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Reproduction, Fertility and Development

Supplementary Material

Effect of melatonin and nitric oxide on capacitation and apoptotic changes induced by epidermal growth factor in ram sperm

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Table S1. Effect of in vitro capacitation with EGF (3h, 39 °C, 5% CO₂, 100% humidity) in the presence or absence of melatonin, L-arginine (L-arg) or/and L-NAME in kinematic parameters of ram spermatozoa. The kinematic parameters recorded for each spermatozoon are curvilinear velocity (VCL, $\mu\text{m/s}$: the average path velocity of the sperm head along its actual trajectory); straight line velocity (VSL, $\mu\text{m/s}$: the average path velocity of the sperm head along a straight line from its first to its last position); average path velocity (VAP, $\mu\text{m/s}$: the average velocity of the sperm head along its average trajectory); percentage of linearity (LIN, %: the ratio between VSL and VCL); percentage of straightness (STR, %: the ratio between VSL and VAP); wobble coefficient (WOB, %: the ratio between VAP and VCL); mean amplitude of lateral head displacement (ALH, μm : the average value of the extreme side-to-side movement of the sperm head in each beat cycle); beat cross-frequency (BCF, Hz: the frequency with which the actual sperm trajectory crosses the average path trajectory); DANCE ($\mu\text{m}^2/\text{s}$), a measure of the pattern of sperm motion (product of VCL and ALH); mean angular displacement (MAD, degrees; time average of absolute values of the instantaneous turning angle of the sperm head along its curvilinear trajectory); and fractal dimension (FD), an estimate of the extent to which a line fills a plane. Values are shown as mean \pm SEM; (n=5).

| | Swim-up | Cap-EGF | L-arg | L-NAME | Mel 100 pM | Mel 100 pM + L-arg | Mel 100 pM + L-NAME | Mel 1 μM | Mel 1 μM + L-arg | Mel 1 μM + L-NAME |
|--|------------------|-------------------|------------------|-------------------|-------------------|--------------------|---------------------|---------------------|-----------------------------|------------------------------|
| VCL ($\mu\text{m/s}$) | 252.5 \pm 9.76 | 234.5 \pm 12.73 | 240.4 \pm 14 | 249.2 \pm 5.15 | 259.4 \pm 13.58 | 234.9 \pm 8.51 | 253.6 \pm 7.96 | 263.1 \pm 6 | 235.9 \pm 9.72 | 257.9 \pm 15.5 |
| VSL ($\mu\text{m/s}$) | 111.8 \pm 8.57 | 102 \pm 8.44 | 108.8 \pm 4.76 | 124.2 \pm 10.20 | 119.1 \pm 7.59 | 110 \pm 9.31 | 124.9 \pm 6.71 | 112.6 \pm 5.13 | 105.5 \pm 9.82 | 126.2 \pm 8.26 |
| VAP ($\mu\text{m/s}$) | 145.6 \pm 6.96 | 133.4 \pm 5.67 | 137 \pm 4.79 | 157.2 \pm 10.56 | 151.8 \pm 5.38 | 137 \pm 9.22 | 156.2 \pm 5.51 | 152.3 \pm 3.4 | 135.2 \pm 7.82 | 155.3 \pm 6.35 |
| LIN (%) | 43.8 \pm 2.19 | 43.5 \pm 5.69 | 45.6 \pm 3.49 | 50.6 \pm 3.75 | 47.5 \pm 4.20 | 47.5 \pm 2.55 | 50.4 \pm 2.86 | 44.0 \pm 2.64 | 45.8 \pm 4.25 | 49.9 \pm 2.72 |
| STR(%) | 73.8 \pm 1.98 | 73.0 \pm 4.81 | 76.7 \pm 2.63 | 77.8 \pm 1.5 | 76.9 \pm 2.93 | 77.5 \pm 2.12 | 78.7 \pm 1.19 | 73.2 \pm 1.71 | 75.5 \pm 4.21 | 78.9 \pm 1.99 |
| WOB (%) | 57.4 \pm 1.38 | 57.6 \pm 3.80 | 57.4 \pm 2.59 | 63.8 \pm 3.81 | 59.7 \pm 3.47 | 59.2 \pm 2.07 | 62.5 \pm 3.11 | 58.9 \pm 2.31 | 58.2 \pm 3.24 | 61.1 \pm 2.54 |
| ALH (μm) | 3.82 \pm 0.31 | 3.52 \pm 0.27 | 3.66 \pm 0.33 | 3.49 \pm 0.19 | 3.81 \pm 0.27 | 3.47 \pm 0.11 | 3.60 \pm 0.24 | 4.04 \pm 0.27 | 3.47 \pm 0.22 | 3.53 \pm 0.29 |
| BCF (Hz) | 38.5 \pm 0.83 | 35.7 \pm 0.86 | 37.7 \pm 1.04 | 40.3 \pm 0.89 | 39.7 \pm 1.24 | 39.3 \pm 1.89 | 40.0 \pm 1.08 | 37.9 \pm 1.07 | 37.1 \pm 2.03 | 40.8 \pm 1.17 |
| DANCE ($\mu\text{m}^2/\text{s}$) | 1130 \pm 99.77 | 1050 \pm 137.6 | 1082 \pm 136.3 | 1026 \pm 81.82 | 1155 \pm 136.6 | 1008 \pm 73.87 | 1122 \pm 115.3 | 1250 \pm 97.34 | 1023 \pm 86.55 | 1094 \pm 141.3 |
| MAD (°) | 183.3 \pm 1.48 | 177.6 \pm 1.76 | 184.2 \pm 2.45 | 180.7 \pm 1.56 | 181.6 \pm 1.40 | 177.5 \pm 5.46 | 185.6 \pm 0.9 | 182.7 \pm 0.44 | 184.6 \pm 1.14 | 182.9 \pm 1.26 |
| Fractal dimension (FD) | 1.34 \pm 0.02 | 1.37 \pm 0.07 | 1.35 \pm 0.05 | 1.27 \pm 0.03 | 1.33 \pm 0.08 | 1.33 \pm 0.04 | 1.29 \pm 0.03 | 1.33 \pm 0.02 | 1.37 \pm 0.08 | 1.31 \pm 0.04 |