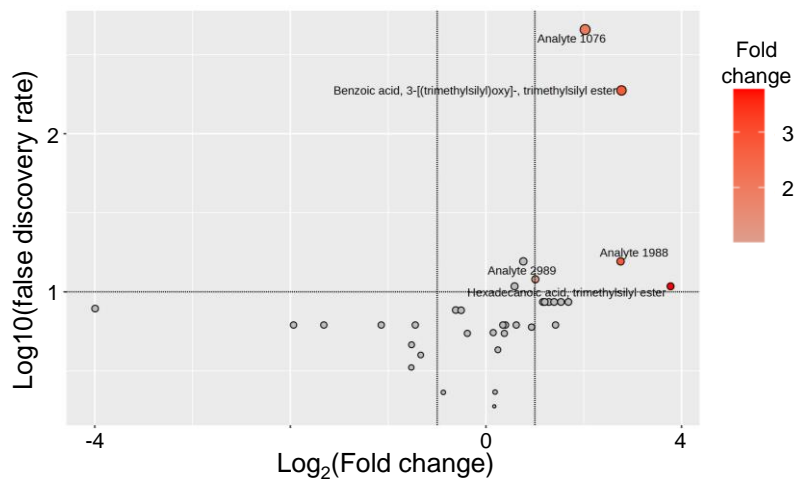


Supplementary Fig. 1. An overview of the data processing and analyses. PCA, principal component analysis; FC, fold change



Supplementary Fig. 2. Volcano plot of the serum metabolic profiles comparing the MF and normal groups.

The red and blue dots represent the up- and down-regulated metabolites in the MF group compared to the normal group. The two vertical lines indicate the  $\log_2$  (fold change)  $\pm 1$ . The horizontal dotted line indicates the false discovery rate cutoff of  $<0.05$ .

Supplementary Table 1. Serum calcium concentrations of the 25 cows.

ID	Health category (Diagnosis)	Serum calcium (mg/dL)	Date of postparturient collected serum
cow1	MF	3.6	-22
cow2	MF	5.4	-20
cow3	Normal	7.9	-23
cow4	Normal	6.4	-23
cow5	MF	4.5	-18
cow6	MF	4	-20
cow7	Normal	7.1	-20
cow8	Normal	7.3	-23
cow9	Normal	6.4	-22
cow10	Normal	9.3	-22
cow11	Normal	8.1	-21
cow12	Normal	6.6	-23
cow13	Normal	6.3	-20
cow14	Normal	8.7	-20
cow15	Normal	7.6	-19
cow16	MF	5	-19
cow17	Normal	7.9	-19
cow18	Normal	6.7	-21
cow19	Normal	8.8	-19
cow20	MF	5.6	-20
cow21	MF	4.8	-19
cow22	MF	5.4	-21
cow23	Normal	7.5	-22
cow24	Normal	7.8	-21
cow25	Normal	10.3	-20





































Supplementary Table 3. The serum compounds with a correlation coefficient of more than 0.5 or less than -0.5 with serum calcium concentrations in the 25 cows.

1st	2nd	Compounds	correlation coefficient
455.6	1.2	Analyte 214	-0.51
502.6	1.0	Disiloxane, hexamethyl-	0.51
505.0	0.9	Analyte 431	-0.54
568.3	0.9	Disiloxane, hexamethyl-	0.51
595.3	1.3	Methylenecyclopropane, 2-(1-hydroxyethyl)-2-trimethylsilyl-	-0.55
599.0	1.5	Analyte 664	-0.52
640.0	1.2	2-Methyl-1,3-propanediol 2TMS	0.51
675.5	1.4	Propanoic acid, 2-(methoxyimino)-, trimethylsilyl ester	-0.50
691.3	1.3	Analyte 814	0.50
709.6	1.3	Analyte 869	-0.59
790.1	1.4	Butanoic acid, 2-methyl-2-[(trimethylsilyloxy)-, trimethylsilyl ester	-0.52
816.3	1.5	Analyte 1039	-0.56
820.5	1.4	Silane, trimethyl(3-methylphenoxy)-	-0.56
833.4	1.4	Butanoic acid, 2-[(trimethylsilyloxy)-, trimethylsilyl ester	-0.57
835.8	1.0	Analyte 1076	-0.60
875.7	2.1	Monomethylphosphate 2TMS	-0.56
1012.1	0.9	Acetamide, 2,2,2-trifluoro-N-methyl-	0.55
1047.2	3.0	Analyte 1590	-0.51
1231.9	1.6	Analyte 1900	0.51
1232.1	0.9	Analyte 1894	0.54
1257.4	1.6	Analyte 1953	-0.51
1282.2	1.4	Analyte 1988	-0.55
1294.3	1.7	Analyte 2007	0.59
1373.8	1.5	Benzoic acid, 3-[(trimethylsilyloxy)-, trimethylsilyl ester	-0.55
1423.9	1.4	Analyte 2212	0.50
1440.3	1.4	Analyte 2245	-0.54
1475.3	1.9	Analyte 2286	-0.51
1675.6	1.6	Analyte 2619	-0.50
1710.0	1.6	Hexadecanoic acid, trimethylsilyl ester	-0.57
1733.5	1.3	Analyte 2806	-0.51
1795.3	1.7	Analyte 2974	0.55
1801.0	1.6	Analyte 2989	-0.54
1803.9	2.5	Analyte 3004	0.55
2491.5	1.6	Analyte 4019	-0.52
2590.0	0.9	Analyte 4279	0.51

1st: The retention time on the first column

2nd: The retention time on the second column

Analytes mean unidentified compounds.

Supplementary Table 4. The serum compounds with statistically significant and two-fold larger or half-fold smaller in the 25 cows.

1st	2nd	Compounds	Fold Change
835.8	1.0	Analyte 1076	4.1
1282.2	1.4	Analyte 1988	6.7
1373.8	1.5	Benzoic acid, 3-[(trimethylsilyl)oxy]-, trimethylsilyl ester	6.8
1710.0	1.6	Hexadecanoic acid, trimethylsilyl ester	13.7
1801.0	1.6	Analyte 2989	2.0

1st: The retention time on the first column

2nd: The retention time on the second column

Analytes mean unidentified compounds.