APBS (Adaptive Poisson-Boltzmann Solver) software, developed by Nathan Baker in collaboration with J. Andrew McCammon and Michael Holst, was used as plug-in implementation to Vega ZZ package. APBS calculates binding free energies (*APBS Binding energy*) by dividing the binding process up into solvation (*APBS Solvation energy*) and Coulombic components. Intermolecular Coulombic contributions to the solvation energy are assessed using either *APBS Electrostatic Interaction energies* or *Molecular electrostatic potential (MEP) interaction energy.*