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Supplementary Material

Effects of long-term dietary supplementation with conjugated linoleic acid on bovine oocyte lipid profile

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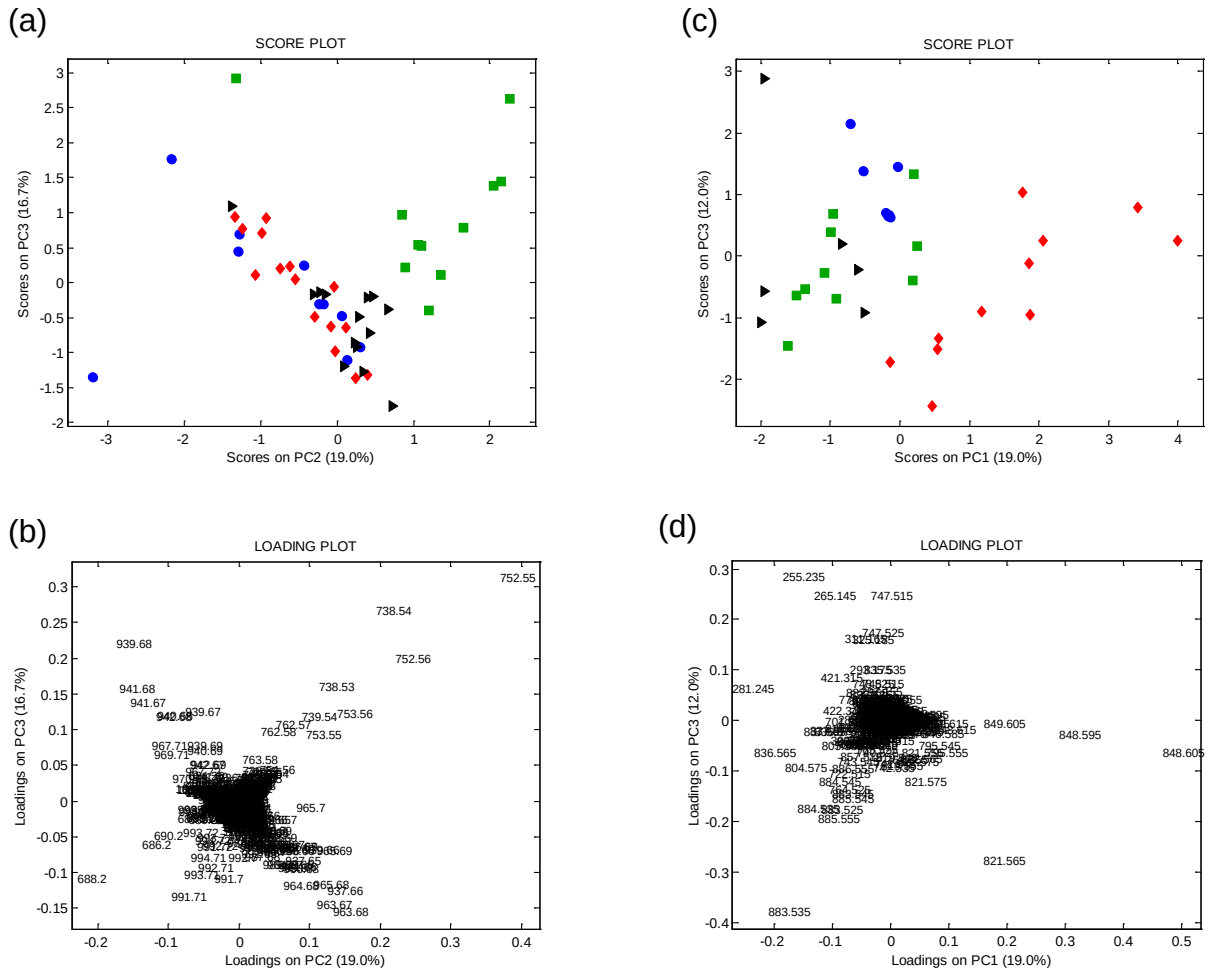


Fig. S1. PCAs from DESI-MS analysis of immature and IVM oocytes collected from 100 g/d CLA and SA supplemented animals. Positive ions: PC2 *vs.* PC3 score plot (**a**). PC2 *vs.* PC3 loading plot labeled in terms of *m/z* ratio (**b**). Negative ions: PC1 *vs.* PC3 score plot (**c**). PC1 *vs.* PC3 loading plot labeled in terms of *m/z* ratio (**d**). Blue circle: SA immature oocytes; red diamonds: CLA immature oocytes; green squares: SA *in vitro* matured oocytes, and black triangles: CLA *in vitro* matured oocytes.

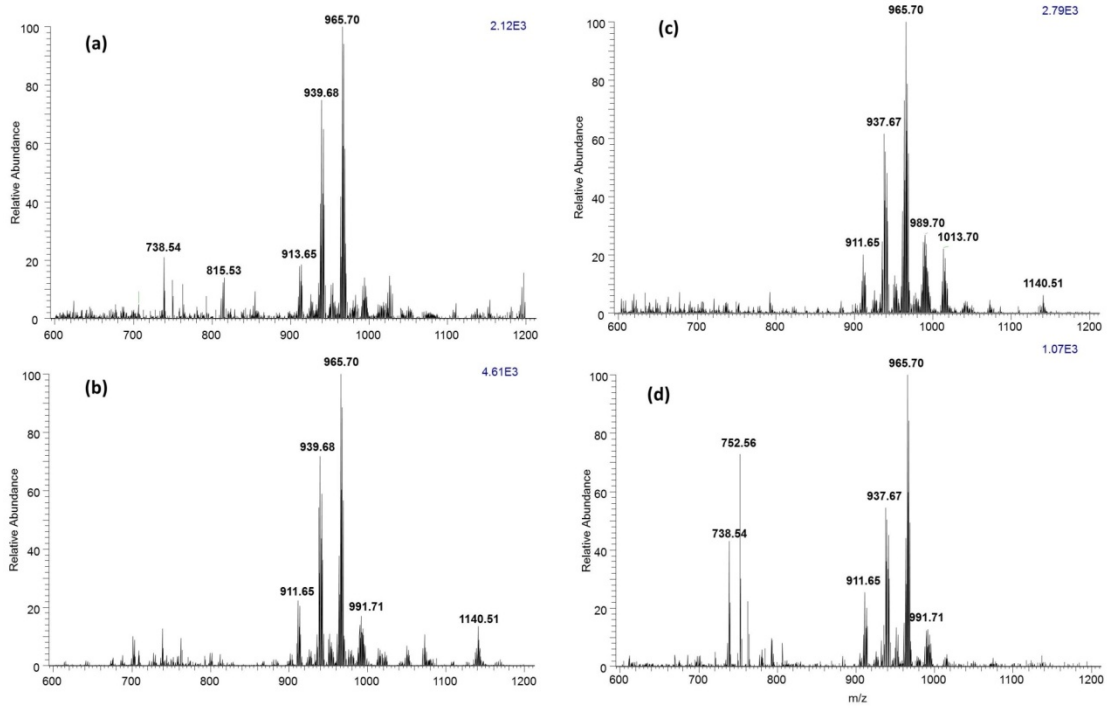


Fig. S2. Representative DESI-MS. Mass spectra in the positive ion mode of CLA-100 immature oocytes (**a**) and SA-100 immature oocytes (**b**); CLA-100 *in vitro* matured oocytes (**c**) and SA-100 *in vitro* matured oocytes (**d**).

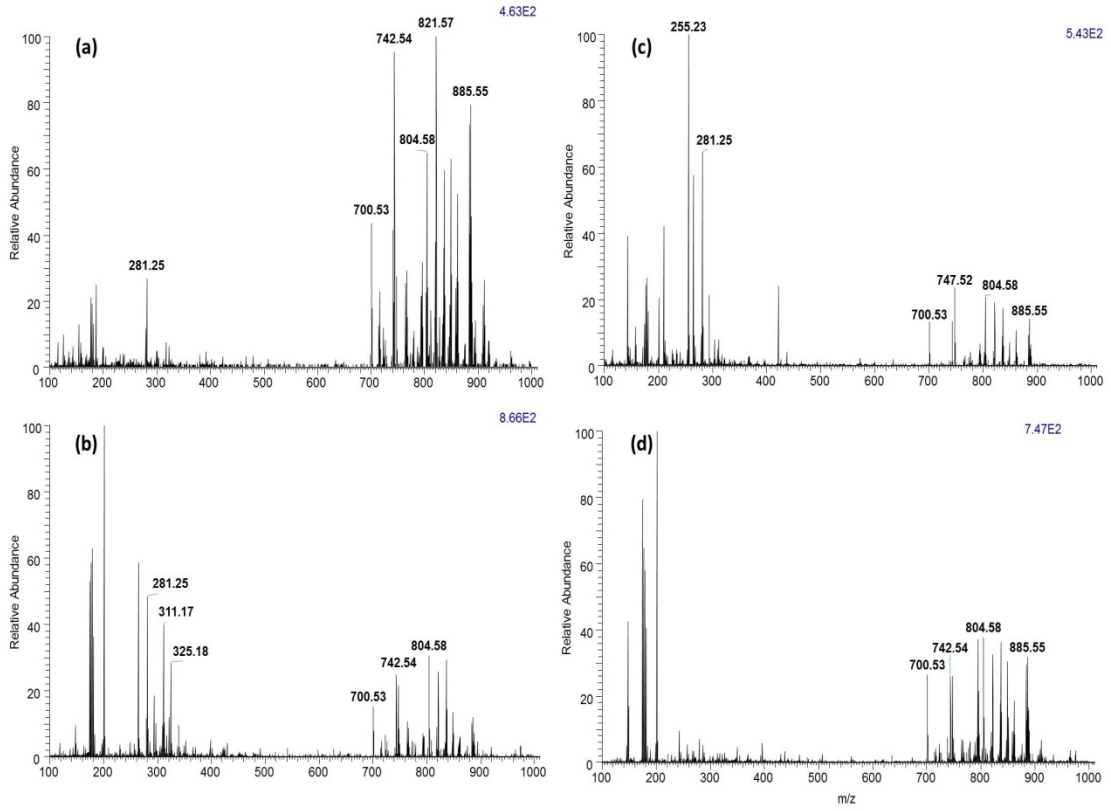
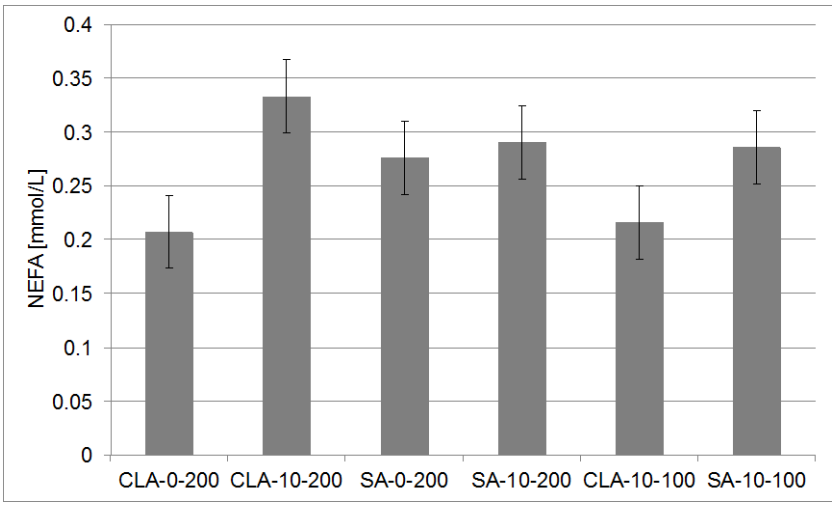
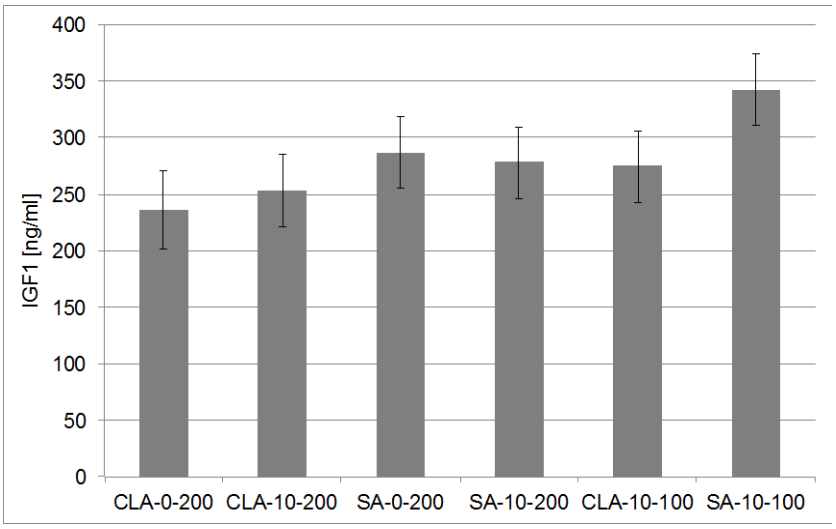


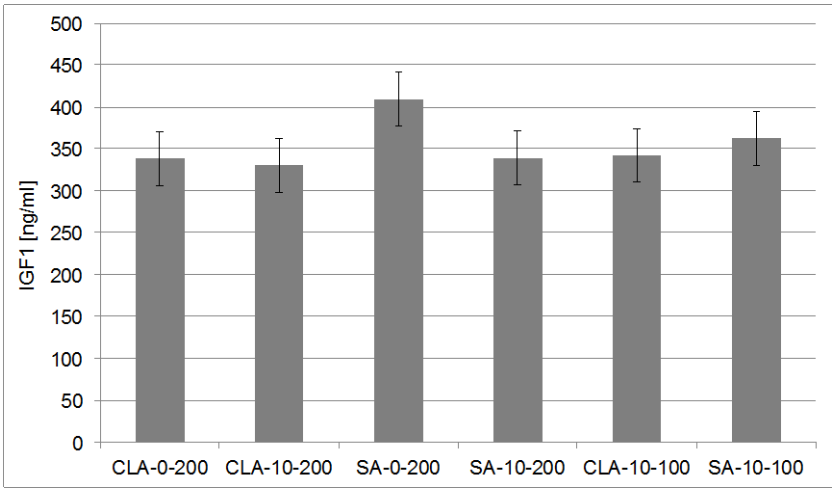
Fig. S3. Representative DESI-MS. Mass spectra in the negative ion mode of CLA-100 immature oocytes (**a**) and SA-100 immature oocytes (**b**); CLA-100 *in vitro* matured oocytes (**c**) and SA-100 *in vitro* matured oocytes (**d**).


NEFA [mmol/L]
0.4
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
CLA-0-200
CLA-10-200
SA-0-200
SA-10-200
CLA-10-100
SA-10-100

(a)


IGF1 [ng/ml]
400
350
300
250
200
150
100
50
0
CLA-0-200
CLA-10-200
SA-0-200
SA-10-200
CLA-10-100
SA-10-100

(b)


IGF1 [ng/ml]
500
450
400
350
300
250
200
150
100
50
0
CLA-0-200
CLA-10-200
SA-0-200
SA-10-200
CLA-10-100
SA-10-100

(c)

Fig. S4. Non-esterified fatty acids and IGF1 analysis. Plasma samples were taken at the onset of 200 g/d fatty acid supplementation (CLA-0-200, SA-0-200) and ten weeks after either 200 g/d supplementation (CLA-10-200, SA-10-200) or 100 g/d supplementation (CLA-10-100, SA-10-100). NEFA plasma concentration after 100 g/d and 200 g/d CLA and SA supplementation (**a**). IGF1 plasma concentration after 100g/d and 200g/d CLA and SA supplementation (**b**). IGF1 follicular fluid concentration (**c**) after 100g/d and 200g/d CLA and SA supplementation.

Table S1. Chemical composition of concentrates and grass silage

	Concentrates				Grass Silage
	CLA-100 [*n*=20]	CLA-200 [*n*=3]	SA-100 [*n*=16]	SA-200 [*n*=3]	[*n*=17]
Dry matter [g/kg]	880 ± 0.4	891 ± 0.5	881 ± 0.3	891 ± 0.6	271 ± 5
Nutrients [g/kg DM]					
Total ash	66 ± 6	70 ± 3	63 ± 3	56 ± 3	98 ± 23
Crude protein	176 ± 10	154 ± 0.2	183 ± 11	153 ± 2	123 ± 9
Ether extract	62 ± 6	102 ± 6	68 ± 13	108 ± 18	36 ± 5
Crude fiber	88 ± 7	97 ± 5	87 ± 6	97 ± 4	239 ± 10
Acid detergent fiber	125 ± 11	130 ± 2	130 ± 6	151 ± 15	279 ± 11
Neutral detergent fiber	261 ± 11	261 ± 2	268 ± 10	276 ± 12	481 ± 20

Table S2. List of primers used for the quantitative PCR analysis

Genes	Primer (Position)	Sequences (5′- 3′)	Amplicon length (bp)	Accession no.	Reference
IGF1R	fw (1589-1611) rev (1660-1642)	CCTCATCAGCTTCACCGTCTACT GCGTCCTGCCCGTCATACT	72	NM_001244612.1	–
GJA1	fw (630-649) rev (709-686)	AGCACGGCAAGGTGAAGATG CACCTCGAAGACAGACTTGAAGAG	80	NM_174068.2	–
FASN	fw (6418-6438) rev (6496-6515)	AGCTTTGTGTTGGCAGAGAAG AGTCACGGATGCCCAGGAT	97	NM_001012669	–
SREBP1	fw (143-162) rev (195-209)	CCAGCTGACAGCTCCATTGA TGCGCGCCACAAGGA	67	AW462480	(Loor *et al.* 2005)
SCAP	fw (332-349) rev (482-501)	GGTCACTTTCCGGGATGG TGGGTAGCAGCAGGCTAAGA	169	NM_001101889.1	(Viturro *et al.* 2009)
Globin	fw (241-260) rev (548-569)	GCAGCCACGGTGGCGAGTAT GTGGGACAGGAGCTTGAAAT	256	X04751	(Cheng *et al.* 1986)

Table S3. Erythrocyte fatty acid profiles by gas chromatographic analysis

Samples were collected at the onset (CLA-0-100 and SA-0-100) and ten weeks after (CLA-10-100 and SA-10-100) 100 g/d of CLA and SA supplementation. Results are expressed as fatty acid methyl esters percentage (% FAME ± s.e.m.). Different superscripts among the same ion indicate significant differences ($P < 0.05$)

	Fatty acid Profile			
	FAME [%]			
Ions	**CLA-0-100**	**CLA-10-100**	**SA-0-100**	**SA-10-100**
C15iso	0.16 ± 0.03^{a}	0.17 ± 0.03^{a}	0.11 ± 0.03^{a}	0.19 ± 0.03^{a}
C16:0	8.74 ± 0.94^{a}	7.87 ± 0.94^{a}	8.63 ± 0.94^{a}	8.89 ± 0.94^{a}
C18:0	12.25 ± 1.11^{a}	12.3 ± 1.11^{a}	12.10 ± 1.11^{a}	13.64 ± 1.11^{a}
C18:1*c9*	43.64 ± 1.96^{a}	37.77 ± 1.96^{a}	44.41 ± 1.96^{a}	37.72 ± 1.96^{a}
C18:2*t9,c12*	0.08 ± 0.01^{a}	0.11 ± 0.01^{a}	0.08 ± 0.01^{a}	0.09 ± 0.01^{a}
C18:3*c9,c12,c15*	1.08 ± 0.40^{a}	3.23 ± 0.40^{b}	1.10 ± 0.40^{a}	2.51 ± 0.40^{ab}
C19:0	0.02 ± 0.01^{ab}	0.09 ± 0.01^{bc}	0.02 ± 0.01^{a}	0.09 ± 0.01^{c}
C20:3*c8,c11,c14*	0.41 ± 0.09^{a}	0.86 ± 0.09^{b}	0.38 ± 0.09^{a}	0.67 ± 0.09^{ab}
C20:4*c5,c8,c11,c14*	3.18 ± 0.89^{a}	5.19 ± 0.89^{a}	3.31 ± 0.89^{a}	3.92 ± 0.89^{a}
C20:4*n3*	0.07 ± 0.04^{a}	0.34 ± 0.04^{b}	0.06 ± 0.04^{a}	0.21 ± 0.044^{ab}
C22:5*n3*	0.07 ± 0.05^{a}	0.07 ± 0.05^{a}	0.04 ± 0.05^{a}	0.07 ± 0.05^{a}
C22:6*n3*	0.09 ± 0.03^{a}	0.21 ± 0.03^{b}	0.11 ± 0.03^{ab}	0.14 ± 0.033^{ab}
C24:0	1.67 ± 0.27^{a}	1.06 ± 0.27^{a}	1.71 ± 0.27^{a}	1.53 ± 0.27^{a}
CLA *c9,t11*	0.79 ± 0.09^{a}	0.55 ± 0.09^{a}	0.78 ± 0.09^{a}	0.62 ± 0.09^{a}
CLA *t10,c12*	0.02 ± 0.005^{a}	0.03 ± 0.005^{a}	0.02 ± 0.005^{a}	0.03 ± 0.005^{a}
SumCLA	0.81 ± 0.09^{a}	0.58 ± 0.09^{a}	0.81 ± 0.09^{a}	0.65 ± 0.09^{a}
MUFA	47.71 ± 1.93^{a}	41.62 ± 1.96^{a}	48.43 ± 1.93^{a}	41.71 ± 1.93^{a}
PUFA	15.78 ± 2.78^{ab}	27.12 ± 2.78^{b}	16.37 ± 2.78^{a}	21.89 ± 2.78^{ab}
SFA	25.95 ± 2.44^{a}	24.16 ± 2.44^{a}	26.57 ± 2.44^{a}	27.48 ± 2.44^{a}
n3	2.02 ± 0.63^{a}	5.63 ± 0.63^{b}	1.99 ± 0.63^{a}	4.14 ± 0.63^{ab}
n6	12.04 ± 2.24^{a}	19.95 ± 2.24^{a}	12.66 ± 2.24^{a}	16.03 ± 2.24^{a}
n3:n6	6.73 ± 0.54^{a}	3.82 ± 0.54^{b}	6.62 ± 0.54^{a}	4.08 ± 0.54^{b}

Table S4. Erythrocyte fatty acid profiles by gas chromatographic analysis

Samples were collected at the onset (CLA-0-200 and SA-0-200) and ten weeks after (CLA-10-200 and SA-10-200) 200 g/d of CLA and SA supplementation. Results are expressed as fatty acid methyl esters percentage (% FAME ± S.E.M.). Different superscripts among the same ion indicate significant differences ($P < 0.05$).

	Fatty acid Profile
	FAME [%]

Ions	CLA-0-200	CLA-10-200	SA-0-200	SA-10-200
C15iso	0.08 ± 0.02^{a}	0.11 ± 0.02^{a}	0.06 ± 0.02^{a}	0.11 ± 0.02^{a}
C16:0	7.25 ± 0.79^{a}	6.61 ± 0.79^{a}	6.72 ± 0.79^{a}	7.14 ± 0.79^{a}
C18:0	9.33 ± 0.61^{a}	12.39 ± 0.61^{a}	11.36 ± 0.61^{ab}	11.87 ± 0.61^{b}
C18:1*c9*	53.23 ± 2.20^{a}	41.22 ± 2.20^{b}	53.28 ± 2.20^{a}	42.14 ± 2.20^{b}
C18:1*t12*	0.06 ± 0.006^{a}	0.08 ± 0.006^{a}	0.09 ± 0.006^{a}	0.06 ± 0.006^{a}
C18:1*t11*	0.16 ± 0.01^{a}	0.24 ± 0.011^{bc}	0.19 ± 0.01^{ab}	0.23 ± 0.01^{bc}
C18:2*c9,c12*	11.44 ± 0.94^{a}	18.07 ± 0.94^{b}	11.45 ± 0.94^{a}	16.55 ± 0.94^{b}
C18:3*c9,c12,c15*	1.46 ± 0.42^{a}	3.77 ± 0.42^{b}	1.29 ± 0.42^{a}	3.36 ± 0.42^{b}
C19:0	0.1 ± 0.02^{a}	0.13 ± 0.02^{a}	0.04 ± 0.02^{a}	0.13 ± 0.02^{a}
C20:3*c8,c11,c14*	0.66 ± 0.08^{ab}	0.96 ± 0.08^{c}	0.60 ± 0.08^{a}	1.002 ± 0.08^{bc}
C20:4*c5,c8,c11,c14*	6 ± 0.55^{a}	6.24 ± 0.55^{a}	5.27 ± 0.55^{a}	6.54 ± 0.55^{a}
C20:4*n3*	0.14 ± 0.06^{ab}	0.40 ± 0.06^{c}	0.12 ± 0.063^{a}	0.36 ± 0.06^{b}
C22:5*n3*	0.63 ± 0.07^{ab}	0.72 ± 0.07^{ab}	0.54 ± 0.07^{b}	0.90 ± 0.07^{a}
C22:6*n3*	0.15 ± 0.02^{a}	0.18 ± 0.01^{ab}	0.13 ± 0.02^{a}	0.25 ± 0.02^{b}
C24:0	0.60 ± 0.03^{a}	0.23 ± 0.03^{b}	0.32 ± 0.03^{b}	0.22 ± 0.03^{b}
CLA *c9,t11*	0.34 ± 0.04^{a}	0.44 ± 0.04^{a}	0.41 ± 0.04^{a}	0.40 ± 0.04^{a}
CLA *t10,c12*	0.008 ± 0.005^{a}	0.04 ± 0.005^{b}	0 ± 0.005^{a}	0.006 ± 0.005^{a}
SumCLA	0.41 ± 0.04^{a}	0.49 ± 0.04^{a}	0.44 ± 0.04^{a}	0.41 ± 0.04^{a}
MUFA	57.12 ± 2.20^{a}	44.70 ± 2.20^{b}	57.21 ± 2.20^{a}	45.66 ± 2.20^{b}
PUFA	22.69 ± 1.52^{a}	33.38 ± 1.52^{b}	21.32 ± 1.52^{a}	32.19 ± 1.52^{b}
SFA	20.19 ± 1.49^{a}	21.92 ± 1.49^{a}	21.47 ± 1.49^{a}	22.15 ± 1.49^{a}
n3	3.84 ± 0.50^{a}	7.11 ± 0.50^{b}	3.25 ± 0.50^{a}	7.14 ± 0.50^{b}
n6	18.44 ± 1.120^{a}	25.79 ± 1.12^{b}	17.62 ± 1.129^{a}	24.63 ± 1.12^{b}
n3:n6	4.85 ±	3.75 ± 0.26^{b}	5.48 ± 0.26^{a}	3.46 ± 0.26^{b}

	0.26[a]

Table S5. Follicular fluid fatty acid profiles by gas chromatographic analysis

Samples were collected at the onset (CLA-0-200 and SA-0-200) and ten weeks after (CLA-10-200 and SA-10-200) diet supplementation with 200 g/d of CLA and SA. Results are expressed as fatty acid methyl esters percentage (% FAME ± s.e.m.). Different superscripts among the same ion indicate significant differences ($P < 0.05$)

	Fatty acid Profile			
	FAME [%]			
Ions	**CLA-0-200**	**CLA-10-200**	**SA-0-200**	**SA-10-200**
C15iso	0.36 ± 0.02^{ab}	0.3 ± 0.02^{c}	0.39 ± 0.02^{a}	0.33 ± 0.02^{bc}
C16:0	12.12 ± 0.41^{a}	11.03 ± 0.41^{a}	12.45 ± 0.41^{a}	11.04 ± 0.41^{a}
C18:0	11.64 ± 0.72^{a}	15.54 ± 0.72^{b}	10.23 ± 0.72^{a}	14.78 ± 0.72^{b}
C18:1*c9*	13.78 ± 0.88^{a}	13.55 ± 0.88^{a}	13.4 ± 0.88^{a}	13 ± 0.88^{a}
C18:1*t12*	0.08 ± 0.01^{ab}	0.12 ± 0.01^{a}	0.08 ± 0.01^{b}	0.08 ± 0.01^{b}
C18:1*t11*	0.23 ± 0.02^{a}	0.36 ± 0.02^{b}	0.22 ± 0.02^{a}	0.28 ± 0.02^{a}
C18:2*c9,c12*	31.74 ± 1.37^{a}	29.93 ± 1.37^{a}	32.53 ± 1.37^{a}	29.61 ± 1.37^{a}
C18:3*c9,c12,c15*	9.52 ± 0.39^{ab}	10.98 ± 0.39^{a}	9.52 ± 0.39^{b}	10.37 ± 0.39^{ab}
C19:0	0.19 ± 0.02^{a}	0.24 ± 0.02^{a}	0.24 ± 0.02^{a}	0.27 ± 0.02^{a}
C20:3*c8,c11,c14*	1.99 ± 0.14^{ab}	1.54 ± 0.14^{b}	2.09 ± 0.14^{ab}	2.32 ± 0.14^{a}
C20:4*c5,c8,c11,c14*	4.55 ± 0.21^{ab}	3.29 ± 0.21^{c}	4.87 ± 0.21^{a}	4.09 ± 0.21^{bc}
C20:4*n3*	0.76 ± 0.07^{ab}	0.86 ± 0.07^{ab}	0.72 ± 0.07^{a}	1.04 ± 0.07^{b}
C22:5*n3*	1.41 ± 0.09^{a}	1.03 ± 0.09^{b}	1.38 ± 0.09^{ab}	1.27 ± 0.09^{ab}
C22:6*n3*	0.41 ± 0.03^{a}	0.28 ± 0.03^{c}	0.44 ± 0.03^{ab}	0.37 ± 0.03^{bc}
C24:0	0.02 ± 0.004^{a}	0.05 ± 0.004^{b}	0.04 ± 0.004^{b}	0.05 ± 0.004^{b}
CLA *c9,t11*	0.1 ± 0.02^{a}	0.16 ± 0.02^{a}	0.11 ± 0.02^{a}	0.12 ± 0.02^{a}
CLA *t10,c12*	0.01 ± 0.02^{a}	0.09 ± 0.02^{b}	0 ± 0.02^{a}	0.04 ± 0.02^{ab}
SumCLA	0.11 ±	0.25 ± 0.03^{b}	0.11 ±	0.16 ± 0.03^{ab}

	0.03[ab]		0.03[a]	
MUFA	18.09 ± 0.96[a]	17.72 ± 0.96[a]	18.22 ± 0.96[a]	17.03 ± 0.96[a]
PUFA	53.36 ± 1.62[a]	50.82 ± 1.62[a]	54.3 ± 1.62[a]	52.42 ± 1.62[a]
SFA	28.55 ± 0.95[ab]	31.45 ± 0.95[b]	27.48 ± 0.95[a]	30.55 ± 0.95[ab]
n3	13.68 ± 0.39[a]	14.72 ± 0.39[a]	13.25 ± 0.39[a]	14.94 ± 0.39[a]
n6	39.57 ± 1.41[a]	35.85 ± 1.41[a]	40.94 ± 1.41[a]	37.32 ± 1.41[a]
n3:n6	2.9 ± 0.09[ab]	2.44 ± 0.09[bc]	3.09 ± 0.09[a]	2.5 ± 0.09[c]