|  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Supplementary Table 3.** Relationships between twin, diamniotic, dichorionic pregnancy (vs. singleton pregnancy) and relevant parameters (with significant variable importance, p<0.05) in maternal serum at labour as evaluated by OPLS and ordinary multiple regression (OMR) models (for details see Statistical analysis) | | | | | | | | | | | | |
|  |  | **OPLS, predictive component** | | | | | | |  | **Multiple**  **regression** | | |
|  | **Variable** | Variable importance | t-statistics | | Component loading | t-statistics | R*a* | |  | Regression coefficient | t-statistics | |
| EXPLAINING VARIABLES | Number of male fetuses | 1.033 | 7.39 | \*\* | 0.115 | 9.56 | 0.524 | \*\* |  | 0.079 | 3.85 | \*\* |
| Gestational age | 1.025 | 3.33 | \*\* | -0.133 | -3.37 | -0.609 | \*\* |  | -0.054 | -1.94 | \* |
| Pregnenolone | 1.564 | 10.74 | \*\* | 0.197 | 12.68 | 0.903 | \*\* |  | 0.083 | 4.14 | \*\* |
| 17-Hydroxypregnenolone | 0.843 | 2.18 | \* | 0.121 | 2.73 | 0.552 | \* |  | 0.030 | 0.85 |  |
| 17-Hydroxypregnenolone, C | 0.585 | 2.27 | \* | 0.072 | 2.10 | 0.328 | \* |  | 0.011 | 0.41 |  |
| 16α-Hydroxypregnenolone | 1.327 | 7.77 | \*\* | 0.165 | 7.95 | 0.756 | \*\* |  | 0.024 | 1.06 |  |
| 20α-Dihydropregnenolone | 1.367 | 7.63 | \*\* | 0.182 | 7.38 | 0.831 | \*\* |  | 0.081 | 6.50 | \*\* |
| Dehydroepiandrosterone | 0.74 | 2.49 | \* | 0.113 | 2.89 | 0.517 | \* |  | 0.041 | 1.43 |  |
| 7-oxo-DHEA | 1.294 | 6.11 | \*\* | 0.164 | 6.25 | 0.751 | \*\* |  | 0.041 | 2.34 | \* |
| 7β-Hydroxy-DHEA | 0.777 | 2.62 | \* | 0.112 | 2.78 | 0.511 | \* |  | 0.015 | 0.45 |  |
| 5-Androstene-3β,16α,17β-triol, C | 1.152 | 4.51 | \*\* | 0.148 | 3.95 | 0.675 | \*\* |  | 0.041 | 1.55 |  |
| Progesterone | 0.619 | 1.97 | \* | 0.092 | 2.46 | 0.423 | \* |  | 0.025 | 1.73 |  |
| 17-Hydroxyprogesterone | 1.152 | 3.59 | \*\* | 0.145 | 3.50 | 0.663 | \*\* |  | 0.018 | 1.13 |  |
| 17,20α-Dihydroxy-4-pregnene-3-one | 1.265 | 6.80 | \*\* | 0.163 | 6.62 | 0.747 | \*\* |  | 0.021 | 1.21 |  |
| 16α-Hydroxyprogesterone | 1.049 | 4.56 | \*\* | 0.123 | 3.99 | 0.565 | \*\* |  | 0.020 | 0.76 |  |
| 20α-Dihydroprogesterone | 1.462 | 12.81 | \*\* | 0.192 | 14.60 | 0.877 | \*\* |  | 0.053 | 4.19 | \*\* |
| 20α-Dihydroprogesterone, C | 1.155 | 6.43 | \*\* | 0.141 | 5.45 | 0.644 | \*\* |  | 0.036 | 1.65 |  |
| 16α-Hydroxytestosterone | 0.749 | 3.04 | \*\* | 0.090 | 2.73 | 0.413 | \* |  | -0.014 | -0.38 |  |
| 16α-Hydroxytestosterone, C | 0.902 | 4.14 | \*\* | 0.104 | 4.04 | 0.477 | \*\* |  | 0.010 | 0.57 |  |
| Epitestosterone, C | 0.712 | 2.47 | \* | 0.103 | 3.28 | 0.470 | \*\* |  | -0.011 | -0.51 |  |
| 5α-Dihydrotestosterone | 0.329 | 3.00 | \* | 0.044 | 1.68 | 0.203 |  |  | -0.038 | -1.66 |  |
| Estrone | 0.621 | 2.07 | \* | 0.091 | 3.41 | 0.416 | \*\* |  | -0.005 | -0.17 |  |
| Estrone, C | 0.653 | 2.67 | \* | 0.076 | 2.59 | 0.350 | \* |  | 0.004 | 0.20 |  |
| Estradiol | 0.973 | 5.44 | \*\* | 0.123 | 5.85 | 0.567 | \*\* |  | 0.013 | 0.88 |  |
| Estradiol, C | 0.75 | 2.01 | \* | 0.098 | 1.63 | 0.448 |  |  | 0.015 | 0.66 |  |
| Estriol | 0.598 | 2.67 | \* | 0.065 | 2.06 | 0.297 | \* |  | 0.008 | 0.29 |  |
| 5α-Dihydroprogesterone | 1.118 | 7.77 | \*\* | 0.140 | 6.06 | 0.639 | \*\* |  | 0.010 | 0.79 |  |
| Allopregnanolone | 1.015 | 4.26 | \*\* | 0.136 | 3.84 | 0.622 | \*\* |  | 0.007 | 0.44 |  |
| Isopregnanolone | 1.526 | 10.91 | \*\* | 0.184 | 9.01 | 0.839 | \*\* |  | 0.056 | 4.12 | \*\* |
| Isopregnanolone, C | 0.582 | 3.59 | \*\* | 0.074 | 3.61 | 0.340 | \*\* |  | -0.035 | -3.68 | \*\* |
| 5β-Dihydroprogesterone | 0.983 | 3.83 | \*\* | 0.124 | 3.22 | 0.569 | \*\* |  | 0.019 | 1.06 |  |
| Pregnanolone | 0.795 | 2.57 | \* | 0.108 | 2.59 | 0.493 | \* |  | 0.008 | 0.41 |  |
| Epipregnanolone | 0.825 | 2.72 | \* | 0.120 | 3.50 | 0.550 | \*\* |  | 0.003 | 0.15 |  |
| Epipregnanolone, C | 0.639 | 5.85 | \*\* | 0.080 | 6.11 | 0.365 | \*\* |  | -0.031 | -2.54 | \* |
| 5α,20α-Tetrahydroprogesterone | 1.4 | 12.97 | \*\* | 0.176 | 11.29 | 0.804 | \*\* |  | 0.037 | 3.53 | \*\* |
| 5α,20α-Tetrahydroprogesterone, C | 0.522 | 2.09 | \* | 0.065 | 1.66 | 0.299 |  |  | -0.011 | -0.51 |  |
| 5α-Pregnane-3α,20α-diol | 1.415 | 9.31 | \*\* | 0.182 | 9.53 | 0.832 | \*\* |  | 0.049 | 5.88 | \*\* |
| 5α-Pregnane-3β,20α-diol | 1.592 | 8.96 | \*\* | 0.193 | 7.55 | 0.884 | \*\* |  | 0.075 | 4.73 | \*\* |
| 5α-Pregnane-3β,20α-diol, C | 0.549 | 2.00 | \* | 0.064 | 1.59 | 0.292 |  |  | 0.016 | 0.73 |  |
| 5β,20α-Tetrahydroprogesterone | 1.273 | 8.74 | \*\* | 0.166 | 7.42 | 0.757 | \*\* |  | 0.049 | 4.10 | \*\* |
| 5β-Pregnane-3α,20α-diol | 1.286 | 6.95 | \*\* | 0.170 | 7.57 | 0.779 | \*\* |  | 0.060 | 4.53 | \*\* |
| 5β-Pregnane-3β,20α-diol | 0.953 | 5.68 | \*\* | 0.134 | 5.78 | 0.614 | \*\* |  | 0.053 | 5.01 | \*\* |
| 17-Hydroxyallopregnanolone | 1 | 4.14 | \*\* | 0.125 | 5.02 | 0.574 | \*\* |  | -0.003 | -0.10 |  |
| 17-Hydroxyallopregnanolone, C | 0.943 | 9.01 | \*\* | 0.109 | 9.37 | 0.498 | \*\* |  | -0.008 | -0.80 |  |
| 17-Hydroxypregnanolone | 0.999 | 3.63 | \*\* | 0.118 | 3.43 | 0.541 | \*\* |  | 0.016 | 0.67 |  |
| 17-Hydroxypregnanolone, C | 0.969 | 4.84 | \*\* | 0.128 | 4.95 | 0.587 | \*\* |  | 0.017 | 1.12 |  |
| 5α-Pregnane-3α,17,20α-triol | 0.884 | 5.82 | \*\* | 0.117 | 6.89 | 0.536 | \*\* |  | -0.011 | -0.90 |  |
| 5β-Pregnane-3α,17,20α-triol | 0.742 | 4.68 | \*\* | 0.099 | 4.97 | 0.455 | \*\* |  | 0.008 | 0.39 |  |
| 5α-Androstane-3,17-dione | 0.586 | 3.08 | \*\* | 0.067 | 2.37 | 0.308 | \* |  | -0.004 | -0.21 |  |
| Androsterone | 0.516 | 2.13 | \* | 0.063 | 1.52 | 0.286 |  |  | -0.005 | -0.26 |  |
| Epiandrosterone | 1.099 | 4.57 | \*\* | 0.143 | 4.28 | 0.652 | \*\* |  | 0.050 | 2.45 | \* |
| Etiocholanolone | 0.312 | 2.19 | \* | 0.052 | 2.67 | 0.239 | \* |  | -0.002 | -0.06 |  |
| 5α-Androstane-3α,17β-diol | 0.735 | 2.67 | \* | 0.093 | 2.32 | 0.425 | \* |  | 0.051 | 1.92 | \* |
| 11-Deoxycortisol | 0.798 | 2.96 | \* | 0.115 | 3.20 | 0.526 | \*\* |  | -0.009 | -0.39 |  |
| 11-Deoxycorticosterone | 1.304 | 6.05 | \*\* | 0.161 | 7.21 | 0.766 | \*\* |  | 0.034 | 1.61 |  |
| 11-Deoxycorticosterone, C | 0.801 | 2.52 | \* | 0.086 | 2.36 | 0.395 | \* |  | 0.009 | 0.40 |  |
| 11β-Hydroxytestosterone | 1.166 | 11.45 | \*\* | 0.146 | 9.51 | 0.711 | \*\* |  | 0.014 | 1.27 |  |
| 11β-Hydroxytestosterone, C | 0.723 | 3.24 | \*\* | 0.082 | 3.42 | 0.400 | \*\* |  | 0.003 | 0.18 |  |
| 3α,5β-Tetrahydrocorticosterone | 1.441 | 10.92 | \*\* | 0.171 | 12.75 | 0.807 | \*\* |  | 0.047 | 3.99 | \*\* |
| 11β-Hydroxyandrosterone | 0.872 | 3.12 | \*\* | 0.114 | 3.68 | 0.560 | \*\* |  | 0.000 | 0.02 |  |
| 11β-Hydroxyepiandrosterone | 1.262 | 5.77 | \*\* | 0.163 | 5.25 | 0.784 | \*\* |  | 0.019 | 1.74 |  |
| 11β-Hydroxyepiandrosterone, C | 0.505 | 2.23 | \* | 0.053 | 1.50 | 0.236 |  |  | 0.000 | -0.01 |  |
|  | Twins (diamniotic dichorionic), LLR |  |  |  | 1.000 | 41.32 | 0.917 | \*\* |  |  | | |
| Explained variability = 84% (68.8% after cross-validation), Sensitivity = 1(1-1), Specificity = 1(1-1) | | | | | | | | | | | | |
| *aR…Component loading expressed as a correlation coefficient with predictive component, \*p<0.05, \*\*p<0.01, LLR…logarithm of likelihood ratio (the ratio of the probability that the phenomenon occurs - twin pregnancy) to the probability that the phenomenon does not occur - singleton pregnancy), C…conjugated steroid* | | | | | | | | | | | | |