**SUPPLEMENTAL TABLES**

**Supplemental Table S1.** Multivariable generalized linear mixed model showing the effect of treatment, order of treatment administration (sequence), log10-transformed somatic cell count (logSCC) and stage of lactation on machine milking-induced short term changes

|  |  |  |  |
| --- | --- | --- | --- |
| Variable1 | ß2 (SE) | *P*-value | aOR3(95% CI) |
| Treatment4 |  | <0.0001 |  |
| TRT | −2.05 (0.22) |  | 0.13 (0.08–0.20) |
| CON | Referent |  | – |
| Sequence |  | 0.79 |  |
| TRT ­­– CON | 0.05 (0.19) |  | 1.05 (0.72–1.52) |
| CON – TRT | Referent |  | – |
| LogSCC | −0.58 (0.16) | 0.0003 | 0.56 (0.41–0.77) |
| Stage of lactation |  | 0.004 |  |
| ≤ 100 DIM | 0.53 (0.23)a |  | 1.70 (1.08–2.67) |
| 101–200 DIM | −0.27 (0.22)b |  | 0.76 (0.49–1.18) |
| > 200 DIM | Referentb |  | – |

a–bGroups with different superscript letters differ at a level of *P* < 0.05 in Tukey-Kramer post hoc test.

1Intercept omitted for clarity.

2Linear regression coefficient.

3Adjusted odds ratio.

4Treatment consisted of 2 different premilking stimulation regimens: latency period till 90-s preparation lag time (TRT) and no latency (CON).

**Supplemental Table S2.** Multivariable general linear mixed model showing the effect of treatment, order of treatment administration (sequence), parity, stage of lactation, log10-transformed somatic cell count (logSCC), and milking session on milk yield (kg/session)

|  |  |  |  |
| --- | --- | --- | --- |
| Variable1 | ß2 (SE) | *P*-value | LSM (95% CI) |
| Treatment3 |  | 0.57 |  |
| TRT | 0.04 (0.1) |  | 15.4 (14.9–15.9) |
| CON | Referent |  | 15.3 (14.8–15.8) |
| Sequence |  | 0.59 |  |
| TRT – CON | −0.3 (0.5) |  | 15.2 (14.5–15.9) |
| CON – TRT | Referent |  | 15.5 (14.8–16.2) |
| Parity |  | <0.0001 |  |
| 1st | −3.0 (0.6)c |  | 13.5 (12.7–14.3) |
| 2nd | −0.5 (0.6)b |  | 16.0 (15.2–16.9) |
| ≥ 3rd | Referenta |  | 16.5 (15.5–17.4) |
| Stage of lactation |  | <0.0001 |  |
| ≤ 100 DIM | 3.6 (0.6)b |  | 16.8 (15.9–17.6) |
| 101–200 DIM | 2.8 (0.6)b |  | 16.0 (15.0–17.0) |
| > 200 DIM | Referenta |  | 13.2 (12.5–14.0) |
| LogSCC | −1.3 (0.5) | 0.006 | – |
| Milking session |  | <0.0001 |  |
| Session 1 | 2.7 (0.1)a |  | 18.0 (17.5–18.5) |
| Session 2 | −2.5 (0.1)c |  | 12.7 (12.2–13.3) |
| Session 3 | Referentb |  | 15.3 (14.8–15.8) |

a–cGroups with different superscript letters differ at a level of *P* < 0.05 in Tukey-Kramer post hoc tests.

1Intercept omitted for clarity.

2Linear regression coefficient.

3Treatment consisted of 2 different premilking stimulation regimens: latency period till 90-s preparation lag time (TRT) and no latency (CON).

**Supplemental Table S3.** Multivariable general linear mixed model showing the effect of treatment, order of treatment administration (sequence), parity, stage of lactation and log10-transformed somatic cell count (logSCC) on machine-on time (s)

|  |  |  |  |
| --- | --- | --- | --- |
| Variable1 | ß2 (SE) | *P*-value | LSM (95% CI) |
| Treatment3 |  | 0.07 |  |
| TRT | −0.01 (0.01) |  | 246 (239–253) |
| CON | Referent |  | 253 (247–260) |
| Sequence |  | 0.81 |  |
| TRT – CON | −0.002 (0.009) |  | 249 (242–257) |
| CON – TRT | Referent |  | 250 (243–258) |
| Parity |  | 0.001 |  |
| 1st | −0.04 (0.01)b |  | 236 (228–244) |
| 2nd | 0.001 (0.01)a |  | 257 (248–266) |
| ≥ 3rd | Referenta |  | 257 (247–267) |
| Stage of lactation |  | 0.006 |  |
| ≤ 100 DIM | 0.03 (0.01)a |  | 257 (248–267) |
| 101–200 DIM | 0.03 (0.01)ab |  | 254 (243–264) |
| > 200 DIM | Referentb |  | 238 (231–246) |
| LogSCC | −0.03 (0.01) | 0.0003 | – |

a–bGroups with different superscript letters differ at a level of *P* < 0.05 in Tukey-Kramer post hoc tests.

1Intercept omitted for clarity.

2Linear regression coefficient.

3Treatment consisted of 2 different premilking stimulation regimens: latency period till 90-s preparation lag time (TRT) and no latency (CON).

**Supplemental Table S4.** Multivariable general linear mixed model showing the effect of treatment, order of treatment administration (sequence), parity, milking session and interaction of treatment and parity on 2-minute milk yield (kg)

|  |  |  |  |
| --- | --- | --- | --- |
| Variable1 | ß2 (SE) | *P*-value | LSM (95% CI) |
| Treatment3 |  | <0.0001 |  |
| TRT | 0.6 (0.1) |  | – |
| CON | Referent |  | – |
| Sequence |  | 0.88 |  |
| TRT – CON | 0.04 (0.3) |  | 5.6 (5.2–6.0) |
| CON – TRT | Referent |  | 5.6 (5.2–6.0) |
| Parity |  | 0.01 |  |
| 1st | −1.2 (0.4)b |  | – |
| 2nd | −0.8 (0.4)ab |  | – |
| ≥ 3rd | Referenta |  | – |
| Stage of lactation |  | 0.04 |  |
| ≤ 100 DIM | 0.7 (0.3)a |  | 5.9 (5.4–6.3) |
| 101–200 DIM | 0.7 (0.3)a |  | 5.8 (5.3–6.4) |
| > 200 DIM | Referentb |  | 5.1 (4.7–5.6) |
| Milking session |  | <0.0001 |  |
| Session 1 | 0.3 (0.1)a |  | 6.2 (5.9–6.5) |
| Session 2 | −1.2 (0.1)c |  | 4.7 (4.4–5.0) |
| Session 3 | Referentb |  | 5.9 (5.6–6.2) |
| Treatment × Parity |  | 0.007 |  |
| TRT × 1st | 0.4 (0.2)b |  | 5.7 (5.3–6.2) |
| CON × 1st | Referentc |  | 4.7 (4.2–5.1) |
| TRT × 2nd | 0.02 (0.2)b |  | 5.7 (5.3–6.2) |
| CON × 2nd | Referentc |  | 5.1 (4.6–5.6) |
| TRT × ≥ 3rd | Referenta |  | 6.5 (6.0–7.0) |
| CON × ≥ 3rd | Referentb |  | 5.9 (5.4–6.4) |

a–cGroups with different superscript letters differ at a level of *P* < 0.05 in Tukey-Kramer post hoc tests.

1Intercept omitted for clarity.

2Linear regression coefficient.

3Treatment consisted of 2 different premilking stimulation regimens: latency period till 90-s preparation lag time (TRT) and no latency (CON).

**Supplemental Table S5.** Multivariable general linear mixed model showing the effect of treatment, order of treatment administration (sequence), parity, stage of lactation, milking session and interaction between parity and treatment on duration of low milk flow rate (s)

|  |  |  |  |
| --- | --- | --- | --- |
| Variable1 | ß2 (SE) | *P*-value | LSM (95% CI) |
| Treatment3 |  | <0.0001 |  |
| TRT | −0.09 (0.03) |  | – |
| CON | Referent |  | – |
| Sequence |  | 0.30 |  |
| TRT – CON | −0.03 (0.03) |  | 18 (17–20) |
| CON – TRT | Referent |  | 19 (18–21) |
| Parity |  | <0.0001 |  |
| 1st | 0.30 (0.04)a |  | – |
| 2nd | 0.17 (0.04)b |  | – |
| ≥ 3rd | Referentc |  | – |
| Stage of lactation |  | 0.01 |  |
| ≤ 100 DIM | −0.10 (0.03)b |  | 17 (15–19) |
| 101–200 DIM | −0.06 (0.04)ab |  | 19 (16–21) |
| > 200 DIM | Referenta |  | 21 (19–23) |
| Milking session |  | <0.0001 |  |
| Session 1 | −0.09 (0.01)c |  | 14 (13–15) |
| Session 2 | 0.19 (0.01)a |  | 27 (25–29) |
| Session 3 | Referentb |  | 17 (16–19) |
| Treatment × Parity |  | 0.001 |  |
| TRT × 1st | −0.12 (0.01)c |  | 19 (17–22) |
| CON × 1st | Referenta |  | 31 (27–35) |
| TRT × 2nd | −0.02 (0.03)c |  | 17 (15–20) |
| CON × 2nd | Referentb |  | 22 (20–25) |
| TRT × ≥ 3rd | Referentd |  | 13 (11–14) |
| CON × ≥ 3rd | Referentc |  | 15 (13–18) |

a–dGroups with different superscript letters differ at a level of *P* < 0.05 in Tukey-Kramer post hoc tests.

1Intercept omitted for clarity.

2Linear regression coefficient.

3Treatment consisted of 2 different premilking stimulation regimens: latency period till 90-s preparation lag time (TRT) and no latency (CON).

**Supplemental Table S6.** Multivariable generalized linear mixed model showing the effect of treatment, order of treatment administration (sequence), parity, log10-transformed somatic cell count (LogSCC), stage of lactation, milking session and interactions of treatment and parity on bimodality

|  |  |  |  |
| --- | --- | --- | --- |
| Variable1 | ß2 (SE) | *P*-value | aOR3(95% CI) |
| Treatment4 |  | <0.0001 |  |
| TRT | −0.9 (0.2) |  | – |
| CON | Referent |  | – |
| Sequence |  | 0.56 |  |
| TRT – CON | 0.05 (0.09) |  | 1.05 (0.89–1.25) |
| CON – TRT | Referent |  | – |
| Parity |  | <0.0001 |  |
| 1st | 0.9 (0.1)a |  | – |
| 2nd | 0.8 (0.1)a |  | – |
| ≥ 3rd | Referentb |  | – |
| LogSCC | 0.23 (0.08) | 0.004 | 1.25 (1.08–1.46) |
| Stage of lactation |  | <0.0001 |  |
| ≤ 100 DIM | −0.8 (0.1)c |  | 0.46 (0.37–0.56) |
| 101–200 DIM | −0.5 (0.1)b |  | 0.59 (0.48–0.73) |
| > 200 DIM | Referenta |  | – |
| Milking session |  | <0.0001 |  |
| Session 1 | −0.1 (0.1)b |  | 0.91 (0.77–1.07) |
| Session 2 | 1.3 (0.1)a |  | 3.73 (3.19–4.37) |
| Session 3 | Referentb |  | – |
| Treatment × Parity |  | 0.002 |  |
| TRT × 1st | −0.7 (0.2)cd |  | 0.19 (0.14–0.24) |
| CON × 1st | Referenta |  | – |
| TRT × 2nd | –0.3 (0.2)c |  | 0.29 (0.22–0.38) |
| CON × 2nd | Referenta |  | – |
| TRT × ≥ 3rd | Referentd |  | 0.39 (0.28–0.53) |
| CON × ≥ 3rd | Referentb |  | – |

a–dGroups with different superscript letters differ at a level of *P* < 0.05 in Tukey-Kramer post hoc tests.

1Intercept omitted for clarity.

2Linear regression coefficient.

3Adjusted odds ratio.

4Treatment consisted of 2 different premilking stimulation regimens: latency period till 90-s preparation lag time (TRT) and no latency (CON).

**Supplemental Table S7.** Multivariable generalized linear mixed model showing the effect of treatment, order of treatment administration (sequence), parity, stage of lactation, and milk yield on milking liner slip

|  |  |  |  |
| --- | --- | --- | --- |
| Variable1 | ß2 (SE) | *P*-value | aOR3(95% CI) |
| Treatment4 |  | 0.14 |  |
| TRT | 0.2 (0.1) |  | 1.21 (0.94–1.56) |
| CON | Referent |  | – |
| Sequence |  | 0.70 |  |
| TRT – CON | –0.05 (0.1) |  | 0.95 (0.73–1.23) |
| CON – TRT | Referent |  | – |
| Parity |  | <0.0001 |  |
| 1st | –0.9 (0.2)c |  | 0.40 (0.29–0.55) |
| 2nd | –0.5 (0.2)b |  | 0.60 (0.44–0.81) |
| ≥ 3rd | Referenta |  | – |
| Stage of lactation |  | <0.0001 |  |
| ≤ 100 DIM | 1.0 (0.2)a |  | 2.82 (2.07–3.83) |
| 101–200 DIM | 0.7 (0.2)a |  | 2.01 (1.43–2.83) |
| > 200 DIM | Referentb |  | – |

a–cGroups with different superscript letters differ at a level of *P* < 0.05 in Tukey-Kramer post hoc tests.

1Intercept omitted for clarity.

2Linear regression coefficient.

3Adjusted odds ratio.

4Treatment consisted of 2 different premilking stimulation regimens: latency period till 90-s preparation lag time (TRT) and no latency (CON).

**Supplemental Table S8.** Multivariable generalized linear mixed model showing the effect of treatment, order of treatment administration (sequence), parity, and stage of lactation on milking unit kick-off

|  |  |  |  |
| --- | --- | --- | --- |
| Variable1 | ß2 (SE) | *P*-value | aOR3(95% CI) |
| Treatment4 |  | 0.34 |  |
| TRT | 0.1 (0.1) |  | 1.14 (0.88–1.47) |
| CON | Referent |  | – |
| Sequence |  | 0.049 |  |
| TRT – CON | –0.3 (0.1) |  | 0.77 (0.59–1.00) |
| CON – TRT | Referent |  | – |
| Parity |  | <0.0001 |  |
| 1st | 1.0 (0.2)a |  | 2.71 (1.92–3.83) |
| 2nd | 0.4 (0.2)a |  | 1.56 (1.07–2.29) |
| ≥ 3rd | Referentb |  | – |
| Stage of lactation |  | <0.0001 |  |
| ≤ 100 DIM | 2.6 (0.3)a |  | 13.3 (8.0–22.1) |
| 101–200 DIM | 2.0 (0.3)b |  | 7.2 (4.2–12.3) |
| > 200 DIM | Referentc |  | – |

a–cGroups with different superscript letters differ at a level of *P* < 0.05 in Tukey-Kramer post hoc tests.

1Intercept omitted for clarity.

2Linear regression coefficient.

3Adjusted odds ratio.

4Treatment consisted of 2 different premilking stimulation regimens: latency period till 90-s preparation lag time (TRT) and no latency (CON).

**Supplemental Table S9.** Multivariable generalized linear mixed model showing the effect of treatment, order of treatment administration (sequence), parity, stage of lactation, and milking session on milking unit reattachment

|  |  |  |  |
| --- | --- | --- | --- |
| Variable1 | ß2 (SE) | *P*-value | aOR3(95% CI) |
| Treatment4 |  | 0.85 |  |
| TRT | −0.03 (0.1) |  | 0.97 (0.72–1.29) |
| CON | Referent |  | – |
| Sequence |  | 0.04 |  |
| TRT – CON | –0.3 (0.1) |  | 0.74 (0.55–0.98) |
| CON – TRT | Referent |  | – |
| Parity |  | 0.01 |  |
| 1st | 0.4 (0.2)a |  | 1.57 (1.01–2.24) |
| 2nd | 0.04 (0.2)b |  | 1.04 (0.71–1.54) |
| ≥ 3rd | Referentb |  | – |
| Stage of lactation |  | 0.01 |  |
| ≤ 100 DIM | 0.5 (0.2)a |  | 1.60 (1.14–2.25) |
| 101–200 DIM | 0.4 (0.2)a |  | 1.56 (1.08–2.24) |
| > 200 DIM | Referentb |  | – |
| Milking session |  | <0.0001 |  |
| Session 1 | −0.6 (0.2)b |  | 0.54 (0.38–0.77) |
| Session 2 | 0.2 (0.2)a |  | 1.19 (0.88–1.61) |
| Session 3 | Referenta |  | – |

a–bGroups with different superscript letters differ at a level of *P* < 0.05 in Tukey-Kramer post hoc tests.

1Intercept omitted for clarity.

2Linear regression coefficient.

3Adjusted odds ratio.

4Treatment consisted of 2 different premilking stimulation regimens: latency period till 90-s preparation lag time (TRT) and no latency (CON).