

**A novel treatment approach of subclinical mastitis in dairy cows –
auriculotherapy of the auricular branch of the vagus nerve**

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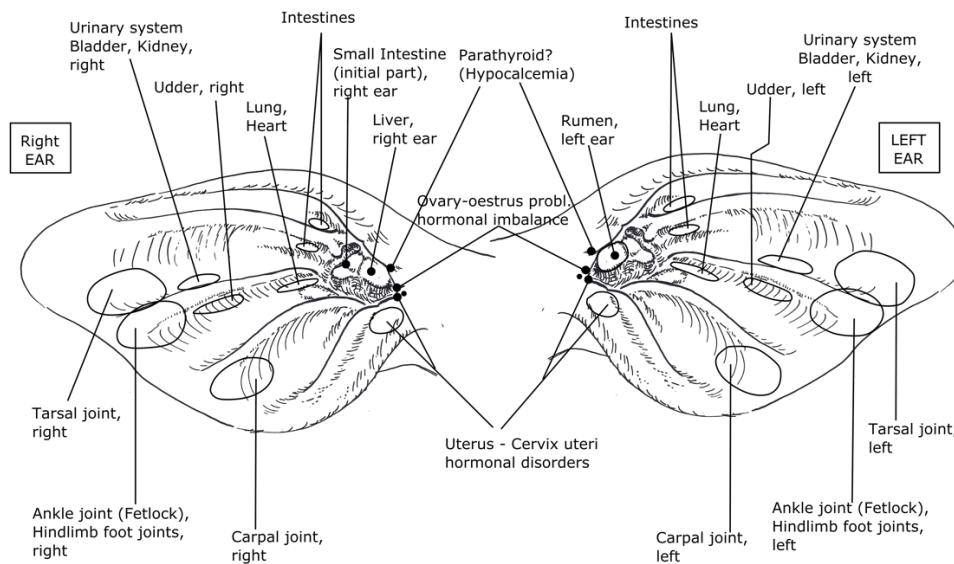
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Figure S1. Auricular Acupuncture Points in cattle according to Kothbauer (Veterinary Acupuncture - Basic Principles and Their Clinical Applications with Ear Acupuncture on Cattle and Some References to the Horse. Zweimühlen-Verlag, Munich, Germany. 1999. page 252).



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Table S1. Descriptive statistics of quarter somatic cell count (in 1.000 cells/mL milk) and independent variables (covariats) included in the linear regression model (ANCOVA) in control group (CON) and treatment group (TRE) on days (d) d0, d2, d4 and d6.

Covariats	Variables	Group	Statistics	d0	d2	d4	d6
Farm	A	CON	n	14	14	14	14
			Quartile 1	201.75	204.5	70.75	60.75
			Median	271	250	306	204.5
			Quartile 3	437.5	364.25	436.75	326.25
		TRE	n	16	16	15	16
			Quartile 1	232.5	221	158	228.75
			Median	381	417.5	356	493.5
			Quartile 3	1249.25	1305.25	2156	1372
	B	CON	n	2	2	2	1
			Quartile 1	118	32	192	168
			Median	1982	1290	1719	168
			Quartile 3*				168
		TRE	n	5	5	5	5
			Quartile 1	182.5	346.5	347.5	272.5
			Median	323	428	394	364
			Quartile 3	995.5	5591	4473	8630.5
	C	CON	n	6	6	6	6
			Quartile 1	344	606	947.25	159
			Median	695	1342.5	1763	330.5
			Quartile 3	2240.5	1714.75	2116.75	1178.5
		TRE	n	14	14	14	14
			Quartile 1	180.75	170	301.5	66
			Median	794.5	1945.5	1725.5	109.5
			Quartile 3	2395.5	8601.5	5546.75	608.25
	D	CON	n	15	15	15	15
			Quartile 1	186	115	247	137
			Median	306	227	305	279
			Quartile 3	468	533	366	337
		TRE	n	9	9	9	9
			Quartile 1	570	339	232	203.5
			Median	1283	878	326	273
			Quartile 3	2601.5	1796	1478	597
E	CON	n	6	6	6	6	
		Quartile 1	277.75	173.75	200.5	247	
		Median	626.5	288.5	295.5	1367.5	

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			Quartile 3	3204.5	1922.5	965.5	2716.75	
		TRE	n	3	3	3	3	
			Quartile 1	132	102	56	73	
			Median	211	252	101	278	
			Quartile 3*					
F	CON		n	11	11	11	11	
			Quartile 1	357	524	311	268	
			Median	575	771	483	531	
			Quartile 3	973	1900	1291	2799	
	TRE		n	5	5	5	5	
			Quartile 1	147	224.5	270.5	135.5	
			Median	494	325	700	165	
			Quartile 3	5043.5	3168.5	2271	5748	
	G	CON		n	4	4	4	4
				Quartile 1	206.75	492.5	312	233.75
				Median	404.5	680	640	450.5
				Quartile 3	507	863	1321.25	746.75
TRE			n	9	9	9	9	
			Quartile 1	154.5	558.5	529.5	188	
			Median	604	1496	959	651	
			Quartile 3	7288.5	3777.5	5946.5	1618	
H**	TRE		n	5	5	5	5	
			Quartile 1	413.5	278	286	268	
			Median	448	466	583	497	
			Quartile 3	701	570.5	816	649	
I	CON		n	40	39	35	40	
			Quartile 1	169	256	281	272.25	
			Median	434.5	719	506	539.5	
			Quartile 3	967.75	1418	1195	1677.5	
	TRE		n	34	34	34	34	
			Quartile 1	150.5	129.5	159.75	119	
			Median	264.5	412	376	353.5	
			Quartile 3	657.5	697.5	1399	1248.75	
Season	Spring	CON		n	12	12	12	
				Quartile 1	193.25	248	54.25	36.25
				Median	423.5	1389	351	265
				Quartile 3	2529.25	4102.75	1080.25	2357
		TRE		n	10	10	10	10
				Quartile 1	171.75	152.5	77.75	59.75
				Median	413.5	299.5	245.5	289.5

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			Quartile 3	540.5	494.5	625.75	498.5
Summer	CON	n	48	47	43	48	
		Quartile 1	169.75	239	248	256.5	
		Median	349	533	340	429	
		Quartile 3	908.25	1343	1052	1198.25	
	TRE	n	34	34	34	34	
		Quartile 1	150,50	129,50	159,75	119,00	
		Median	150.5	129.5	159.75	119	
		Quartile 3	657,50	697,50	1399,00	1248,75	
Fall	CON	n	16	16	16	15	
		Quartile 1	201.25	176.75	305.5	168	
		Median	411	294	477	245	
		Quartile 3	803.5	1421.75	1858	349	
	TRE	n	36	36	35	36	
		Quartile 1	270.25	342.75	270	223.25	
		Median	763	913	708	370	
		Quartile 3	1915.75	5391.75	3029	1025	
Winter	CON	n	22	22	22	22	
		Quartile 1	311.75	190.25	295.5	249	
		Median	419.5	417.5	397.5	335.5	
		Quartile 3	687	788.75	773	668	
	TRE	n	20	20	20	20	
		Quartile 1	168.25	232.5	268.25	150.25	
		Median	788	668	797	250	
		Quartile 3	3572.5	2041.5	1874.5	1479.25	
Ear tag position	6	CON	n	2	2	2	2
			Quartile 1	373	351	259	260
			Median	1309.5	1611.5	808	1990.5
			Quartile 3*				
	TRE	n	13	13	13	13	
		Quartile 1	168	107	98.5	88	
		Median	258	186	174	280	
		Quartile 3	920	644.5	799.5	578	
	7***	TRE	n	3	3	2	3
			Quartile 1	175	224	3347	206
			Median	1151	3027	6708.5	1699
			Quartile 3*				
	9	CON	n	18	18	18	18
Quartile 1			152.25	250.25	309.25	288	
Median			502.5	912	554.5	692	

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Bacteriological Culture	10	TRE	Quartile 3	1017	1825	1397.25	3030
			n	8	8	8	8
			Quartile 1	296.75	108.75	179	95.75
			Median	1189	374.5	353	198
			Quartile 3	1924.75	5646.5	2767	327.5
	10	CON	n	69	69	64	68
			Quartile 1	190	202.5	255.75	175.5
			Median	396	487	394.5	298.5
			Quartile 3	673	1070	813	693.75
		TRE	n	72	72	72	72
			Quartile 1	192.75	259.5	251.25	184.5
			Median	446.5	569	583.5	364.5
			Quartile 3	1320.25	2052	1821.25	1284.25
	13***	TRE	n	2	2	2	2
			Quartile 1	165	97	111	63
			Median	169.5	127.5	245.5	80
			Quartile 3*				
	14	CON	n	6	6	6	6
			Quartile 1	277.75	173.75	200.5	379.75
			Median	924.5	784.5	583.5	1232.5
Quartile 3			2281.25	1703.25	940	2319	
TRE		n	2	2	2	2	
		Quartile 1	612	596	1522	782	
		Median	3977.5	2856	3194.5	6339.5	
		Quartile 3*					
Bacteriological Culture	Bacteriological negative	CON	n	24	23	22	24
			Quartile 1	154	138	233.25	161.25
			Median	244.5	241	309	267.5
			Quartile 3	394.75	524	453.75	322.5
		TRE	n	28	28	28	28
			Quartile 1	155	160.5	159.25	112
			Median	282.5	403.5	294	254.5
			Quartile 3	538.25	834.75	677	537.25
	Bacteriological positive	CON	n	74	74	71	73
			Quartile 1	237.75	237.5	269	249.5
			Median	475.5	707.5	433	437
			Quartile 3	965.25	1527.25	1291	1669
		TRE	n	72	72	71	72
Quartile 1	200.5		224.5	237	167.25		
Median	574		594.5	705	387		

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			Quartile 3	1611.5	2095.5	2567	1430.25
Udder side	Left	CON	n	47	46	45	46
			Quartile 1	223	237.5	259.5	225.75
			Median	397	525.5	447	354.5
			Quartile 3	816	1532.5	1175	1058.75
		TRE	n	48	48	47	48
			Quartile 1	177	192.75	217	153
			Median	470	417	513	356.5
			Quartile 3	1412.5	1238.75	2003	750
	Right	CON	n	51	51	48	51
			Quartile 1	186	191	263	183
			Median	396	459	336.5	323
			Quartile 3	912	1151	834.5	952
		TRE	n	52	52	52	52
			Quartile 1	228.25	260.75	228.5	156.25
			Median	439.5	588.5	463	329.5
			Quartile 3	1260	2768.25	1963.25	1630
Parity	Primiparous	CON	n	13	13	11	13
			Quartile 1	157	335.5	162	295.5
			Median	240	751	419	705
			Quartile 3	1383	1338	2331	2375.5
		TRE	n	25	25	25	25
			Quartile 1	209	155.5	246.5	132.5
			Median	527	466	754	409
			Quartile 3	2798.5	3158	2602	963.5
	two lactations	CON	n	22	22	20	22
			Quartile 1	221	231.5	145.25	85
			Median	361.5	760	395.5	291.5
			Quartile 3	991.5	1817.75	1115.75	1529.25
		TRE	n	22	22	22	22
			Quartile 1	180.75	160.5	289.5	157.75
			Median	518	634	531	549.5
			Quartile 3	1564.5	1859.5	3466	1534.25
	> two lactations	CON	n	63	62	62	62
			Quartile 1	172	191	296.75	225.75
			Median	425	383	372	327.5
			Quartile 3	699	940.25	886.25	782
		TRE	n	53	53	52	53
			Quartile 1	180	222	194.5	164
			Median	369	490	372	280
			Quartile 3				

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Days in Milk	≤ 100	CON	Quartile 3	1170	1526	1436.25	785.5
			n	13	13	13	13
			Quartile 1	167	112	166.5	133.5
			Median	254	233	340	280
		TRE	Quartile 3	1064	919	612	1584.5
			n	25	25	25	25
			Quartile 1	165.5	232.5	188.5	164
			Median	448	508	754	298
	100 - 199	CON	Quartile 3	1557.5	3390	2203.5	1128.5
			n	23	23	20	23
			Quartile 1	215	316	245.75	226
			Median	326	696	414	779
		TRE	Quartile 3	1015	1750	1159.25	1652
			n	27	27	26	27
			Quartile 1	168	161	170	122
			Median	307	282	315.5	277
	≥ 200	CON	Quartile 3	1189	1556	2760	569
			n	62	61	60	61
			Quartile 1	190.5	214.5	280.25	217
			Median	427.5	489	401	303
		TRE	Quartile 3	719.5	1221	1150.5	653.5
			n	48	48	48	48
			Quartile 1	225	296.25	251.25	184.5
			Median	472	588.5	429	364.5
Quartile 3	1320.25	1305.25	1813	1306.25			

* Missing values due to low number of cases.

** Cows from this farm participated to TRE only.

*** Ear tag position found in TRE only.

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Table S2. Pairwise Comparisons of median quarter somatic cell counts (QSCC) over time on days (d) d0, d2, d4 and d6 in treatment (TRE) and control (CON) group.

Study Group	Time (I)	Time (J)	Mean Difference (I-J)	Standard Error	P-value	95% Confidence Interval for Difference	
						Lower Bound	Upper Bound
CON	d0	d2	-0.032	0.05	1.000	-0.16	0.09
		d4	-0.008	0.05	1.000	-0.13	0.11
		d4	0.010	0.05	1.000	-0.12	0.14
	d2	d0	0.032	0.05	1.000	-0.09	0.16
		d4	0.024	0.05	1.000	-0.11	0.15
		d6	0.041	0.05	1.000	-0.10	0.18
	d4	d0	0.008	0.05	1.000	-0.11	0.13
		d2	-0.024	0.05	1.000	-0.15	0.11
		d6	0.018	0.05	1.000	-0.11	0.15
	d6	d0	-0.010	0.05	1.000	-0.14	0.12
		d2	-0.041	0.05	1.000	-0.18	0.10
		d4	-0.018	0.05	1.000	-0.15	0.11
TRE	d0	d2	-0.410	0.05	1.000	-0.17	0.09
		d4	-0.017	0.04	1.000	-0.13	0.10
		d6	0.165*	0.05	0.009	0.03	0.30
	d2	d0	0.041	0.05	1.000	-0.09	0.17
		d4	0.024	0.05	1.000	-0.10	0.15
		d6	0.206*	0.05	0.000	0.07	0.34
	d4	d0	0.017	0.04	1.000	-0.10	0.13
		d2	-0.024	0.05	1.000	-0.15	0.10
		d6	0.182*	0.05	0.005	0.04	0.33
	d6	d0	-0.165*	0.05	0.009	-0.30	-0.03
		d2	-0.206*	0.05	0.010	-0.34	-0.07
		d4	-0.182*	0.05	0.005	-0.33	-0.04

* The mean difference is significant $P < 0.05$.

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Table S3. ANCOVA of quarter somatic cell counts over the time (days 0, 2, 4 and 6) in treatment (TRE) and control (CON) group including covariates (udder side, bacteriological analysis, ear tag position, parity, farm, season and days in milk (DIM)).

Study Group	Covariates	Type III Sum of Squares	Degrees of Freedom	Mean Square	F-value	P-value	Noncentrality Parameter	Observed Power
CON	Time	0.71	3	0.24	2.63	0.051	7.90	0.640
	Udder Side	0.19	3	0.06	0.69	0.562	2.05	0.194
	Bacteriological analysis	0.07	3	0.02	0.26	0.852	0.79	0.100
	Ear tag position	0.64	3	0.21	2.36	0.072	7.07	0.587
	Parity	0.65	3	0.22	2.40	0.068	7.20	0.596
	Farm	1.55	3	0.52	5.75	0.001*	17.25	0.948
	Season	0.90	3	0.30	3.32	0.020*	9.97	0.752
	Group DIM	0.66	3	0.22	2.43	0.066	7.30	0.602
TRE	Time	0.73	3	0.24	2.27	0.081	6.81	0.570
	Udder Side	0.64	3	0.21	1.98	0.118	5.93	0.506
	Bacteriological analysis	0.26	3	0.09	0.81	0.491	2.42	0.223
	Ear tag position	1.26	3	0.42	3.90	0.009*	11.71	0.824
	Parity	0.81	3	0.27	2.49	0.060	7.48	0.615
	Farm	0.70	3	0.23	2.16	0.093	6.48	0.546
	Season	0.39	3	0.13	1.20	0.310	3.60	0.321
	Group DIM	0.22	3	0.07	0.67	0.569	2.02	0.191

* The mean difference is significant $P < 0.05$.

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Table S4. Proportion of bacteriological negative and positive samples and identified pathogens in positive samples from quarter milk samples of control (CON) group and treatment (TRE) group on days (d) d0 and d6.

Study Group	Bacteriological results	d0		d6	
		n (%)	Proportion of the positive (%)	n (%)	Proportion of the positive (%)
CON	negative samples	47 (48.0)		38 (38.8)	
	positive samples	51 (52.0)		60 (61.1)	
	multiple isolates	12	23.5	7	11.7
	<i>E. coli</i>	1	2	2	3.3
	<i>Staph. aureus</i>	4	7.8	3	5
	environmental	1	2	11	18.3
	minor contagious	23	45.1	22	36.7
	major contagious	9	17.6	14	23.3
	fungus	1	2	1	1.7
	total	98 (100.0)	100	98 (100.0)	100
TRE	negative samples	41 (41.0)		40 (40.0)	
	positive samples	59 (59.0)		60 (60.0)	
	multiple isolates	21	35.6	25	41.7
	<i>E. coli</i>	2	3.4	3	5
	<i>Staph. aureus</i>	7	11.9	3	5
	environmental	2	3.4	10	16.7
	minor contagious	23	39	16	26.7
	major contagious	4	6.8	3	5
	fungus	0	0	0	0
	total	100 (100.0)	100	100 (100.0)	100

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Table S5. Pairwise Comparisons of quarter somatic cell counts over time on days (d) d0, d2, d4, and d6 in bacteriological positive samples in treatment (TRE) and control (CON) group.

Study Group	Time (I)	Time (J)	Mean Difference (I-J)	Std. Error	P-value	95% Confidence Interval for Difference	
						Lower Bound	Upper Bound
CON	d0	d2	-0.078	0.05	0.785	-0.22	0.06
		d4	-0.018	0.05	1.000	-0.16	0.12
		d6	-0.04	0.06	1.000	-0.20	0.12
	d2	d0	0.078	0.05	0.785	-0.06	0.22
		d4	0.06	0.06	1.000	-0.09	0.21
		d6	0.038	0.06	1.000	-0.12	0.20
	d4	d0	0.018	0.05	1.000	-0.12	0.16
		d2	-0.06	0.06	1.000	-0.21	0.09
		d6	-0.022	0.06	1.000	-0.18	0.14
	d6	d0	0.04	0.06	1.000	-0.12	0.20
		d2	-0.038	0.06	1.000	-0.20	0.12
		d4	0.022	0.06	1.000	-0.14	0.18
TRE	d0	d2	0.006	0.05	1.000	-0.14	0.15
		d4	-0.019	0.05	1.000	-0.15	0.11
		d6	0.179*	0.06	0.020	0.02	0.34
	d2	d0	-0.006	0.05	1.000	-0.15	0.14
		d4	-0.024	0.05	1.000	-0.16	0.11
		d6	0.174*	0.06	0.030	0.01	0.34
	d4	d0	0.019	0.05	1.000	-0.11	0.15
		d2	0.024	0.05	1.000	-0.11	0.16
		d6	0.198*	0.06	0.017	0.03	0.37
	d6	d0	-0.179*	0.06	0.020	-0.34	-0.02
		d2	-0.174*	0.06	0.030	-0.34	-0.01
		d4	-0.198*	0.06	0.017	-0.37	-0.03

* The mean difference is significant $P < 0.05$

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Table S6. Pairwise Comparisons of quarter somatic cell counts in bacteriological negative samples of treatment (TRE) and control (CON) group over time on days (d) d0, d2, d4, and d6.

Study Group	Time (I)	Time (J)	Mean Difference (I-J)	Standard Error	P-value	95% Confidence Interval for Difference	
						Lower Bound	Upper Bound
CON	d0	d2	0.122	0.10	1.000	-0.18	0.42
		d4	0.026	0.09	1.000	-0.23	0.28
		d6	0.175	0.09	0.316	-0.07	0.42
	d2	d0	-0.122	0.10	1.000	-0.42	0.18
		d4	-0.097	0.10	1.000	-0.38	0.18
		d6	0.053	0.10	1.000	-0.23	0.33
	d4	d0	-0.026	0.09	1.000	-0.28	0.23
		d2	0.097	0.10	1.000	-0.18	0.38
		d6	0.149	0.06	0.104	-0.02	0.32
	d6	d0	0.175	0.09	0.316	-0.42	0.07
		d2	0.053	0.10	1.000	-0.33	0.23
		d4	-0.149	0.06	0.104	-0.32	0.02
TRE	d0	d2	-0.161	0.10	0.674	-0.44	0.12
		d4	-0.014	0.09	1.000	-0.26	0.24
		d6	0.128	0.10	1.000	-0.16	0.41
	d2	d0	0.161	0.10	0.674	-0.12	0.44
		d4	0.146	0.09	0.733	-0.12	0.41
		d6	0.289*	0.09	0.014	0.05	0.53
	d4	d0	0.014	0.09	1.000	-0.24	0.26
		d2	-0.146	0.09	0.733	-0.41	0.12
		d6	0.142	0.10	0.908	-0.13	0.42
	d6	d0	-0.128	0.10	1.000	-0.41	0.16
		d2	-0.289*	0.09	0.014	-0.53	-0.05
		d4	-0.142	0.10	0.908	-0.42	0.13

* The mean difference is significant $P < 0.05$

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Table S7. Pairwise Comparisons of quarter somatic cell counts over time on days (d) d0, d2, d4 and d6 of ear tag position 10 in treatment (TRE) and control (CON) group.

Study Group	Time (I)	Time (J)	Mean Difference (I-J)	Std. Error	P-value	95% Confidence Interval for Difference	
						Lower Bound	Upper Bound
CON	d0	d2	-0.01	0.06	1.000	-0.18	0.16
		d4	-0.01	0.06	1.000	-0.17	0.15
		d6	0.10	0.06	0.474	-0.05	0.25
	d2	d0	0.01	0.06	1.000	-0.16	0.18
		d4	0.00	0.07	1.000	-0.18	0.18
		d6	0.11	0.06	0.505	-0.06	0.28
	d4	d0	0.01	0.06	1.000	-0.15	0.17
		d2	0.00	0.07	1.000	-0.18	0.18
		d6	0.11	0.05	0.224	-0.03	0.25
	d6	d0	-0.10	0.06	0.474	-0.25	0.05
		d2	-0.11	0.06	0.505	-0.28	0.06
		d4	-0.11	0.05	0.224	-0.25	0.03
TRE	d0	d2	-0.12	0.05	0.198	-0.26	0.03
		d4	-0.06	0.05	1.000	-0.20	0.08
		d6	0.10	0.06	0.664	-0.07	0.27
	d2	d0	0.12	0.05	0.198	-0.03	0.26
		d4	0.06	0.06	1.000	-0.10	0.22
		d6	0.218*	0.06	0.003	0.06	0.38
	d4	d0	0.06	0.05	1.000	-0.08	0.20
		d2	-0.06	0.06	1.000	-0.22	0.10
		d6	0.16	0.07	0.124	-0.02	0.34
	d6	d0	-0.10	0.06	0.664	-0.27	0.07
		d2	-0.218*	0.06	0.003	-0.38	-0.06
		d4	-0.16	0.07	0.124	-0.34	0.02

* The mean difference is significant $P < 0.05$

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SUPPLEMENTARY FILE

Table S8. Pairwise Comparisons of quarter somatic cell counts over time on days (d) d0, d2, d4 and d6 of ear tag position 9 in treatment (TRE) and control (CON) group.

Study Group	Time (I)	Time (J)	Mean Difference (I-J)	Std. Error	P-value	95% Confidence Interval for Difference	
						Lower Bound	Upper Bound
CON	d0	d2	-0.164	0.060	0.088	-0.344	0.016
		d4	-0.093	0.082	1.000	-0.338	0.151
		d6	-0.268	0.109	0.153	-0.594	0.059
	d2	d0	0.164	0.060	0.088	-0.016	0.344
		d4	0.070	0.068	1.000	-0.132	0.272
		d6	-0.104	0.101	1.000	-0.406	0.198
	d4	d0	0.093	0.082	1.000	-0.151	0.338
		d2	-0.070	0.068	1.000	-0.272	0.132
		d6	-0.174	0.126	1.000	-0.550	0.201
	d6	d0	0.268	0.109	0.153	-0.059	0.594
		d2	0.104	0.101	1.000	-0.198	0.406
		d4	0.174	0.126	1.000	-0.201	0.550
TRE	d0	d2	0.161	0.258	1.000	-0.776	1.098
		d4	0.197	0.190	1.000	-0.495	0.890
		d6	0.730*	0.138	0.007	0.228	1.233
	d2	d0	-0.161	0.258	1.000	-1.098	0.776
		d4	0.037	0.081	1.000	-0.259	0.332
		d6	0.570	0.216	0.200	-0.214	1.353
	d4	d0	-0.197	0.190	1.000	-0.890	0.495
		d2	-0.037	0.081	1.000	-0.332	0.259
		d6	0.533	0.159	0.073	-0.045	1.111
	d6	d0	-0.730*	0.138	0.007	-1.233	-0.228
		d2	-0.570	0.216	0.200	-1.353	0.214
		d4	-0.533	0.159	0.073	-1.111	0.045

* The mean difference is significant $P < 0.05$

Bibliography

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