

Title: Sperm-borne miR-216b modulates cell proliferation during early embryo development via *K-RAS*

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Supplementary Table S1. Mean and SEM of sperm quality characteristics evaluated in three commercial semen batches from each bull. ^{a,b} Different letters on the same line indicate statistical difference ($P < 0.05$) between fertility groups. ¹High fertility; ² Low fertility; ³Sperm with plasma and acrosome membrane integrity and high mitochondrial membrane potential; ⁴Production of ROS (reactive oxygen species). SEM: standard error of the mean.

| Sperm quality analyses of all straws | Fertility groups | |
|--------------------------------------------------------------------------------------|------------------------|------------------------|
| | HF ¹ (n=9) | LF ² (n=9) |
| <i>Sperm conventional analyses</i> | | |
| Straw volume (mL) | 0.25 | 0.25 |
| Subjective motility (%) | 66.4±1.1 | 63.3±1.1 |
| Vigor (1-5) | 3.4±0.1 | 3.3±0.1 |
| Sperm concentration (×10 ⁶ sperm/mL) | 100.9±5.4 | 90.8±4.7 |
| Major defects (%) | 18.7±2.0 ^b | 25.0±0.9 ^a |
| Minor defects (%) | 4.6±1.1 | 4.2±0.7 |
| Total defects (%) | 23.4±2.6 | 29.2±1.1 |
| Total of sperm/straw (×10 ⁶) | 25.2±1.4 | 22.7±1.2 |
| Total of motility sperm/straw (×10 ⁶) | 17.1±0.8 ^a | 13.5±0.8 ^b |
| <i>Sperm motility characteristics</i> | | |
| Total motility (%) | 76.7±1.9 | 71.9±1.7 |
| Progressive motility (%) | 54.7±2.0 | 52.5±1.7 |
| Rapid cells (%) | 70.5±1.7 | 66.7±1.6 |
| Curvilinear velocity (VCL; μm/s) | 145.8±2.6 ^b | 172.8±3.7 ^a |
| Progressive velocity (VSL; μm/s) | 78.3±2.7 | 83.9±1.9 |
| Path velocity (VAP; μm/s) | 97.4±1.5 ^b | 105.4±1.6 ^a |
| Linearity (LIN; %) | 53.7±1.6 ^a | 48.7±1.0 ^b |
| Straightness (STR; %) | 81.1±1.4 | 80.6±0.9 |
| Wobble (WOB; %) | 66.0±1.0 ^a | 60.4±0.8 ^b |
| Lateral head displacement (ALH; μm) | 2.7±0.04 ^b | 3.4±0.1 ^a |
| Beat cross frequency (BCF; Hz) | 22.3±0.3 | 23.1±0.5 |
| <i>Sperm membranes integrity, mitochondrial membrane function and ROS production</i> | | |
| PIAIH ³ (%) | 42.7±2.4 | 42.1±1.8 |
| Plasma membrane integrity (%) | 44.4±2.4 | 46.8±2.6 |
| Acrosome membrane integrity (%) | 53.4±2.4 | 57.7±1.4 |
| High mitochondrial membrane potential (%) | 58.0±3.2 | 52.6±2.8 |
| ROS ⁴ (%) | 6.1±1.3 ^b | 10.6±1.5 ^a |

Supplementary Table S2. Individual characteristics and values obtained for all three commercial semen batches from each bull in order to select the best batch from each bull. Bold text indicates the selected batch of each bull. HF: high fertility; LF: low fertility. ¹Progressive motility; ²Major defects; ³Minor defects; ⁴Sperm with plasma and acrosome membranes integrity and high mitochondrial membrane potential; ⁵Value obtained by the formula: $(1 \times \text{PROG}) + [(3 \times (100 - \text{MAJ}))] + [(2 \times (100 - \text{MIN}))] + (3 \times \text{PIAIH})$.

| Bull | Batch | PROG ¹ (%) | MAJ ² (%) | MIN ³ (%) | PIAIH ⁴ (%) | Value ⁵ |
|------|----------|-----------------------|----------------------|----------------------|------------------------|--------------------|
| HF-1 | 1 | 54.1 | 20.7 | 9.2 | 58.2 | 648.2 |
| | 2 | 52.8 | 13.2 | 2.4 | 34.2 | 611.0 |
| | 3 | 43.2 | 22.0 | 7.2 | 37.2 | 574.4 |
| HF-2 | 1 | 55.0 | 7.7 | 1.5 | 45.7 | 666.0 |
| | 2 | 59.3 | 16.2 | 1.7 | 49.0 | 654.3 |
| | 3 | 68.5 | 9.0 | 3.0 | 43.5 | 666.0 |
| HF-3 | 1 | 49.2 | 20.0 | 7.2 | 45.2 | 610.4 |
| | 2 | 60.0 | 33.0 | 4.7 | 41.7 | 576.7 |
| | 3 | 50.0 | 26.7 | 4.7 | 29.7 | 549.6 |
| LF-1 | 1 | 49.7 | 23.2 | 3.7 | 37.7 | 585.8 |
| | 2 | 48.4 | 19.7 | 4.0 | 33.7 | 582.4 |
| | 3 | 46.0 | 23.2 | 3.0 | 35.7 | 577.5 |
| LF-2 | 1 | 49.4 | 27.7 | 3.5 | 39.2 | 576.9 |
| | 2 | 57.5 | 26.7 | 6.0 | 38.5 | 580.9 |
| | 3 | 63.1 | 27.2 | 6.0 | 40.5 | 591.0 |
| LF-3 | 1 | 51.1 | 30.7 | 4.0 | 46.7 | 591.1 |
| | 2 | 44.5 | 24.2 | 4.2 | 49.2 | 611.1 |
| | 3 | 62.5 | 22. | 3.2 | 57.2 | 661.7 |

Supplementary Table S3. Mean and SEM of sperm quality characteristics evaluated in each of the three commercial semen batches from HF-1, HF-2 and HF-3 bulls. Bold text indicates the selected batch. ¹High fertility; ²Sperm with plasma and acrosome membranes integrity and high mitochondrial membrane potential; ³Production of ROS (reactive oxygen species). SEM: standard error of the mean.

| Sperm quality | Semen batches from high fertility bulls | | | | | | | | |
|-----------------------------------------------|-----------------------------------------|----------------------------|----------------------------|-----------------------------|------------------------------|-----------------------------|------------------------------|-------------------------------|------------------------------|
| | HF¹-1-1 | HF ¹ -1-2 | HF ¹ -1-3 | HF ¹ -2-1 | HF ¹ -2-2 | HF¹-2-3 | HF¹-3-1 | HF ¹ -3-2 | HF ¹ -3-3 |
| Total sperm ($\times 10^6$) | 19.4 \pm 5.0 | 33.7 \pm 1.9 | 26.2 \pm 3.1 | 29.9 \pm 4.1 | 21.6 \pm 2.2 | 28.1 \pm 5.0 | 20.0 \pm 1.2 | 27.5 \pm 3.1 | 24.4 \pm 5.0 |
| Total sperm with motility ($\times 10^6$) | 12.9 \pm 2.9 | 21.0 \pm 0.3 | 16.3 \pm 1.3 | 18.2 \pm 4.2 | 13.9 \pm 0.3 | 19.1 \pm 4.1 | 14.0 \pm 0.9 | 19.2 \pm 2.2 | 15.6 \pm 2.03 |
| Subjective motility (%) | 67.5 \pm 2.5 | 62.5 \pm 2.5 | 62.5 \pm 2.5 | 67.5 \pm 7.5 | 65.0 \pm 5.0 | 67.5 \pm 2.5 | 70.0 \pm 0.0 | 70.0 \pm 0.0 | 65.0 \pm 5.0 |
| Vigor (1-5) | 3.2 \pm 0.2 ^{ab} | 3.5 \pm 0.0 ^a | 2.5 \pm 0.0 ^b | 3.5 \pm 0.0 | 3.5 \pm 0.0 | 3.7 \pm 0.2 | 3.5 \pm 0.0 | 3.5 \pm 0.0 | 3.5 \pm 0.0 |
| Sperm concentration ($\times 10^6$ sperm/mL) | 77.5 \pm 20.0 | 135.0 \pm 7.5 | 105.0 \pm 12.5 | 103.7 \pm 16.2 | 86.2 \pm 8.7 | 112.5 \pm 20.0 | 80.0 \pm 5.0 | 110.0 \pm 12.5 | 97.5 \pm 2 |
| Major defects (%) | 20.7 \pm 3.2 | 13.2 \pm 2.2 | 22.0 \pm 4.0 | 7.7 \pm 0.2 ^b | 16.2 \pm 1.2 ^a | 8.7 \pm 2.2 ^{ab} | 20.0 \pm 5.0 | 33.0 \pm 1.5 | 26.7 \pm 2.2 |
| Minor defects (%) | 9.2 \pm 6.7 | 2.4 \pm 1.4 | 7.2 \pm 5.7 | 1.5 \pm 0.0 | 1.7 \pm 0.7 | 3.0 \pm 2.0 | 7.2 \pm 5.2 | 4.7 \pm 0.7 | 4.7 \pm 3.2 |
| Total defects (%) | 30.0 \pm 3.5 | 15.5 \pm 3.5 | 29.2 \pm 9.7 | 9.2 \pm 0.2 | 18.0 \pm 2.0 | 11.7 \pm 4.2 | 25.2 \pm 10.2 | 37.7 \pm 2.2 | 31.5 \pm 5.5 |
| Total motility (%) | 81.2 \pm 4.6 | 75.1 \pm 6.0 | 72.2 \pm 4.0 | 70.5 \pm 1.6 | 76.5 \pm 2.9 | 80.5 \pm 2.2 | 84.9 \pm 1.6 | 85.4 \pm 0.8 | 63.5 \pm 7.5 |
| Progressive motility (%) | 54.1 \pm 0.8 | 52.8 \pm 1.3 | 43.2 \pm 12.5 | 55.0 \pm 0.9 ^b | 59.3 \pm 3.1 ^{ab} | 68.2 \pm 1.5 ^a | 49.2 \pm 2.1 | 60.0 \pm 4.5 | 50.0 \pm 1.3 |
| Rapid cells (%) | 76.0 \pm 4.2 | 70.1 \pm 4.5 | 65.2 \pm 1.0 | 64.1 \pm 0.9 ^b | 69.9 \pm 2.4 ^{ab} | 75.5 \pm 0.4 ^a | 75.1 \pm 1.6 | 79.3 \pm 2.5 | 59.0 \pm 7.7 |
| Curvilinear velocity (VCL; μ m/s) | 160.3 \pm 2.8 | 158.1 \pm 7.1 | 136.5 \pm 11.1 | 149.7 \pm 12.7 | 146.9 \pm 1.3 | 139.8 \pm 0.7 | 133.9 \pm 2.7 ^b | 140.0 \pm 1.1 ^{ab} | 146.7 \pm 0.4 ^a |

| | | | | | | | | | |
|-------------------------------------------------|------------------------------|-----------------------------|-----------------------------|-----------------|----------------|-----------------|------------------------------|-----------------------------|-----------------------------|
| Progressive velocity (VSL; $\mu\text{m/s}$) | 75.9 \pm 1.9 | 80.0 \pm 80.1 | 62.6 \pm 12.9 | 88.5 \pm 4.9 | 81.9 \pm 0.5 | 87.2 \pm 4.6 | 63.8 \pm 1.1 | 80.5 \pm 5.7 | 84.5 \pm 7.8 |
| Path velocity (VAP; $\mu\text{m/s}$) | 99.1 \pm 1.0 | 100.5 \pm 5.2 | 81.7 \pm 7.7 | 102.7 \pm 5.9 | 97.0 \pm 0.2 | 99.4 \pm 2.9 | 86.4 \pm 0.05 | 99.5 \pm 4.5 | 98.7 \pm 4.9 |
| Linearity (LIN; %) | 47.3 \pm 0.3 | 50.4 \pm 2.8 | 45.3 \pm 5.7 | 59.3 \pm 1.7 | 55.8 \pm 0.8 | 62.3 \pm 2.9 | 47.7 \pm 1.8 | 57.5 \pm 3.6 | 57.6 \pm 5.2 |
| Straightness (STR; %) | 76.5 \pm 1.1 | 79.4 \pm 3.9 | 75.7 \pm 8.6 | 86.2 \pm 0.2 | 84.4 \pm 0.3 | 87.6 \pm 2.0 | 73.8 \pm 1.3 | 80.8 \pm 2.1 | 85.4 \pm 3.6 |
| Wobble (WOB; %) | 61.8 \pm 0.4 ^{ab} | 63.5 \pm 0.4 ^a | 59.8 \pm 0.7 ^b | 68.7 \pm 1.8 | 66.0 \pm 0.7 | 71.1 \pm 1.7 | 64.7 \pm 1.1 | 71.0 \pm 2.6 | 67.2 \pm 3.1 |
| Lateral head displacement (ALH; μm) | 2.8 \pm 0.05 | 2.7 \pm 0.05 | 2.7 \pm 0.1 | 2.7 \pm 0.2 | 2.7 \pm 0.0 | 2.4 \pm 0.05 | 2.6 \pm 0.1 | 2.4 \pm 0.0 | 2.7 \pm 0.1 |
| Beat cross frequency (BCF; Hz) | 21.8 \pm 0.8 | 23.6 \pm 1.4 | 21.0 \pm 1.4 | 23.6 \pm 0.4 | 23.2 \pm 0.5 | 23.0 \pm 0.05 | 20.5 \pm 0.05 ^b | 20.9 \pm 0.2 ^b | 22.7 \pm 0.1 ^a |
| PIAIH ² (%) | 58.2 \pm 2.7 ^a | 34.2 \pm 3.2 ^b | 37.2 \pm 1.7 ^b | 45.7 \pm 8.7 | 49.0 \pm 8.0 | 43.2 \pm 4.7 | 45.2 \pm 3.7 ^a | 41.7 \pm 1.7 ^a | 29.7 \pm 1.7 ^b |
| Plasma membrane integrity (%) | 61.5 \pm 0.0 ^a | 35.2 \pm 3.2 ^b | 39.0 \pm 2.5 ^b | 47.0 \pm 1 | 49.7 \pm 8.7 | 44.7 \pm 4.7 | 48.2 \pm 3.7 ^a | 43.7 \pm 1.7 ^a | 30.5 \pm 1.0 ^b |
| Acrosome membrane integrity (%) | 67.2 \pm 6.7 ^a | 41.2 \pm 0.7 ^b | 44.7 \pm 0.7 ^b | 55.2 \pm 4.7 | 60.2 \pm 9.7 | 55.0 \pm 1.0 | 60.0 \pm 6.0 | 56.0 \pm 2.0 | 41.0 \pm 2.0 |
| High mitochondrial membrane potential (%) | 78.0 \pm 6.5 | 54.7 \pm 11.2 | 73.0 \pm 3.5 | 53.2 \pm 11.2 | 56.7 \pm 8.2 | 52.0 \pm 9.5 | 57.7 \pm 5.2 | 57.5 \pm 6.0 | 39.0 \pm 3.5 |
| ROS ³ (%) | 5.5 \pm 0.5 | 16.5 \pm 5.5 | 5.5 \pm 1.5 | 5.5 \pm 3.5 | 4.5 \pm 3.5 | 3.5 \pm 2.5 | 6.50 \pm 5.5 | 39.5 \pm 39.5 | 4.5 \pm 1.5 |

Supplementary Table S4. Mean and SEM of sperm quality characteristics evaluated in each of the three commercial semen batches from LF-1, LF-2 and LF-3 bulls. Bold text indicates the selected batch. ¹Low fertility; ²Sperm with plasma and acrosome membranes integrity and high mitochondrial membrane potential; ³Production of ROS (reactive oxygen species). SEM: standard error of the mean.

| Sperm quality | Semen batches from low fertility bulls | | | | | | | | |
|-----------------------------------------------|----------------------------------------|----------------------|----------------------|----------------------|----------------------|---------------------------|-----------------------------|-----------------------------|-----------------------------|
| | LF¹-1-1 | LF ¹ -1-2 | LF ¹ -1-3 | LF ¹ -2-1 | LF ¹ -2-2 | LF¹-2-3 | LF ¹ -3-1 | LF ¹ -3-2 | LF¹-3-3 |
| Total sperm ($\times 10^6$) | 21.9 \pm 1.9 | 27.5 \pm 5.6 | 23.7 \pm 3.1 | 15.3 \pm 0.3 | 20.3 \pm 2.2 | 17.2 \pm 1.6 | 26.2 \pm 1.2 | 25.0 \pm 1.2 | 27.2 \pm 1.6 |
| Total sperm with motility ($\times 10^6$) | 11.9 \pm 0.1 | 16.8 \pm 4.7 | 13.9 \pm 3.6 | 9.2 \pm 0.2 | 11.5 \pm 0.3 | 10.3 \pm 0.93 | 17.1 \pm 0.8 | 16.8 \pm 0.2 | 18.3 \pm 0.4 |
| Subjective motility (%) | 55.0 \pm 5.0 | 60.0 \pm 5.0 | 57.5 \pm 7.5 | 60.0 \pm 0.0 | 57.5 \pm 7.5 | 60.0 \pm 0.0 | 65.0 \pm 0.0 | 67.5 \pm 2.5 | 67.5 \pm 2.5 |
| Vigor (1-5) | 3.5 \pm 0.0 | 3.2 \pm 0.2 | 3.2 \pm 0.2 | 3.2 \pm 0.2 | 2.7 \pm 0.2 | 3.0 \pm 0.0 | 3.5 \pm 0.0 | 3.5 \pm 0.0 | 3.5 \pm 0.0 |
| Sperm concentration ($\times 10^6$ sperm/mL) | 87.5 \pm 7.5 | 110.0 \pm 22.5 | 95.0 \pm 12.5 | 61.25 \pm 1.2 | 81.2 \pm 8.7 | 68.7 \pm 6.2 | 105.0 \pm 5.0 | 100.0 \pm 5.0 | 108.7 \pm 6.2 |
| Major defects (%) | 23.2 \pm 1.2 | 19.7 \pm 0.7 | 23.2 \pm 1.2 | 27.7 \pm 1.2 | 26.7 \pm 2.7 | 27.2 \pm 4.2 | 30.7 \pm 0.2 ^a | 24.2 \pm 0.2 ^b | 22.4 \pm 1.5 ^b |
| Minor defects (%) | 3.7 \pm 1.2 | 4.0 \pm 1.5 | 3.00 \pm 1.5 | 3.5 \pm 2.5 | 6.0 \pm 4.5 | 6.0 \pm 4.0 | 4.0 \pm 2.0 | 4.2 \pm 2.7 | 3.2 \pm 1.30.0 |
| Total defects (%) | 27.0 \pm 0.0 | 23.7 \pm 2.2 | 26.25 \pm 2.7 | 31.2 \pm 1.2 | 32.7 \pm 7.2 | 33.2 \pm 0.2 | 34.7 \pm 1.7 | 28.5 \pm 2.5 | 25.6 \pm 2.8 |
| Total motility (%) | 63.8 \pm 2.4 | 67.4 \pm 3.9 | 63.35 \pm 2.1 | 74.5 \pm 0.9 | 69.6 \pm 1.7 | 76.4 \pm 3.7 | 76.8 \pm 6.2 | 73.8 \pm 6.2 | 81.6 \pm 2.9 |
| Progressive motility (%) | 49.7 \pm 3.9 | 48.4 \pm 5.5 | 46.05 \pm 1.8 | 49.4 \pm 3.5 | 57.5 \pm 0.4 | 63.1 \pm 2.7 | 51.1 \pm 0.3 ^b | 44.5 \pm 0.5 ^c | 62.5 \pm 1.6 ^a |
| Rapid cells (%) | 60.7 \pm 3.3 | 61.5 \pm 7.3 | 59.50 \pm 2.1 | 66.0 \pm 2.1 | 67.5 \pm 2.2 | 70.3 \pm 4.0 | 70.3 \pm 4.1 | 67.4 \pm 3.2 | 76.7 \pm 2.5 |
| Curvilinear velocity (VCL; μ m/s) | 185.5 \pm 2.7 | 184.9 \pm 5.1 | 186.9 \pm 7.6 | 150.4 \pm 7.4 | 160.0 \pm 7.3 | 150.7 \pm 3.7 | 181.1 \pm 6.5 | 175.6 \pm 0.4 | 179.8 \pm 6.6 |

| | | | | | | | | | |
|-------------------------------------------------|-----------------|-----------------|-----------------|-----------------|-----------------|----------------|-------------------------------|-----------------------------|-----------------------------|
| Progressive velocity (VSL; $\mu\text{m/s}$) | 86.9 \pm 0.8 | 84.6 \pm 3.0 | 87.7 \pm 6.1 | 70.5 \pm 7.0 | 87.9 \pm 0.6 | 81.6 \pm 3.9 | 86.9 \pm 7.9 | 75.4 \pm 1.5 | 93.5 \pm 1.8 |
| Path velocity (VAP; $\mu\text{m/s}$) | 105.3 \pm 0.4 | 104.8 \pm 3.0 | 108.5 \pm 6.4 | 90.8 \pm 9.0 | 105.2 \pm 2.0 | 94.7 \pm 3.1 | 110.4 \pm 4.4 | 102.8 \pm 1.1 | 114.2 \pm 3.1 |
| Linearity (LIN; %) | 46.8 \pm 0.2 | 45.7 \pm 0.3 | 46.9 \pm 1.4 | 46.7 \pm 2.3 | 55.1 \pm 2.9 | 54.1 \pm 1.2 | 47.90 \pm 2.0 | 43.0 \pm 0.8 | 52.0 \pm 0.8 |
| Straightness (STR; %) | 82.6 \pm 0.4 | 80.7 \pm 0.6 | 80.8 \pm 0.85 | 77.6 \pm 0.05 | 83.6 \pm 2.1 | 86.1 \pm 1.2 | 78.60 \pm 4.0 | 73.4 \pm 0.7 | 81.9 \pm 0.6 |
| Wobble (WOB; %) | 56.8 \pm 0.6 | 57.7 \pm 0.1 | 58.0 \pm 1.1 | 60.2 \pm 3.0 | 65.8 \pm 1.8 | 62.8 \pm 0.5 | 60.90 \pm 0.2 ^{ab} | 58.6 \pm 0.5 ^b | 63.5 \pm 0.6 ^a |
| Lateral head displacement (ALH; μm) | 3.8 \pm 0.1 | 4.0 \pm 0.2 | 3.9 \pm 0.2 | 2.8 \pm 0.05 | 2.7 \pm 0.05 | 2.8 \pm 0.0 | 3.80 \pm 0.2 | 3.5 \pm 0.2 | 3.3 \pm 0.1 |
| Beat cross frequency (BCF; Hz) | 23.8 \pm 0.5 | 21.7 \pm 1.3 | 23.0 \pm 1.2 | 22.0 \pm 1.1 | 26.6 \pm 1.1 | 26.3 \pm 0.8 | 21.10 \pm 1.1 | 20.9 \pm 0.8 | 22.4 \pm 0.2 |
| PIAIH ² (%) | 37.7 \pm 0.2 | 33.7 \pm 1.7 | 35.7 \pm 2.7 | 39.2 \pm 2.7 | 38.5 \pm 0.0 | 40.5 \pm 0.5 | 46.75 \pm 5.7 | 49.2 \pm 0.2 | 57.2 \pm 3.7 |
| Plasma membrane integrity (%) | 43.0 \pm 3.5 | 34.7 \pm 2.7 | 38.7 \pm 2.2 | 41.2 \pm 4.2 | 40.2 \pm 1.2 | 41.2 \pm 0.7 | 66.25 \pm 5.7 | 54.2 \pm 2.2 | 61.2 \pm 0.7 |
| Acrosome membrane integrity (%) | 60.5 \pm 1.5 | 57.7 \pm 1.2 | 57.7 \pm 1.7 | 56.0 \pm 2.5 | 53.5 \pm 4.5 | 51.7 \pm 0.2 | 55.25 \pm 8.2 | 60.5 \pm 3.5 | 66.0 \pm 7.0 |
| High mitochondrial membrane potential (%) | 41.2 \pm 1.2 | 35.7 \pm 1.7 | 42.0 \pm 2.5 | 49.5 \pm 3.0 | 49.5 \pm 4.5 | 57.2 \pm 3.2 | 68.50 \pm 2.5 | 64.0 \pm 3.0 | 65.7 \pm 1.2 |
| ROS ³ (%) | 7.0 \pm 0.0 | 7.5 \pm 4.5 | 8.7 \pm 6.2 | 11.5 \pm 0.5 | 6.0 \pm 5.0 | 4.5 \pm 1.5 | 15.5 \pm 4.5 | 14.5 \pm 1.5 | 20.5 \pm 3.5 |

Supplementary Table S5. Mean and SEM of relative levels of the panel of 380 miRNAs detected in bovine gametes: sperm cells from high (HF) and low fertility (LF) bulls and in mature oocytes. SEM: standard error of the mean.

| miRNAs | Sperm cells | | | | Mature oocytes (n=3) | | |
|-----------------------------------------------------------|-------------|----------|----------|----------|----------------------|---|--------|
| | HF (n=3) | | LF (n=3) | | | | |
| <i>Detected in both groups (HF and LF) of sperm cells</i> | | | | | | | |
| bta-let-7a-3p | 0.0082 | ± 0.0029 | 0.0052 | ± 0.0064 | . | ± | . |
| bta-let-7a-5p | 0.0787 | ± 0.0399 | 0.0820 | ± 0.0179 | 0.0060 | ± | 0.0017 |
| bta-let-7c | 0.0655 | ± 0.0376 | 0.0462 | ± 0.0203 | 0.0056 | ± | 0.0034 |
| bta-let-7d | 0.0275 | ± 0.0122 | 0.0283 | ± 0.0087 | 0.0067 | ± | 0.0018 |
| bta-let-7e | 0.1503 | ± 0.0720 | 0.1566 | ± 0.0185 | 0.0198 | ± | 0.0155 |
| bta-let-7f | 0.0064 | ± 0.0042 | 0.0104 | ± 0.0054 | 0.0021 | ± | 0.0015 |
| bta-let-7g | 0.0245 | ± 0.0080 | 0.0114 | ± 0.0138 | 0.0042 | ± | 0.0022 |
| bta-let-7i | 0.0116 | ± 0.0091 | 0.0065 | ± 0.0037 | 0.0072 | ± | 0.0045 |
| bta-miR-7 | 0.0403 | ± 0.0391 | 0.0234 | ± 0.0192 | 0.0049 | ± | 0.0012 |
| bta-miR-9-5p | 0.0225 | ± 0.0145 | 0.0211 | ± 0.0146 | 0.0100 | ± | 0.0074 |
| bta-miR-10a | 0.3017 | ± 0.1970 | 0.4891 | ± 0.0931 | 0.0454 | ± | 0.0070 |
| bta-miR-10b | 0.0049 | ± 0.0028 | 0.0059 | ± 0.0032 | 0.0429 | ± | 0.0074 |
| bta-miR-15a | 0.7730 | ± 0.7098 | 1.0576 | ± 0.9029 | 0.0422 | ± | 0.0035 |
| bta-miR-15b | 1.5945 | ± 1.5854 | 1.7931 | ± 1.6892 | 0.0134 | ± | 0.0051 |
| bta-miR-16a | 2.2002 | ± 1.8295 | 2.5497 | ± 2.7160 | 0.1019 | ± | 0.0280 |
| bta-miR-16b | 5.3977 | ± 4.7208 | 4.0805 | ± 2.1031 | 0.2727 | ± | 0.2049 |
| bta-miR-17-5p | 0.0725 | ± 0.0696 | 0.0549 | ± 0.0341 | 0.0171 | ± | 0.0024 |
| bta-miR-18a | 0.0863 | ± 0.0674 | 0.0838 | ± 0.0811 | 0.0102 | ± | 0.0044 |
| bta-miR-18b | 0.0289 | ± 0.0261 | 0.0212 | ± 0.0202 | 0.0023 | ± | 0.0016 |
| bta-miR-19a | 0.0286 | ± 0.0337 | 0.0134 | ± 0.0198 | 0.0377 | ± | 0.0034 |
| bta-miR-19b | 0.0349 | ± 0.0414 | 0.0158 | ± 0.0150 | 0.0462 | ± | 0.0296 |
| bta-miR-20a | 0.7040 | ± 0.6306 | 0.4886 | ± 0.3146 | 0.0443 | ± | 0.0072 |
| bta-miR-20b | 0.2230 | ± 0.2159 | 0.2580 | ± 0.1985 | 0.0225 | ± | 0.0039 |
| bta-miR-21-5p | 0.0134 | ± 0.0128 | 0.0133 | ± 0.0020 | 0.0037 | ± | 0.0028 |

| | | | |
|----------------|-----------------|-----------------|-----------------|
| bta-miR-22-5p | 0.0018 ± 0.0014 | 0.0026 ± 0.0037 | 0.0120 ± 0.0038 |
| bta-miR-23a | 0.0625 ± 0.0436 | 0.0895 ± 0.0414 | 0.0119 ± 0.0055 |
| bta-miR-23b-3p | 0.0017 ± 0.0014 | 0.0031 ± 0.0013 | 0.0526 ± 0.0702 |
| bta-miR-24-3p | 0.0235 ± 0.0144 | 0.0190 ± 0.0238 | 0.0142 ± 0.0032 |
| bta-miR-25 | 0.1769 ± 0.0393 | 0.2059 ± 0.1237 | 0.0092 ± 0.0045 |
| bta-miR-26a | 0.0563 ± 0.0559 | 0.0460 ± 0.0361 | 0.0848 ± 0.0746 |
| bta-miR-26b | 0.0032 ± 0.0003 | 0.0032 ± 0.0031 | 0.0060 ± 0.0071 |
| bta-miR-27a-3p | 0.2037 ± 0.2382 | 0.1972 ± 0.2456 | 0.0522 ± 0.0674 |
| bta-miR-27b | 0.0973 ± 0.0979 | 0.0931 ± 0.0757 | 0.0795 ± 0.0772 |
| bta-miR-28 | 0.0047 ± 0.0014 | 0.0039 ± 0.0017 | . ± . |
| bta-miR-29a | 0.1277 ± 0.1156 | 0.1502 ± 0.1266 | . ± . |
| bta-miR-29b | 0.0215 ± 0.0137 | 0.0236 ± 0.0292 | 0.0027 ± 0.0023 |
| bta-miR-29c | 0.1176 ± 0.1196 | 0.1621 ± 0.1269 | 0.0027 ± 0.0008 |
| bta-miR-29d-3p | 0.0157 ± 0.0066 | 0.0176 ± 0.0181 | . ± . |
| bta-miR-29d-5p | 0.0012 ± 0.0014 | 0.0027 ± 0.0025 | 0.0027 ± 0.0012 |
| bta-miR-29e | 0.0059 ± 0.0033 | 0.0094 ± 0.0063 | . ± . |
| bta-miR-30a-5p | 0.0515 ± 0.0559 | 0.0891 ± 0.0726 | 0.0143 ± 0.0075 |
| bta-miR-30b-5p | 0.0627 ± 0.0710 | 0.0600 ± 0.0516 | 0.0468 ± 0.0234 |
| bta-miR-30c | 0.0508 ± 0.0584 | 0.0414 ± 0.0491 | 0.1560 ± 0.2161 |
| bta-miR-30d | 0.0606 ± 0.0541 | 0.0517 ± 0.0568 | 0.0136 ± 0.0089 |
| bta-miR-30e-5p | 0.0652 ± 0.0615 | 0.0634 ± 0.0689 | 0.0164 ± 0.0013 |
| bta-miR-30f | 0.0131 ± 0.0024 | 0.0037 ± 0.0030 | 0.0151 ± 0.0088 |
| bta-miR-33b | 0.0004 ± 0.0002 | 0.0002 ± 0.0000 | 0.0007 ± 0.0006 |
| bta-miR-34a | 0.0342 ± 0.0412 | 0.0146 ± 0.0034 | 0.0070 ± 0.0070 |
| bta-miR-34b | 0.3081 ± 0.3086 | 0.5956 ± 0.6895 | 0.0157 ± 0.0056 |
| bta-miR-34c | 0.3413 ± 0.3560 | 0.6209 ± 0.7448 | 0.0203 ± 0.0195 |
| bta-miR-92a | 0.0264 ± 0.0112 | 0.0332 ± 0.0128 | 0.0624 ± 0.0259 |
| bta-miR-92b | 0.0906 ± 0.0335 | 0.0621 ± 0.0093 | 9.8715 ± 2.7442 |
| bta-miR-93 | 0.6598 ± 0.5074 | 0.8192 ± 0.6432 | 0.0103 ± 0.0007 |
| bta-miR-95 | 0.0026 ± 0.0011 | 0.0041 ± 0.0043 | . ± . |
| bta-miR-99a-5p | 1.0856 ± 0.7910 | 1.7422 ± 1.0775 | 0.0043 ± 0.0037 |

| | | | |
|----------------|-------------------|------------------|-------------------|
| bta-miR-99b | 0.9146 ± 0.3110 | 0.7662 ± 0.2567 | 4.4615 ± 0.8911 |
| bta-miR-99b | 0.9223 ± 0.2811 | 0.8128 ± 0.2058 | 5.7071 ± 0.4555 |
| bta-miR-100 | 0.9619 ± 0.6599 | 1.2005 ± 0.9492 | 0.0136 ± 0.0020 |
| bta-miR-101 | 0.0882 ± 0.1035 | 0.1185 ± 0.1503 | 0.0057 ± 0.0034 |
| bta-miR-103 | 0.0598 ± 0.0553 | 0.0660 ± 0.0652 | 0.0065 ± 0.0047 |
| bta-miR-105a | 0.0014 ± 0.0015 | 0.0014 ± 0.0010 | 0.0075 ± 0.0089 |
| bta-miR-105b | 0.0034 ± 0.0011 | 0.0015 ± 0.0011 | 0.0137 ± 0.0083 |
| bta-miR-106a | 0.6168 ± 0.6309 | 0.5302 ± 0.4100 | 0.0466 ± 0.0238 |
| bta-miR-106b | 0.1893 ± 0.1984 | 0.1591 ± 0.1428 | 0.0124 ± 0.0061 |
| bta-miR-122 | 0.0064 ± 0.0040 | 0.0140 ± 0.0030 | 0.0044 ± 0.0033 |
| bta-miR-122 | 0.0079 ± 0.0052 | 0.0121 ± 0.0042 | 0.0036 ± 0.0031 |
| bta-miR-124a | 0.0002 ± 0.0001 | 0.0009 ± 0.0008 | 0.0065 ± 0.0030 |
| bta-miR-124b | 0.0009 ± 0.0006 | 0.0003 ± 0.0001 | 0.0073 ± 0.0037 |
| bta-miR-125a | 0.0045 ± 0.0027 | 0.0079 ± 0.0099 | 0.0117 ± 0.0169 |
| bta-miR-125b | 0.5888 ± 0.5419 | 0.7015 ± 0.8170 | 0.0044 ± 0.0007 |
| bta-miR-1260b | 39.7555 ± 33.8572 | 35.3772 ± 7.6421 | 22.6233 ± 17.8693 |
| bta-miR-126-3p | 0.0090 ± 0.0019 | 0.0062 ± 0.0026 | 0.0919 ± 0.1301 |
| bta-miR-126-5p | 0.0103 ± 0.0030 | 0.0063 ± 0.0008 | 0.0869 ± 0.1112 |
| bta-miR-127 | 0.0080 ± 0.0044 | 0.0045 ± 0.0010 | 0.0416 ± 0.0009 |
| bta-miR-128 | 0.0091 ± 0.0079 | 0.0087 ± 0.0104 | 0.0016 ± 0.0011 |
| bta-miR-1281 | 0.0342 ± 0.0186 | 0.0377 ± 0.0167 | 2.7083 ± 0.2929 |
| bta-miR-1287 | 0.0022 ± 0.0012 | 0.0008 ± 0.0005 | 0.0622 ± 0.0610 |
| bta-miR-129 | 0.0025 ± 0.0016 | 0.0040 ± 0.0027 | 0.0253 ± 0.0076 |
| bta-miR-129-5p | 0.0023 ± 0.0010 | 0.0035 ± 0.0029 | 0.0136 ± 0.0014 |
| bta-miR-130a | 0.0068 ± 0.0050 | 0.0036 ± 0.0009 | 0.0135 ± 0.0029 |
| bta-miR-130b | 0.0101 ± 0.0027 | 0.0079 ± 0.0030 | 0.0222 ± 0.0077 |
| bta-miR-132 | 0.1163 ± 0.0001 | 0.1441 ± 0.0320 | 0.1328 ± 0.2099 |
| bta-miR-133a | 0.0012 ± 0.0008 | 0.0013 ± 0.0013 | 0.1335 ± 0.1083 |
| bta-miR-134 | 0.0033 ± 0.0003 | 0.0024 ± 0.0012 | 0.1272 ± 0.2080 |
| bta-miR-135a | 0.0134 ± 0.0061 | 0.0220 ± 0.0126 | . ± . |
| bta-miR-135b | 0.0062 ± 0.0045 | 0.0147 ± 0.0144 | 0.0003 ± 0.0000 |

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|-----------------|-----------------|-----------------|--------|---|--------|
| bta-miR-136 | 0.0030 ± 0.0029 | 0.0005 ± 0.0000 | . | ± | . |
| bta-miR-138 | 0.0119 ± 0.0042 | 0.0090 ± 0.0041 | 0.2403 | ± | 0.2825 |
| bta-miR-139 | 0.0093 ± 0.0071 | 0.0043 ± 0.0031 | 0.0222 | ± | 0.0208 |
| bta-miR-140 | 0.0084 ± 0.0109 | 0.0072 ± 0.0100 | 0.0238 | ± | 0.0370 |
| bta-miR-141 | 0.0048 ± 0.0065 | 0.0065 ± 0.0067 | 0.0392 | ± | 0.0361 |
| bta-miR-143 | 0.0697 ± 0.0383 | 0.0843 ± 0.0201 | 0.0253 | ± | 0.0310 |
| bta-miR-148b | 0.0292 ± 0.0384 | 0.0332 ± 0.0408 | 0.0658 | ± | 0.0205 |
| bta-miR-149-3p | 0.5544 ± 0.1471 | 0.5893 ± 0.4360 | 0.3350 | ± | 0.0541 |
| bta-miR-150 | 0.0068 ± 0.0009 | 0.0063 ± 0.0059 | 0.0364 | ± | 0.0450 |
| bta-miR-151-3p | 0.0404 ± 0.0098 | 0.0342 ± 0.0054 | 0.0288 | ± | 0.0273 |
| bta-miR-151-5p | 0.2233 ± 0.1935 | 0.3127 ± 0.2565 | 0.0280 | ± | 0.0040 |
| bta-miR-152 | 0.0021 ± 0.0012 | 0.0027 ± 0.0003 | 0.0572 | ± | 0.0963 |
| bta-miR-153 | 0.0075 ± 0.0101 | 0.0091 ± 0.0098 | 0.0094 | ± | 0.0068 |
| bta-miR-154a | 0.0008 ± 0.0008 | 0.0008 ± 0.0001 | 0.0605 | ± | 0.0887 |
| bta-miR-154b | 0.0355 ± 0.0122 | 0.0224 ± 0.0081 | 0.2430 | ± | 0.3430 |
| bta-miR-181a | 0.0010 ± 0.0008 | 0.0026 ± 0.0023 | 0.0006 | ± | 0.0002 |
| bta-miR-181b | 0.0012 ± 0.0005 | 0.0022 ± 0.0027 | 0.0078 | ± | 0.0023 |
| bta-miR-181d | 0.0010 ± 0.0010 | 0.0013 ± 0.0015 | 0.0344 | ± | 0.0446 |
| bta-miR-184 | 0.0016 ± 0.0010 | 0.0008 ± 0.0008 | . | ± | . |
| bta-miR-185 | 0.0008 ± 0.0004 | 0.0029 ± 0.0031 | 0.0050 | ± | 0.0022 |
| bta-miR-186 | 0.0385 ± 0.0329 | 0.0441 ± 0.0437 | 0.0322 | ± | 0.0373 |
| bta-miR-187 | 0.0095 ± 0.0028 | 0.0061 ± 0.0017 | 0.0954 | ± | 0.0535 |
| bta-miR-188 | 0.0128 ± 0.0056 | 0.0122 ± 0.0043 | 0.0277 | ± | 0.0103 |
| bta-miR-190a | 0.0010 ± 0.0001 | 0.0014 ± 0.0006 | 0.0080 | ± | 0.0085 |
| bta-miR-190b | 0.0326 ± 0.0126 | 0.0361 ± 0.0072 | 0.0058 | ± | 0.0023 |
| bta-miR-191 | 0.0526 ± 0.0461 | 0.0566 ± 0.0330 | 0.0654 | ± | 0.0763 |
| bta-miR-192 | 0.0160 ± 0.0122 | 0.0180 ± 0.0156 | 0.0069 | ± | 0.0010 |
| bta-miR-193a-3p | 0.0006 ± 0.0004 | 0.0008 ± 0.0004 | 0.0029 | ± | 0.0002 |
| bta-miR-193a-5p | 0.0027 ± 0.0020 | 0.0026 ± 0.0028 | 0.0102 | ± | 0.0014 |
| bta-miR-193b | 0.0004 ± 0.0001 | 0.0004 ± 0.0004 | 0.0020 | ± | 0.0020 |
| bta-miR-194 | 0.0202 ± 0.0093 | 0.0463 ± 0.0416 | 0.0073 | ± | 0.0051 |

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|-----------------|-------------------|--------------------|--------------------|
| bta-miR-196a | 0.0067 ± 0.0020 | 0.0099 ± 0.0053 | 0.0633 ± 0.0871 |
| bta-miR-196b | 0.0040 ± 0.0046 | 0.0033 ± 0.0013 | 0.0054 ± 0.0083 |
| bta-miR-199a-3p | 0.0010 ± 0.0003 | 0.0015 ± 0.0010 | . ± . |
| bta-miR-199c | 0.0023 ± 0.0006 | 0.0037 ± 0.0022 | 0.0487 ± 0.0740 |
| bta-miR-200a | 0.0550 ± 0.0092 | 0.0607 ± 0.0690 | . ± . |
| bta-miR-200b | 0.2199 ± 0.1115 | 0.3415 ± 0.0697 | 0.0109 ± 0.0144 |
| bta-miR-200c | 0.0097 ± 0.0051 | 0.0141 ± 0.0025 | 0.0119 ± 0.0172 |
| bta-miR-204 | 0.2858 ± 0.2761 | 0.3263 ± 0.2577 | 0.0809 ± 0.1048 |
| bta-miR-205 | 0.0018 ± 0.0002 | 0.0006 ± 0.0002 | 0.0512 ± 0.0420 |
| bta-miR-206 | 0.0021 ± 0.0016 | 0.0014 ± 0.0008 | 0.0838 ± 0.1251 |
| bta-miR-210 | 0.0135 ± 0.0061 | 0.0119 ± 0.0082 | 0.0353 ± 0.0061 |
| bta-miR-211 | 0.2784 ± 0.2428 | 0.2881 ± 0.2613 | 0.7258 ± 1.1919 |
| bta-miR-214 | 0.0033 ± 0.0005 | 0.0059 ± 0.0020 | 0.0202 ± 0.0012 |
| bta-miR-215 | 0.0084 ± 0.0054 | 0.0107 ± 0.0087 | 0.0050 ± 0.0030 |
| bta-miR-216a | 0.0017 ± 0.0018 | 0.0047 ± 0.0024 | 0.0256 ± 0.0060 |
| bta-miR-216b | 0.0100 ± 0.0045 | 0.0166 ± 0.0022 | 0.0016 ± 0.0014 |
| bta-miR-217 | 0.0003 ± 0.0001 | 0.0004 ± 0.0003 | . ± . |
| bta-miR-219-3p | 0.0015 ± 0.0014 | 0.0008 ± 0.0004 | 0.0245 ± 0.0300 |
| bta-miR-222 | 0.0009 ± 0.0005 | 0.0009 ± 0.0007 | 0.0355 ± 0.0280 |
| bta-miR-296-3p | 0.0088 ± 0.0074 | 0.0075 ± 0.0020 | 0.1602 ± 0.1695 |
| bta-miR-296-5p | 0.0358 ± 0.0255 | 0.0362 ± 0.0210 | 1.4022 ± 1.0605 |
| bta-miR-302c | 0.0022 ± 0.0017 | 0.0006 ± 0.0002 | 0.0214 ± 0.0358 |
| bta-miR-320a | 0.0427 ± 0.0173 | 0.0325 ± 0.0064 | 0.0776 ± 0.0262 |
| bta-miR-320b | 0.0052 ± 0.0015 | 0.0058 ± 0.0015 | 0.0178 ± 0.0134 |
| bta-miR-323 | 133.069 ± 68.9476 | 101.8617 ± 48.3551 | 535.5672 ± 82.9623 |
| bta-miR-324 | 0.0065 ± 0.0045 | 0.0062 ± 0.0026 | 0.0116 ± 0.0124 |
| bta-miR-326 | 0.0124 ± 0.0061 | 0.0154 ± 0.0118 | 0.0669 ± 0.0070 |
| bta-miR-328 | 0.0076 ± 0.0037 | 0.0088 ± 0.0068 | 0.0182 ± 0.0136 |
| bta-miR-330 | 0.0050 ± 0.0053 | 0.0040 ± 0.0030 | 0.0194 ± 0.0080 |
| bta-miR-331-5p | 0.0026 ± 0.0014 | 0.0020 ± 0.0008 | 0.0272 ± 0.0165 |
| bta-miR-335 | 0.0520 ± 0.0597 | 0.0476 ± 0.0568 | . ± . |

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| bta-miR-338 | 0.0017 ± 0.0000 | 0.0055 ± 0.0049 | 0.0092 ± 0.0055 |
| bta-miR-339a | 0.0082 ± 0.0030 | 0.0142 ± 0.0039 | 0.0796 ± 0.0611 |
| bta-miR-339b | 0.0110 ± 0.0035 | 0.0135 ± 0.0120 | 0.2950 ± 0.3178 |
| bta-miR-345-3p | 0.0021 ± 0.0015 | 0.0024 ± 0.0010 | 0.0209 ± 0.0037 |
| bta-miR-345-5p | 0.0028 ± 0.0017 | 0.0042 ± 0.0020 | 0.0200 ± 0.0108 |
| bta-miR-346 | 0.0240 ± 0.0121 | 0.0217 ± 0.0147 | 0.3168 ± 0.0560 |
| bta-miR-363 | 0.0024 ± 0.0024 | 0.0033 ± 0.0034 | 0.0140 ± 0.0041 |
| bta-miR-365-5p | 0.0034 ± 0.0002 | 0.0057 ± 0.0012 | 0.0174 ± 0.0041 |
| bta-miR-369-3p | 0.0034 ± 0.0024 | 0.0020 ± 0.0014 | . ± . |
| bta-miR-370 | 0.0033 ± 0.0027 | 0.0031 ± 0.0010 | 0.0255 ± 0.0075 |
| bta-miR-374a | 0.0017 ± 0.0012 | 0.0021 ± 0.0001 | . ± . |
| bta-miR-374b | 0.0022 ± 0.0009 | 0.0041 ± 0.0008 | . ± . |
| bta-miR-375 | 0.2240 ± 0.0873 | 0.2036 ± 0.0566 | 0.0753 ± 0.1085 |
| bta-miR-378 | 0.0024 ± 0.0022 | 0.0025 ± 0.0014 | 0.1925 ± 0.1854 |
| bta-miR-378b | 0.0016 ± 0.0008 | 0.0015 ± 0.0009 | 0.1624 ± 0.1365 |
| bta-miR-378d | 0.0023 ± 0.0025 | 0.0013 ± 0.0003 | 0.2972 ± 0.2940 |
| bta-miR-380-5p | 0.0214 ± 0.0056 | 0.0099 ± 0.0087 | 0.2800 ± 0.2844 |
| bta-miR-381 | 0.0043 ± 0.0031 | 0.0023 ± 0.0028 | 0.0056 ± 0.0016 |
| bta-miR-382 | 0.0304 ± 0.0095 | 0.0207 ± 0.0184 | 0.1174 ± 0.0504 |
| bta-miR-383 | 0.0022 ± 0.0006 | 0.0014 ± 0.0008 | 0.0198 ± 0.0199 |
| bta-miR-409a | 0.0045 ± 0.0072 | 0.0006 ± 0.0002 | . ± . |
| bta-miR-410 | 0.0004 ± 0.0001 | 0.0004 ± 0.0001 | 0.0049 ± 0.0044 |
| bta-miR-411a | 0.0036 ± 0.0018 | 0.0022 ± 0.0017 | 0.0403 ± 0.0310 |
| bta-miR-421 | 0.0070 ± 0.0064 | 0.0067 ± 0.0021 | 0.1600 ± 0.1417 |
| bta-miR-423-3p | 0.0029 ± 0.0023 | 0.0036 ± 0.0030 | 0.0216 ± 0.0044 |
| bta-miR-423-5p | 0.0100 ± 0.0034 | 0.0126 ± 0.0044 | 0.0031 ± 0.0010 |
| bta-miR-425-5p | 1.1448 ± 0.3236 | 1.2706 ± 0.2768 | 0.0051 ± 0.0006 |
| bta-miR-429 | 0.0144 ± 0.0073 | 0.0109 ± 0.0036 | 0.0388 ± 0.0065 |
| bta-miR-431 | 0.0026 ± 0.0014 | 0.0027 ± 0.0019 | 0.0149 ± 0.0038 |
| bta-miR-432 | 0.0029 ± 0.0015 | 0.0008 ± 0.0008 | 0.0796 ± 0.0897 |
| bta-miR-433 | 0.0099 ± 0.0026 | 0.0081 ± 0.0053 | 0.1321 ± 0.0795 |

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| bta-miR-449a | 0.3221 ± 0.3608 | 0.2782 ± 0.3492 | 0.0034 ± 0.0012 |
| bta-miR-449b | 0.0206 ± 0.0301 | 0.0301 ± 0.0446 | 0.0101 ± 0.0078 |
| bta-miR-449d | 0.0132 ± 0.0058 | 0.0108 ± 0.0074 | 0.0335 ± 0.0128 |
| bta-miR-452 | 0.0013 ± 0.0012 | 0.0002 ± 0.0000 | 0.0328 ± 0.0395 |
| bta-miR-453 | 0.0069 ± 0.0001 | 0.0039 ± 0.0005 | 0.0046 ± 0.0035 |
| bta-miR-484 | 0.0007 ± 0.0007 | 0.0012 ± 0.0010 | 0.0332 ± 0.0101 |
| bta-miR-485 | 0.0005 ± 0.0002 | 0.0004 ± 0.0004 | 0.0082 ± 0.0103 |
| bta-miR-486 | 0.0694 ± 0.0216 | 0.0555 ± 0.0103 | 0.1009 ± 0.0403 |
| bta-miR-487b | 0.0012 ± 0.0008 | 0.0006 ± 0.0006 | 0.0027 ± 0.0023 |
| bta-miR-489 | 0.0016 ± 0.0011 | 0.0010 ± 0.0004 | 0.0482 ± 0.0301 |
| bta-miR-490 | 0.0039 ± 0.0007 | 0.0047 ± 0.0028 | 0.0339 ± 0.0150 |
| bta-miR-491 | 0.0012 ± 0.0007 | 0.0029 ± 0.0037 | 0.0221 ± 0.0168 |
| bta-miR-493 | 0.0106 ± 0.0053 | 0.0105 ± 0.0037 | 0.0527 ± 0.0298 |
| bta-miR-494 | 1.3068 ± 0.7157 | 2.2816 ± 1.0446 | 10.5780 ± 3.0809 |
| bta-miR-497 | 0.0097 ± 0.0084 | 0.0113 ± 0.0112 | 0.0055 ± 0.0041 |
| bta-miR-500 | 0.0011 ± 0.0002 | 0.0008 ± 0.0001 | 0.1472 ± 0.0597 |
| bta-miR-503-3p | 0.0199 ± 0.0085 | 0.0115 ± 0.0028 | 0.2895 ± 0.1169 |
| bta-miR-504 | 0.0027 ± 0.0024 | 0.0033 ± 0.0008 | 0.0994 ± 0.0753 |
| bta-miR-505 | 0.0033 ± 0.0001 | 0.0009 ± 0.0003 | 0.0320 ± 0.0253 |
| bta-miR-532 | 0.0139 ± 0.0207 | 0.0002 ± 0.0002 | 0.0095 ± 0.0027 |
| bta-miR-541 | 0.0150 ± 0.0027 | 0.0141 ± 0.0050 | 0.1668 ± 0.0058 |
| bta-miR-542-5p | 0.0110 ± 0.0059 | 0.0034 ± 0.0017 | 0.0005 ± 0.0002 |
| bta-miR-545-5p | 0.0024 ± 0.0012 | 0.0007 ± 0.0003 | . ± . |
| bta-miR-574 | 0.1758 ± 0.0904 | 0.1467 ± 0.0745 | 1.8761 ± 0.5947 |
| bta-miR-584 | 0.0031 ± 0.0017 | 0.0031 ± 0.0017 | 0.0298 ± 0.0138 |
| bta-miR-631 | 29.1166 ± 16.7781 | 13.9559 ± 3.3240 | 86.5707 ± 17.6398 |
| bta-miR-654 | 0.0025 ± 0.0002 | 0.0026 ± 0.0037 | 0.0218 ± 0.0137 |
| bta-miR-656 | 0.0030 ± 0.0006 | 0.0059 ± 0.0075 | 0.0232 ± 0.0123 |
| bta-miR-658 | 0.0019 ± 0.0018 | 0.0011 ± 0.0007 | 0.0024 ± 0.0009 |
| bta-miR-660 | 0.0076 ± 0.0010 | 0.0089 ± 0.0055 | 0.0347 ± 0.0334 |
| bta-miR-664a | 0.0113 ± 0.0042 | 0.0186 ± 0.0155 | 0.0312 ± 0.0099 |

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|-----------------|-----------------|-----------------|-------------------|
| bta-miR-664b | 0.0277 ± 0.0202 | 0.0274 ± 0.0151 | 0.0520 ± 0.0315 |
| bta-miR-665 | 0.0852 ± 0.0075 | 0.0491 ± 0.0148 | 1.8669 ± 0.5909 |
| bta-miR-669 | 0.0136 ± 0.0064 | 0.0152 ± 0.0086 | 0.2193 ± 0.0717 |
| bta-miR-677 | 0.0036 ± 0.0029 | 0.0011 ± 0.0002 | 0.0120 ± 0.0074 |
| bta-miR-708 | 0.0009 ± 0.0002 | 0.0007 ± 0.0004 | 0.0137 ± 0.0096 |
| bta-miR-744 | 0.0005 ± 0.0001 | 0.0011 ± 0.0007 | 0.0034 ± 0.0011 |
| bta-miR-760-3p | 0.0084 ± 0.0033 | 0.0064 ± 0.0049 | 0.0605 ± 0.0178 |
| bta-miR-760-5p | 0.1440 ± 0.1134 | 0.2701 ± 0.2594 | 0.2879 ± 0.0473 |
| bta-miR-761 | 0.0017 ± 0.0005 | 0.0017 ± 0.0015 | 0.0019 ± 0.0020 |
| bta-miR-763 | 0.0047 ± 0.0032 | 0.0170 ± 0.0118 | 0.0537 ± 0.0226 |
| bta-miR-764 | 0.0296 ± 0.0388 | 0.0177 ± 0.0243 | 0.0095 ± 0.0053 |
| bta-miR-767 | 0.0068 ± 0.0045 | 0.0056 ± 0.0016 | 0.0156 ± 0.0003 |
| bta-miR-769 | 0.0010 ± 0.0003 | 0.0007 ± 0.0006 | 0.0265 ± 0.0138 |
| bta-miR-873 | 0.0009 ± 0.0012 | 0.0003 ± 0.0003 | 0.0258 ± 0.0200 |
| bta-miR-877 | 0.0348 ± 0.0057 | 0.0213 ± 0.0024 | 0.1232 ± 0.0367 |
| bta-miR-885 | 0.0025 ± 0.0026 | 0.0031 ± 0.0020 | 0.0446 ± 0.0129 |
| bta-miR-935 | 0.0072 ± 0.0025 | 0.0080 ± 0.0044 | 0.2452 ± 0.0586 |
| bta-miR-940 | 0.9009 ± 0.3382 | 0.7745 ± 0.1527 | 32.4275 ± 12.2599 |
| bta-miR-1224 | 3.2663 ± 1.3077 | 3.1231 ± 0.5453 | 2.3409 ± 0.6572 |
| bta-miR-1246 | 0.9191 ± 0.4005 | 1.2088 ± 0.4008 | 12.6860 ± 4.3288 |
| bta-miR-1247-3p | 0.0213 ± 0.0070 | 0.0135 ± 0.0013 | 0.2544 ± 0.1685 |
| bta-miR-1247-5p | 0.3421 ± 0.1552 | 0.3060 ± 0.1354 | 0.4988 ± 0.2081 |
| bta-miR-1249 | 0.0018 ± 0.0013 | 0.0014 ± 0.0012 | 0.0401 ± 0.0039 |
| bta-miR-1291 | 0.0005 ± 0.0003 | 0.0014 ± 0.0008 | 0.0118 ± 0.0070 |
| bta-miR-1296 | 0.0181 ± 0.0065 | 0.0193 ± 0.0015 | . ± . |
| bta-miR-1306 | 0.0258 ± 0.0200 | 0.0246 ± 0.0120 | 0.3889 ± 0.3027 |
| bta-miR-1307 | 0.0654 ± 0.0223 | 0.1022 ± 0.0375 | 0.7877 ± 0.2135 |
| bta-miR-1343-3p | 0.0224 ± 0.0115 | 0.0252 ± 0.0090 | 0.3794 ± 0.0953 |
| bta-miR-1343-5p | 1.2755 ± 0.6539 | 0.9952 ± 0.1259 | 0.4247 ± 0.1212 |
| bta-miR-1388-3p | 0.0090 ± 0.0056 | 0.0060 ± 0.0019 | 0.1593 ± 0.0131 |
| bta-miR-4523 | 0.0113 ± 0.0053 | 0.0088 ± 0.0035 | 0.0115 ± 0.0047 |

| | | | |
|-----------------|-----------------|-----------------|-------------------|
| Hm/Ms/RtT1snRNA | 0.9175 ± 0.2472 | 1.6149 ± 0.5084 | 53.6440 ± 17.0054 |
| RNT43 snoRNA | 0.0041 ± 0.0025 | 0.0045 ± 0.0006 | 0.1462 ± 0.0139 |

Exclusively detected in HF group of sperm cells

| | | | |
|-----------------|-----------------|-------|------------------|
| bta-miR-27a-5p | 0.0010 ± 0.0005 | . ± . | 0.0035 ± 0.0021 |
| bta-miR-31 | 0.0022 ± 0.0003 | . ± . | 0.0146 ± 0.0072 |
| bta-miR-32 | 0.0006 ± 0.0005 | . ± . | . ± . |
| bta-miR-133b | 0.0004 ± 0.0003 | . ± . | 0.0074 ± 0.0071 |
| bta-miR-142-5p | 0.0009 ± 0.0009 | . ± . | . ± . |
| bta-miR-145 | 0.0008 ± 0.0001 | . ± . | 0.1698 ± 0.1044 |
| bta-miR-146a | 0.0142 ± 0.0129 | . ± . | 0.0051 ± 0.0037 |
| bta-miR-147 | 0.0028 ± 0.0011 | . ± . | 0.0008 ± 0.0009 |
| bta-miR-149-5p | 0.0011 ± 0.0008 | . ± . | 1.5298 ± 1.5311 |
| bta-miR-154c | 0.0012 ± 0.0005 | . ± . | 0.0114 ± 0.0187 |
| bta-miR-212 | 0.0017 ± 0.0000 | . ± . | 0.0071 ± 0.0088 |
| bta-miR-219 | 0.0027 ± 0.0011 | . ± . | 0.0703 ± 0.0348 |
| bta-miR-301b | 0.0002 ± 0.0002 | . ± . | . ± . |
| bta-miR-302b | 0.0007 ± 0.0004 | . ± . | . ± . |
| bta-miR-331-3p | 0.0009 ± 0.0001 | . ± . | . ± . |
| bta-miR-380-3p | 0.0013 ± 0.0001 | . ± . | 0.0206 ± 0.0159 |
| bta-miR-411b | 0.0003 ± 0.0002 | . ± . | 0.0041 ± 0.0028 |
| bta-miR-425-3p | 0.0827 ± 0.0364 | . ± . | 14.1388 ± 5.8179 |
| bta-miR-495 | 0.0022 ± 0.0009 | . ± . | . ± . |
| bta-miR-502b | 0.0023 ± 0.0001 | . ± . | 0.0378 ± 0.0310 |
| bta-miR-545-3p | 0.0012 ± 0.0004 | . ± . | . ± . |
| bta-miR-655 | 0.0004 ± 0.0003 | . ± . | 0.0054 ± 0.0044 |
| bta-miR-874 | 0.0227 ± 0.0104 | . ± . | 0.0836 ± 0.0124 |
| bta-miR-1225-3p | 0.3219 ± 0.1637 | . ± . | 26.8475 ± 0.9828 |

Exclusively detected in LF group of sperm cells

| | | | |
|---------------|-------|-----------------|-----------------|
| bta-let-7b | . ± . | 0.0878 ± 0.1248 | 0.0021 ± 0.0016 |
| bta-miR-17-3p | . ± . | 0.0016 ± 0.0012 | 0.0060 ± 0.0038 |
| bta-miR-21-3p | . ± . | 0.0001 ± 0.0000 | 0.0051 ± 0.0020 |

| | | | | | | | | | |
|----------------|---|---|---|--------|---|--------|--------|---|--------|
| bta-miR-107 | . | ± | . | 0.0006 | ± | 0.0001 | 0.1329 | ± | 0.1836 |
| bta-miR-129-3p | . | ± | . | 0.0005 | ± | 0.0002 | 0.0038 | ± | 0.0020 |
| bta-miR-137 | . | ± | . | 0.0027 | ± | 0.0015 | 0.0011 | ± | 0.0002 |
| bta-miR-146b | . | ± | . | 0.0066 | ± | 0.0039 | 0.0080 | ± | 0.0064 |
| bta-miR-148a | . | ± | . | 0.0271 | ± | 0.0317 | 0.1130 | ± | 0.0373 |
| bta-miR-181c | . | ± | . | 0.0017 | ± | 0.0006 | . | ± | . |
| bta-miR-195 | . | ± | . | 0.7969 | ± | 0.7520 | 0.0367 | ± | 0.0193 |
| bta-miR-301a | . | ± | . | 0.0003 | ± | 0.0003 | . | ± | . |
| bta-miR-302a | . | ± | . | 0.0017 | ± | 0.0020 | . | ± | . |
| bta-miR-361 | . | ± | . | 0.0002 | ± | 0.0001 | 0.0065 | ± | 0.0043 |
| bta-miR-362-3p | . | ± | . | 0.0013 | ± | 0.0012 | 0.0144 | ± | 0.0157 |
| bta-miR-362-5p | . | ± | . | 0.0003 | ± | 0.0003 | 0.0025 | ± | 0.0010 |
| bta-miR-371 | . | ± | . | 0.0005 | ± | 0.0005 | 0.0018 | ± | 0.0013 |
| bta-miR-376a | . | ± | . | 0.0003 | ± | 0.0001 | . | ± | . |
| bta-miR-377 | . | ± | . | 0.0010 | ± | 0.0006 | 0.0007 | ± | 0.0005 |
| bta-miR-378c | . | ± | . | 0.0013 | ± | 0.0009 | 0.0020 | ± | 0.0020 |
| bta-miR-379 | . | ± | . | 0.0007 | ± | 0.0004 | 0.0023 | ± | 0.0011 |
| bta-miR-424-3p | . | ± | . | 0.0007 | ± | 0.0003 | 0.0135 | ± | 0.0046 |
| bta-miR-424-5p | . | ± | . | 0.0010 | ± | 0.0012 | 0.0239 | ± | 0.0151 |
| bta-miR-448 | . | ± | . | 0.0001 | ± | 0.0000 | 0.0028 | ± | 0.0008 |
| bta-miR-455-5p | . | ± | . | 0.0011 | ± | 0.0013 | 0.0716 | ± | 0.0709 |
| bta-miR-483 | . | ± | . | 0.0010 | ± | 0.0008 | 0.0061 | ± | 0.0033 |
| bta-miR-488 | . | ± | . | 0.0008 | ± | 0.0004 | 0.1997 | ± | 0.1782 |
| bta-miR-499 | . | ± | . | 0.0007 | ± | 0.0005 | . | ± | . |
| bta-miR-502a | . | ± | . | 0.0005 | ± | 0.0003 | 0.0033 | ± | 0.0031 |
| bta-miR-503-5p | . | ± | . | 0.0004 | ± | 0.0002 | 0.0414 | ± | 0.0183 |
| bta-miR-543 | . | ± | . | 0.0021 | ± | 0.0001 | 0.0046 | ± | 0.0038 |
| bta-miR-670 | . | ± | . | 0.0012 | ± | 0.0006 | . | ± | . |
| bta-miR-671 | . | ± | . | 0.0005 | ± | 0.0000 | 0.0005 | ± | 0.0002 |

Not detected in either group (HF and LF) of sperm cells

| | | | | | | | | | |
|-----------|---|---|---|---|---|---|---|---|---|
| bta-miR-1 | . | ± | . | . | ± | . | . | ± | . |
|-----------|---|---|---|---|---|---|---|---|---|

| | | | | | | | | | |
|-----------------|---|---|---|---|---|---|--------|---|--------|
| bta-miR-9-3p | . | ± | . | . | ± | . | . | ± | . |
| bta-miR-22-3p | . | ± | . | . | ± | . | . | ± | . |
| bta-miR-23b-5p | . | ± | . | . | ± | . | 0.0016 | ± | 0.0009 |
| bta-miR-24 | . | ± | . | . | ± | . | . | ± | . |
| bta-miR-26c | . | ± | . | . | ± | . | . | ± | . |
| bta-miR-30b-3p | . | ± | . | . | ± | . | . | ± | . |
| bta-miR-33a | . | ± | . | . | ± | . | 0.0021 | ± | 0.0002 |
| bta-miR-96 | . | ± | . | . | ± | . | . | ± | . |
| bta-miR-98 | . | ± | . | . | ± | . | . | ± | . |
| bta-miR-99a-3p | . | ± | . | . | ± | . | 0.0049 | ± | 0.0021 |
| bta-miR-133c | . | ± | . | . | ± | . | 0.0857 | ± | 0.1468 |
| bta-miR-142-3p | . | ± | . | . | ± | . | . | ± | . |
| bta-miR-144 | . | ± | . | . | ± | . | . | ± | . |
| bta-miR-155 | . | ± | . | . | ± | . | 0.0170 | ± | 0.0238 |
| bta-miR-182 | . | ± | . | . | ± | . | 0.0238 | ± | 0.0328 |
| bta-miR-183 | . | ± | . | . | ± | . | 0.0519 | ± | 0.0370 |
| bta-miR-193a | . | ± | . | . | ± | . | 0.0006 | ± | 0.0004 |
| bta-miR-197 | . | ± | . | . | ± | . | 0.4294 | ± | 0.5134 |
| bta-miR-199a-5p | . | ± | . | . | ± | . | 0.0009 | ± | 0.0007 |
| bta-miR-199b | . | ± | . | . | ± | . | 0.0319 | ± | 0.0304 |
| bta-miR-202 | . | ± | . | . | ± | . | 0.0033 | ± | 0.0014 |
| bta-miR-208a | . | ± | . | . | ± | . | . | ± | . |
| bta-miR-208b | . | ± | . | . | ± | . | . | ± | . |
| bta-miR-219-5p | . | ± | . | . | ± | . | . | ± | . |
| bta-miR-221 | . | ± | . | . | ± | . | 0.0199 | ± | 0.0074 |
| bta-miR-223 | . | ± | . | . | ± | . | 0.0089 | ± | 0.0064 |
| bta-miR-224 | . | ± | . | . | ± | . | . | ± | . |
| bta-miR-299 | . | ± | . | . | ± | . | . | ± | . |
| bta-miR-302d | . | ± | . | . | ± | . | 0.0025 | ± | 0.0006 |
| bta-miR-329a | . | ± | . | . | ± | . | . | ± | . |
| bta-miR-329b | . | ± | . | . | ± | . | . | ± | . |

| | | | | | | | | | |
|-----------------|---|---|---|---|---|---|--------|---|--------|
| bta-miR-340 | . | ± | . | . | ± | . | 0.1876 | ± | 0.2809 |
| bta-miR-342 | . | ± | . | . | ± | . | 0.0220 | ± | 0.0215 |
| bta-miR-365-3p | . | ± | . | . | ± | . | . | ± | . |
| bta-miR-367 | . | ± | . | . | ± | . | . | ± | . |
| bta-miR-369-5p | . | ± | . | . | ± | . | 0.0388 | ± | 0.0515 |
| bta-miR-376b | . | ± | . | . | ± | . | . | ± | . |
| bta-miR-376c | . | ± | . | . | ± | . | . | ± | . |
| bta-miR-376d | . | ± | . | . | ± | . | . | ± | . |
| bta-miR-376e | . | ± | . | . | ± | . | . | ± | . |
| bta-miR-409b | . | ± | . | . | ± | . | 0.0360 | ± | 0.0278 |
| bta-miR-411c-3p | . | ± | . | . | ± | . | . | ± | . |
| bta-miR-411c-5p | . | ± | . | . | ± | . | . | ± | . |
| bta-miR-412 | . | ± | . | . | ± | . | 0.0023 | ± | 0.0015 |
| bta-miR-449c | . | ± | . | . | ± | . | 0.0181 | ± | 0.0242 |
| bta-miR-450a | . | ± | . | . | ± | . | . | ± | . |
| bta-miR-450b | . | ± | . | . | ± | . | . | ± | . |
| bta-miR-451 | . | ± | . | . | ± | . | . | ± | . |
| bta-miR-454 | . | ± | . | . | ± | . | 0.0457 | ± | 0.0539 |
| bta-miR-455-3p | . | ± | . | . | ± | . | 0.0085 | ± | 0.0061 |
| bta-miR-487a | . | ± | . | . | ± | . | 0.0021 | ± | 0.0008 |
| bta-miR-496 | . | ± | . | . | ± | . | 0.0978 | ± | 0.0761 |
| bta-miR-539 | . | ± | . | . | ± | . | 0.0014 | ± | 0.0016 |
| bta-miR-544a | . | ± | . | . | ± | . | 0.0100 | ± | 0.0129 |
| bta-miR-544b | . | ± | . | . | ± | . | 0.0132 | ± | 0.0056 |
| bta-miR-551a | . | ± | . | . | ± | . | 0.0043 | ± | 0.0038 |
| bta-miR-551b | . | ± | . | . | ± | . | . | ± | . |
| bta-miR-562 | . | ± | . | . | ± | . | 0.0042 | ± | 0.0010 |
| bta-miR-568 | . | ± | . | . | ± | . | 0.0020 | ± | 0.0017 |
| bta-miR-582 | . | ± | . | . | ± | . | 0.1427 | ± | 0.0503 |
| bta-miR-592 | . | ± | . | . | ± | . | 0.0020 | ± | 0.0002 |
| bta-miR-599 | . | ± | . | . | ± | . | . | ± | . |

| | | | | | | | | | |
|--------------|---|---|---|---|---|---|------------|---|-----------|
| bta-miR-615 | . | ± | . | . | ± | . | 40790.7953 | ± | 2884.1670 |
| bta-miR-628 | . | ± | . | . | ± | . | 0.0363 | ± | 0.0567 |
| bta-miR-652 | . | ± | . | . | ± | . | 0.0110 | ± | 0.0048 |
| bta-miR-653 | . | ± | . | . | ± | . | . | ± | . |
| bta-miR-758 | . | ± | . | . | ± | . | 0.1031 | ± | 0.0769 |
| bta-miR-759 | . | ± | . | . | ± | . | . | ± | . |
| bta-miR-875 | . | ± | . | . | ± | . | 0.0028 | ± | 0.0014 |
| bta-miR-876 | . | ± | . | . | ± | . | 0.4186 | ± | 0.3493 |
| bta-miR-1179 | . | ± | . | . | ± | . | 0.0090 | ± | 0.0124 |
| bta-miR-1185 | . | ± | . | . | ± | . | . | ± | . |
| bta-miR-1193 | . | ± | . | . | ± | . | 0.0185 | ± | 0.0213 |
| bta-miR-1197 | . | ± | . | . | ± | . | . | ± | . |
| bta-miR-1248 | . | ± | . | . | ± | . | 0.0205 | ± | 0.0186 |
| bta-miR-1271 | . | ± | . | . | ± | . | . | ± | . |
| bta-miR-1277 | . | ± | . | . | ± | . | . | ± | . |
| bta-miR-1282 | . | ± | . | . | ± | . | 0.0031 | ± | 0.0026 |
| bta-miR-1284 | . | ± | . | . | ± | . | 0.0202 | ± | 0.0191 |
| bta-miR-1298 | . | ± | . | . | ± | . | 0.0032 | ± | 0.0022 |
| bta-miR-1301 | . | ± | . | . | ± | . | 0.0099 | ± | 0.0094 |
| bta-miR-3064 | . | ± | . | . | ± | . | 0.0025 | ± | 0.0006 |

Supplementary Table S6. Mean and SEM of relative levels of the nine miRNAs investigated in zygotes from high fertility (HF), low fertility (LF) and parthenogenesis (PA) groups as well as mature oocytes (OO). ¹High fertility; ²Low fertility; ³Parthenogenetic; ⁴Mature oocytes. SEM: standard error of the mean.

| Zygotes | HF ¹ (n=9) | LF ² (n=9) | PA ³ (n=9) | OO ⁴ (n=3) |
|----------------|----------------------------|----------------------------|----------------------------|----------------------------|
| bta-miR-33b | 0.001±0.0001 | 0.002±0.0009 | 0.001±0.0002 | 0.001±0.0004 |
| bta-miR-126-5p | 0.0008±0.0001 | 0.002±0.001 | 0.001±0.0006 | 0.0004±0.0001 |
| bta-miR-205 | 0.027±0.007 | 0.06±0.03 | 0.02±0.003 | 0.02±0.01 |
| bta-miR-216b | 0.0006±0.0001 ^b | 0.002±0.00003 ^a | 0.001±0.0003 ^{ab} | 0.0006±0.0003 ^b |
| bta-miR-339a | 0.020±0.002 | 0.01±0.002 | 0.01±0.003 | 0.01±0.003 |
| bta-miR-500 | 0.001±0.0004 | 0.005±0.005 | 0.0007±0.0001 | 0.001±0.0005 |
| bta-miR-505 | 0.013±0.002 | 0.02±0.01 | 0.01±0.001 | 0.01±0.003 |
| bta-miR-532 | 0.001±0.0004 | 0.006±0.005 | 0.002±0.0006 | 0.0009±0.0002 |
| bta-miR-542-5p | 0.0009±0.0003 | 0.006±0.006 | 0.0005±0.0002 | 0.0006±0.0003 |

Supplementary Table S7. Mean and SEM of relative levels of the nine miRNAs investigated in two-cell embryos from high fertility (HF), low fertility (LF) and parthenogenesis (PA) groups as well as mature oocytes (OO). ¹High fertility; ²Low fertility; ³Parthenogenetic; ⁴Mature oocytes. SEM: standard error of the mean.

| Two-cell embryos | HF ¹ (n=9) | LF ² (n=9) | PA ³ (n=9) | OO ⁴ (n=3) |
|------------------|------------------------|------------------------|-------------------------|----------------------------|
| bta-miR-33b | 0.001±0.0003 | 0.002±0.005 | 0.001±0.0004 | 0.001±0.0004 |
| bta-miR-126-5p | 0.05±0.02 ^a | 0.07±0.04 ^a | 0.02±0.01 ^a | 0.004±0.0001 ^b |
| bta-miR-205 | 0.04±0.01 | 0.05±0.01 | 0.05±0.01 | 0.02±0.01 |
| bta-miR-216b | 0.002±0.0005 | 0.001±0.0002 | 0.001±0.0005 | 0.0006±0.0003 |
| bta-miR-339a | 0.12±0.02 ^a | 0.12±0.03 ^a | 0.08±0.01 ^a | 0.01±0.003 ^b |
| bta-miR-500 | 0.13±0.06 | 0.10±0.05 | 0.15±0.08 | 0.001±0.0005 |
| bta-miR-505 | 0.05±0.01 | 0.06±0.02 | 0.05±0.01 | 0.01±0.003 |
| bta-miR-532 | 0.02±0.01 ^a | 0.02±0.01 ^a | 0.01±0.007 ^a | 0.0009±0.0002 ^b |
| bta-miR-542-5p | 0.002±0.0005 | 0.003±0.0006 | 0.001±0.0005 | 0.0006±0.0003 |

Supplementary Table S8. Pre-implantation development rates of bovine embryos produced *in vitro* with IVF of 8 hours for kinetics evaluation.

Two-cell (evaluated 28 to 30 hpi) and four-cell (evaluated 40 to 42 hpi) rates were evaluated in embryos produced *in vitro* using high and low

fertility semen samples. ^{*a,b} Different letters on the same column indicate tendency to statistical difference (P=0.07) between groups.

¹High fertility; ²Low fertility. Hpi: hours post insemination. Mean and SEM are presented as percentages. SEM: standard error of the mean.

| Fertility Groups | Two-cell embryos | | | Four-cell embryos | | |
|------------------|------------------|-----|------------------------|-------------------|-----|----------|
| | Oocytes | N | Mean±SEM | Oocytes | N | Mean±SEM |
| HF ¹ | 328 | 115 | 35.1±6.8 ^{*a} | 489 | 124 | 25.4±4.2 |
| LF ² | 365 | 104 | 28.5±6.7 ^{*b} | 482 | 129 | 26.8±3.2 |

Supplementary Table S9. Classification of morphologically abnormal spermatozoa with regard to major and minor defects adapted from Blom ⁴⁶.

| Major Defects | Minor Defects |
|-------------------------------------------------------------------------------|-------------------------------------------|
| Acrosome defects | Head defects: |
| Proximal Droplet | Narrow |
| Head defects: | Giant, short, broad, and small normal |
| Underdeveloped | Free normal |
| Tail curled in the head | Abaxial, Retroaxial, Oblique implantation |
| Free pathological heads | Simple bent tail |
| Narrow at base | Distal droplet |
| Pear-shaped defect | |
| Small abnormal heads | |
| Abnormal contour | |
| Pouch formation | |
| Double forms | |
| Midpiece defects: | |
| Corkscrew defect, Pseudodroplet, Tail stump, Broken neck, Swelling and others | |
| Tail defects: | |
| Strongly coiled or folded tail | |
| Coiled with distal droplet attached | |

Supplementary Table S10. Set up for bovine sperm kinetic evaluation on Sperm Class Analyzer (Microptic, Barcelona, Spain).

| Characteristic | Adjusted to |
|-----------------------|------------------------------------------|
| Particle area | 15 to 70 μm^2 |
| Fast sperm | VAP (path velocity) > 40 $\mu\text{m/s}$ |
| Progressive motility | STR (straightness) > 70% |
| Circular movement | LIN (linearity) < 50% |
| VAP points | 5 |
| Number of images | 30 |
| Images per second | 50 |

Supplementary Table S11. Panel of 380 miRNAs investigated by qPCR in sperm cells.

| # | miRNA | # | miRNA | # | miRNA | # | miRNA | # | miRNA |
|----|---------------|----|----------------|-----|----------------|-----|----------------|-----|----------------|
| 1 | bta-let-7a-3p | 77 | bta-miR-142-5p | 153 | bta-miR-216a | 229 | bta-miR-361 | 305 | bta-miR-496 |
| 2 | bta-let-7a-5p | 78 | bta-miR-143 | 154 | bta-miR-216b | 230 | bta-miR-362-3p | 306 | bta-miR-497 |
| 3 | bta-let-7b | 79 | bta-miR-144 | 155 | bta-miR-217 | 231 | bta-miR-362-5p | 307 | bta-miR-499 |
| 4 | bta-let-7c | 80 | bta-miR-145 | 156 | bta-miR-218 | 232 | bta-miR-363 | 308 | bta-miR-500 |
| 5 | bta-let-7d | 81 | bta-miR-146a | 157 | bta-miR-219 | 233 | bta-miR-365-3p | 309 | bta-miR-502a |
| 6 | bta-let-7e | 82 | bta-miR-146b | 158 | bta-miR-219-3p | 234 | bta-miR-365-5p | 310 | bta-miR-502b |
| 7 | bta-let-7f | 83 | bta-miR-147 | 159 | bta-miR-219-5p | 235 | bta-miR-367 | 311 | bta-miR-503-3p |
| 8 | bta-let-7g | 84 | bta-miR-148a | 160 | bta-miR-221 | 236 | bta-miR-369-3p | 312 | bta-miR-503-5p |
| 9 | bta-let-7i | 85 | bta-miR-148b | 161 | bta-miR-222 | 237 | bta-miR-369-5p | 313 | bta-miR-504 |
| 10 | bta-miR-1 | 86 | bta-miR-149-3p | 162 | bta-miR-223 | 238 | bta-miR-370 | 314 | bta-miR-505 |
| 11 | bta-miR-100 | 87 | bta-miR-149-5p | 163 | bta-miR-22-3p | 239 | bta-miR-371 | 315 | bta-miR-532 |
| 12 | bta-miR-101 | 88 | bta-miR-150 | 164 | bta-miR-224 | 240 | bta-miR-374a | 316 | bta-miR-539 |
| 13 | bta-miR-103 | 89 | bta-miR-151-3p | 165 | bta-miR-22-5p | 241 | bta-miR-374b | 317 | bta-miR-541 |
| 14 | bta-miR-105a | 90 | bta-miR-151-5p | 166 | bta-miR-23a | 242 | bta-miR-375 | 318 | bta-miR-542-5p |
| 15 | bta-miR-105b | 91 | bta-miR-152 | 167 | bta-miR-23b-3p | 243 | bta-miR-376a | 319 | bta-miR-543 |
| 16 | bta-miR-106a | 92 | bta-miR-153 | 168 | bta-miR-23b-5p | 244 | bta-miR-376b | 320 | bta-miR-544a |
| 17 | bta-miR-106b | 93 | bta-miR-154a | 169 | bta-miR-24 | 245 | bta-miR-376c | 321 | bta-miR-544b |

| | | | | | | | | | |
|----|-----------------|-----|---------------|-----|----------------|-----|-----------------|-----|----------------|
| 18 | bta-miR-107 | 94 | bta-miR-154b | 170 | bta-miR-24-3p | 246 | bta-miR-376d | 322 | bta-miR-545-3p |
| 19 | bta-miR-10a | 95 | bta-miR-154c | 171 | bta-miR-25 | 247 | bta-miR-376e | 323 | bta-miR-545-5p |
| 20 | bta-miR-10b | 96 | bta-miR-155 | 172 | bta-miR-26a | 248 | bta-miR-377 | 324 | bta-miR-551a |
| 21 | bta-miR-1179 | 97 | bta-miR-15a | 173 | bta-miR-26b | 249 | bta-miR-378 | 325 | bta-miR-551b |
| 22 | bta-miR-1185 | 98 | bta-miR-15b | 174 | bta-miR-26c | 250 | bta-miR-378b | 326 | bta-miR-562 |
| 23 | bta-miR-1193 | 99 | bta-miR-16a | 175 | bta-miR-27a-3p | 251 | bta-miR-378c | 327 | bta-miR-568 |
| 24 | bta-miR-1197 | 100 | bta-miR-16b | 176 | bta-miR-27a-5p | 252 | bta-miR-378d | 328 | bta-miR-574 |
| 25 | bta-miR-122 | 101 | bta-miR-17-3p | 177 | bta-miR-27b | 253 | bta-miR-379 | 329 | bta-miR-582 |
| 26 | bta-miR-122 | 102 | bta-miR-17-5p | 178 | bta-miR-28 | 254 | bta-miR-380-3p | 330 | bta-miR-584 |
| 27 | bta-miR-1224 | 103 | bta-miR-181a | 179 | bta-miR-296-3p | 255 | bta-miR-380-5p | 331 | bta-miR-592 |
| 28 | bta-miR-1225-3p | 104 | bta-miR-181b | 180 | bta-miR-296-5p | 256 | bta-miR-381 | 332 | bta-miR-599 |
| 29 | bta-miR-1246 | 105 | bta-miR-181c | 181 | bta-miR-299 | 257 | bta-miR-382 | 333 | bta-miR-615 |
| 30 | bta-miR-1247-3p | 106 | bta-miR-181d | 182 | bta-miR-29a | 258 | bta-miR-383 | 334 | bta-miR-628 |
| 31 | bta-miR-1247-5p | 107 | bta-miR-182 | 183 | bta-miR-29b | 259 | bta-miR-409a | 335 | bta-miR-631 |
| 32 | bta-miR-1248 | 108 | bta-miR-183 | 184 | bta-miR-29c | 260 | bta-miR-409b | 336 | bta-miR-652 |
| 33 | bta-miR-1249 | 109 | bta-miR-184 | 185 | bta-miR-29d-3p | 261 | bta-miR-410 | 337 | bta-miR-653 |
| 34 | bta-miR-124a | 110 | bta-miR-185 | 186 | bta-miR-29d-5p | 262 | bta-miR-411a | 338 | bta-miR-654 |
| 35 | bta-miR-124b | 111 | bta-miR-186 | 187 | bta-miR-29e | 263 | bta-miR-411b | 339 | bta-miR-655 |
| 36 | bta-miR-125a | 112 | bta-miR-187 | 188 | bta-miR-301a | 264 | bta-miR-411c-3p | 340 | bta-miR-656 |

| | | | | | | | | | |
|----|----------------|-----|-----------------|-----|----------------|-----|-----------------|-----|----------------|
| 37 | bta-miR-125b | 113 | bta-miR-188 | 189 | bta-miR-301b | 265 | bta-miR-411c-5p | 341 | bta-miR-658 |
| 38 | bta-miR-1260b | 114 | bta-miR-18a | 190 | bta-miR-302a | 266 | bta-miR-412 | 342 | bta-miR-660 |
| 39 | bta-miR-126-3p | 115 | bta-miR-18b | 191 | bta-miR-302b | 267 | bta-miR-421 | 343 | bta-miR-664a |
| 40 | bta-miR-126-5p | 116 | bta-miR-190a | 192 | bta-miR-302c | 268 | bta-miR-423-3p | 344 | bta-miR-664b |
| 41 | bta-miR-127 | 117 | bta-miR-190b | 193 | bta-miR-302d | 269 | bta-miR-423-5p | 345 | bta-miR-665 |
| 42 | bta-miR-1271 | 118 | bta-miR-191 | 194 | bta-miR-3064 | 270 | bta-miR-424-3p | 346 | bta-miR-669 |
| 43 | bta-miR-1277 | 119 | bta-miR-192 | 195 | bta-miR-30a-5p | 271 | bta-miR-424-5p | 347 | bta-miR-670 |
| 44 | bta-miR-128 | 120 | bta-miR-193a | 196 | bta-miR-30b-3p | 272 | bta-miR-425-3p | 348 | bta-miR-671 |
| 45 | bta-miR-1281 | 121 | bta-miR-193a-3p | 197 | bta-miR-30b-5p | 273 | bta-miR-425-5p | 349 | bta-miR-677 |
| 46 | bta-miR-1282 | 122 | bta-miR-193a-5p | 198 | bta-miR-30c | 274 | bta-miR-429 | 350 | bta-miR-7 |
| 47 | bta-miR-1284 | 123 | bta-miR-193b | 199 | bta-miR-30d | 275 | bta-miR-431 | 351 | bta-miR-708 |
| 48 | bta-miR-1287 | 124 | bta-miR-194 | 200 | bta-miR-30e-5p | 276 | bta-miR-432 | 352 | bta-miR-744 |
| 49 | bta-miR-129 | 125 | bta-miR-195 | 201 | bta-miR-30f | 277 | bta-miR-433 | 353 | bta-miR-758 |
| 50 | bta-miR-1291 | 126 | bta-miR-196a | 202 | bta-miR-31 | 278 | bta-miR-448 | 354 | bta-miR-759 |
| 51 | bta-miR-129-3p | 127 | bta-miR-196b | 203 | bta-miR-32 | 279 | bta-miR-449a | 355 | bta-miR-760-3p |
| 52 | bta-miR-129-5p | 128 | bta-miR-197 | 204 | bta-miR-320a | 280 | bta-miR-449b | 356 | bta-miR-760-5p |
| 53 | bta-miR-1296 | 129 | bta-miR-199a-3p | 205 | bta-miR-320b | 281 | bta-miR-449c | 357 | bta-miR-761 |
| 54 | bta-miR-1298 | 130 | bta-miR-199a-5p | 206 | bta-miR-323 | 282 | bta-miR-449d | 358 | bta-miR-763 |
| 55 | bta-miR-1301 | 131 | bta-miR-199b | 207 | bta-miR-324 | 283 | bta-miR-450a | 359 | bta-miR-764 |

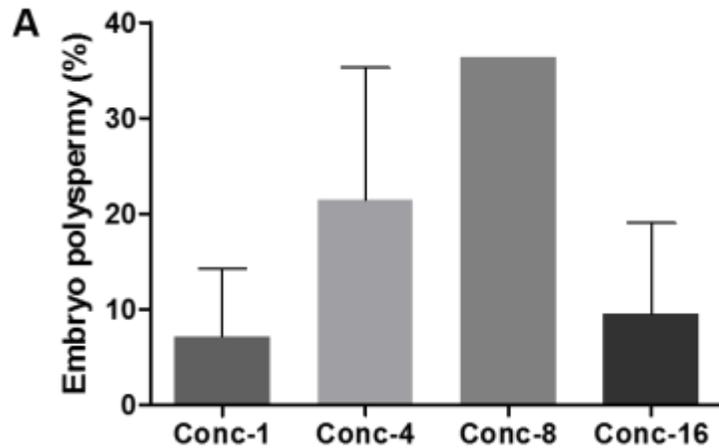
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| 56 | bta-miR-1306 | 132 | bta-miR-199c | 208 | bta-miR-326 | 284 | bta-miR-450b | 360 | bta-miR-767 |
| 57 | bta-miR-1307 | 133 | bta-miR-19a | 209 | bta-miR-328 | 285 | bta-miR-451 | 361 | bta-miR-769 |
| 58 | bta-miR-130a | 134 | bta-miR-19b | 210 | bta-miR-329a | 286 | bta-miR-452 | 362 | bta-miR-873 |
| 59 | bta-miR-130b | 135 | bta-miR-200a | 211 | bta-miR-329b | 287 | bta-miR-4523 | 363 | bta-miR-874 |
| 60 | bta-miR-132 | 136 | bta-miR-200b | 212 | bta-miR-330 | 288 | bta-miR-453 | 364 | bta-miR-875 |
| 61 | bta-miR-133a | 137 | bta-miR-200c | 213 | bta-miR-331-3p | 289 | bta-miR-454 | 365 | bta-miR-876 |
| 62 | bta-miR-133b | 138 | bta-miR-202 | 214 | bta-miR-331-5p | 290 | bta-miR-455-3p | 366 | bta-miR-877 |
| 63 | bta-miR-133c | 139 | bta-miR-204 | 215 | bta-miR-335 | 291 | bta-miR-455-5p | 367 | bta-miR-885 |
| 64 | bta-miR-134 | 140 | bta-miR-205 | 216 | bta-miR-338 | 292 | bta-miR-483 | 368 | bta-miR-92a |
| 65 | bta-miR-1343-3p | 141 | bta-miR-206 | 217 | bta-miR-339a | 293 | bta-miR-484 | 369 | bta-miR-92b |
| 66 | bta-miR-1343-5p | 142 | bta-miR-208a | 218 | bta-miR-339b | 294 | bta-miR-485 | 370 | bta-miR-93 |
| 67 | bta-miR-135a | 143 | bta-miR-208b | 219 | bta-miR-33a | 295 | bta-miR-486 | 371 | bta-miR-935 |
| 68 | bta-miR-135b | 144 | bta-miR-20a | 220 | bta-miR-33b | 296 | bta-miR-487a | 372 | bta-miR-9-3p |
| 69 | bta-miR-136 | 145 | bta-miR-20b | 221 | bta-miR-340 | 297 | bta-miR-487b | 373 | bta-miR-940 |
| 70 | bta-miR-137 | 146 | bta-miR-210 | 222 | bta-miR-342 | 298 | bta-miR-488 | 374 | bta-miR-95 |
| 71 | bta-miR-138 | 147 | bta-miR-211 | 223 | bta-miR-345-3p | 299 | bta-miR-489 | 375 | bta-miR-9-5p |
| 72 | bta-miR-1388-3p | 148 | bta-miR-212 | 224 | bta-miR-345-5p | 300 | bta-miR-490 | 376 | bta-miR-96 |
| 73 | bta-miR-139 | 149 | bta-miR-21-3p | 225 | bta-miR-346 | 301 | bta-miR-491 | 377 | bta-miR-98 |
| 74 | bta-miR-140 | 150 | bta-miR-214 | 226 | bta-miR-34a | 302 | bta-miR-493 | 378 | bta-miR-99a-3p |

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|----|----------------|-----|---------------|-----|-------------|-----|-------------|-----|----------------|
| 75 | bta-miR-141 | 151 | bta-miR-215 | 227 | bta-miR-34b | 303 | bta-miR-494 | 379 | bta-miR-99a-5p |
| 76 | bta-miR-142-3p | 152 | bta-miR-21-5p | 228 | bta-miR-34c | 304 | bta-miR-495 | 380 | bta-miR-99b |

Supplementary Table S12. Sequences of forward and reverse miR-216b target gene primers used for qPCR.

| Gene | Product size | Forward primer (5'-3') | Reverse primer (5'-3') | NCBI accession number |
|----------------|---------------------|-------------------------------|-------------------------------|------------------------------|
| <i>K-RAS</i> | 133 | GCCAGTTAGCTTGGTAGGTGT | AACCAAGGCATGGCAACAAT | NM_001110001.2 |
| <i>BECN1</i> | 182 | GCCTCTGAAACTGGACACGA | GGGGGATGAATCTGCGAGAG | NM_001033627.2 |
| <i>JUN</i> | 140 | TGCAAACGTTTTGAGGCGAG | GGGCTTTAGTCCTCGGACAC | NM_001077827.1 |
| <i>β-ACTIN</i> | 158 | TCGGTTGGATCGAGCATTCC | CCTTAGAGAGAAGCGGGGTG | NM_173979.3 |

Supplementary Figure S1. Preliminary results of polyspermic embryo induction. A. Zygote polyspermy rates by different sperm concentrations in *in vitro* fertilization drops. **B.** Number of oocytes and mean and SEM (%) of zygote polyspermy rates by different sperm concentrations in *in vitro* fertilization drops. Groups: Conc-1: 1×10^6 sperm/mL (n = 2); Conc-4: 4×10^6 sperm/mL (n = 2); Conc-8: 8×10^6 sperm/mL (n = 2); Conc-16: 16×10^6 sperm/mL (n = 2). For this experiment, two straws from one frozen-thawed commercial semen batch were used to perform two replicates of *in vitro* embryo production. Zygotes were collected 12 hours post insemination (hpi) to evaluate embryo polyspermy rate. SEM: standard error of the mean.



B

| Groups | Number of oocytes | Total of polyspermy (%) |
|------------------------------|-------------------|-------------------------|
| Conc-1 (1×10^6) | 29 | 7.1±7.1 |
| Conc-4 (4×10^6) | 30 | 21.5±13.8 |
| Conc-8 (8×10^6) | 33 | 36.4±0 |
| Conc-16 (16×10^6) | 34 | 9.5±9.5 |