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Dietary α-linolenic acid from flaxseed oil improved folliculogenesis and IVF performance in dairy cows, similar to eicosapentaenoic and docosahexaenoic acids from fish oil

U Moallem[1], A Shafran[1,2], M Zachut[1], I Dekel[1], Y Portnick[1] and A Arieli[2]

[1]*Department of Ruminant Science, Institute of Animal Sciences, the Volcani Center, PO Box 6, Bet-Dagan 50250, Israel and* [2]*Department of Animal Science, the Robert H. Smith Faculty of Agriculture, Food and Environmental Quality Sciences, the Hebrew University of Jerusalem, Rehovot 76100, Israel*

Correspondence should be addressed to U Moallem; Email: uzim@volcani.agri.gov.il

Abstract

The objectives of this study were to determine the differential incorporation of various omega-3 (n-3) fatty acids (FAs) supplemented to dairy cows into ovarian compartments and assess the effects on IVF. Forty-two 256-day pregnant cows were supplemented with encapsulated fats, in treatments designated as i) SFA – saturated fat at 240 and 560 g/day per cow, *prepartum* and *post partum* (PP) respectively; ii) FLX – flaxseed oil at 300 and 700 g/day per cow *prepartum* and PP respectively; and iii) FO – fish oil at 300 and 700 g/day per cow *prepartum* and PP respectively. Commencing at 60 days in lactation, ovum pickup (OPU) was performed twice weekly (20 sessions; five cows per group) and *in vitro* maturation and IVF were conducted. The proportion of α-linolenic acid (ALA) was greater in follicular fluid (FF), granulosa cells, and cumulus–oocyte complexes (COCs) of FLX cows than in other groups ($P<0.001$). The proportion of docosahexaenoic acid (DHA) was 6.7 times as great in FF of FO as in other groups ($P<0.001$); docosapentaenoic acid n-3 and DHA were detected in COCs of FO but not in others. The follicle number during OPU was higher in FLX and FO than in SFA ($P<0.05$), and the oocyte cleavage rate was higher in FLX and FO than in SFA ($P<0.01$). Also, the percentage of oocytes that developed to blastocysts tended to be higher in both n-3 groups than in SFA ($P<0.1$). In conclusion, both dietary n-3 FAs similarly improved folliculogenesis and IVF performance; therefore, ALA-rich botanical n-3 seems to be a satisfactory approach to improve oocyte quality.
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Introduction

In recent years, it has become well accepted that dietary fat may have beneficial effects on fertility of dairy cows directly rather than by improving the cows' energy balance (Staples *et al*. 1998, Mattos *et al*. 2000). Dietary fatty acids (FAs) may influence the functionality of reproductive tissues by changing cell membrane composition and, consequently, membrane fluidity. Membrane fluidity can affect the transfer of nutrients and other biological factors into the cell and thereby influence physiological functions of the tissue, as shown by Zeron *et al*. (2002). FAs may also be involved in reproductive processes as precursors of steroid hormones (via cholesterol) and of prostaglandins (via arachidonic acid (AA)). Among FA families, the polyunsaturated FAs (PUFAs) were demonstrated to be mediators in various reproductive processes, and in the last decade, major research was focused on the effects of omega-6 (n-6) and especially omega-3 (n-3) FAs.

Several studies have investigated the effects of specific PUFAs on follicular development and preovulatory follicle characteristics, and they yielded inconsistent results: Robinson *et al*. (2002) observed an increased number of medium-sized follicles in cows fed diets rich in C18:2n-6 or C18:3n-3, whereas Ponter *et al*. (2006) reported fewer small follicles in cows supplemented with flaxseed (rich in C18:3n-3) than in cows fed soybean (rich in C18:2n-6). In other studies, cows (Heravi Moussavi *et al*. 2007) and ewes (Zeron *et al*. 2002) supplemented with fish oil (FO) demonstrated an increased number of small follicles than control animals. However, Petit *et al*. (2004) found no effect on follicle growth in cows fed flaxseed, which was in agreement with the findings of Wonnacott *et al*. (2010) in ewes fed n-3 or n-6 PUFAs.

The effects of long-chain FAs on oocyte quality and on fertilization were also examined in several previous studies. Leroy *et al*. (2005) demonstrated adverse effects of saturated FAs – palmitic (C16:0) and stearic (C18:0) acids, on cleavage and development rates of blastocysts *in vitro*. In a study in which cows were fed with a variety of mono- and PUFAs during the summer, Bilby *et al*. (2006) observed no influence on quality of oocytes collected by ovum pickup (OPU), but Cerri *et al*. (2009)

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found a tendency for increased fertilization of oocytes and improved embryo quality in cows fed C18:2n-6 and C18:1-*trans*. In a study by Fouladi-Nashta *et al.* (2009*b*), inert fat containing predominantly palmitic (C:16) and oleic (C:18:1n-9) acids increased the proportion of cleaved embryos compared with soya or flaxseed oil (FLX).

In a recent study conducted in our laboratory, dietary microencapsulated FLX (rich in α-linolenic acid (ALA)) increased the numbers of small follicles and follicles collected by OPU, and also enhanced the cleavage rate of IVF oocytes compared with the control group (Zachut *et al.* 2010). Animals cannot synthesize n-3 FAs *de novo*; therefore, these FAs need to be supplied in the diet (Wathes *et al.* 2007); short-chain n-3 FAs can be elongated and desaturated to form long-chain n-3 FAs (Mattos *et al.* 2000). In humans, FO forms the basis of the most popular nutritional supplements; it provides the long-chain n-3 FAs – eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) – which are considered more effective than ALA (Simopoulos 2002). However, in ruminants, FO has been found to suppress feed intake in many studies (Donovan *et al.* 2000, AbuGhazaleh *et al.* 2002, Whitlock *et al.* 2002); therefore, botanical n-3 sources, such as ALA from flaxseed, might be preferable to animal sources in ruminant nutrition. Therefore, the objectives of this study were to determine the differential incorporation of two dietary n-3 sources – FLX rich in ALA and FO rich in EPA and DHA – into plasma, milk, and ovarian compartments and to assess the effects on folliculogenesis, oocyte quality, and IVF performance.

Materials and methods

Cows and diets

The procedures used were approved by the Volcani Center Animal Care Committee. The study was conducted at the Volcani Center experimental farm in Bet Dagan, Israel, and was scheduled from September through May to avoid impacts of heat stress. Forty-two multiparous, 256-day pregnant, Israeli-Holstein dry cows were stratified randomly within stratum, and strata were defined according to the following parameters: previous lactation milk and fat yields, parity, and body weight. The dietary treatments continued until 100 days in lactation and were as follows: i) SFA (n=14) – fed a basal diet and supplemented with encapsulated saturated fat at 240 and 560 g/day per cow *prepartum* and *post partum* (PP) respectively; ii) FLX (n=14) – fed a basal diet and supplemented *prepartum* at 300 g/day per cow with fat providing ALA (C18:3n-3) at 56.1 g/day and PP at 700 g/day per cow providing 131.0 g/day ALA from FLX; and iii) FO – fed a basal diet and supplemented *prepartum* at 300 g/day per cow with fat providing EPA (C20:5n-3) at 5.8 g/day and DHA (C22:6n-3) at 4.3 g/day and PP at 700 g/day per cow, providing EPA at 13.5 g/day and DHA at 10.0 g/day from FO. The fat content of the SFA supplement was 99% compared to 80% in the FLX and FO; therefore, the supplemented amounts were different among groups to maintain similar content of fat in all diets. The fat supplements were specially prepared and supplied by SILA (Venice, Italy). The ingredients and chemical composition of the dry cow rations of all treatment groups are presented in Table 1 and of the milking cow is presented in Table 2. The FA compositions of the fat supplements are presented in Table 3 and those of the whole diet in Table 4. The cows were milked three times daily and individually fed total mixed ration daily at 1100 h.

Aspiration of large follicles

Seven cows from each group were subjected to synchronization of estrous cycles followed by aspiration of large follicles. These cows were i.m. administered with GNRH analog (200 μg gonadorelin; Gonabreed, Parnell Australia PTY, Alexandria, Australia), and 7 days later, their ovaries were monitored by ultrasound for the presence of corpus luteum (CL). Cows with CL on the ovaries were treated with $PGF_{2\alpha}$ analog (500 μg cloprostenol; Estroplan, Parnell Australia PTY) and estrus was visually monitored. Fourteen to 15 days after behavioral estrus, the cows received another $PGF_{2\alpha}$ injection, and 48 h later, the follicular fluid (FF) from follicles >7 mm in diameter was aspirated according to Moallem *et al.* (2007). Follicles were aspirated individually with the aid of an ultrasound scanner

Table 1 Ingredients and chemical composition of the experimental dry cow's diets.

	Treatments		
	SFA	FLX	FO
Ingredients (% of DM)			
Corn grain (ground)	5.5	5.4	5.4
Barley grain (rolled)	5.5	5.5	5.5
Wheat grain (rolled)	0.7	0.7	0.7
Soybean meal	3.2	3.2	3.2
Sunflower meal	3.4	3.4	3.4
Rapeseed meal	1.3	1.2	1.2
Cottonseed	0.9	0.9	0.9
Wheat bran	5.5	5.5	5.5
Wheat silage	8.6	8.6	8.6
Corn silage	8.3	8.3	8.3
Clover hay	1.2	1.2	1.2
Oats hay	51.4	51.1	51.1
Soybean molasses	1.0	1.0	1.0
Urea	0.2	0.2	0.2
Limestone	0.3	0.3	0.3
Calcium bicarbonate	0.4	0.4	0.4
Salt	0.7	0.7	0.7
Encapsulated saturated FA	1.9	–	–
Encapsulated flaxseed oil	–	2.4	–
Encapsulated fish oil	–	–	2.5
Vitamins and minerals[a]	0.03	0.03	0.03
Chemical composition			
NE_L[b] (MJ/kg DM)	6.15	6.15	6.15
Crude protein	13.3	13.3	13.3
NDF	51.0	51.0	51.0
Forage NDF	42.9	42.6	42.6
Ether extract	3.0	3.1	3.2
Ca	0.3	0.3	0.3
P	0.1	0.1	0.1

[a]Contained 20 000 000 IU vitamin A/kg, 2 000 000 IU vitamin D/kg, 15 000 IU/kg vitamin E, 6000 mg/kg Mn, 6000 mg/kg Zn, 2000 mg/kg Fe, 1500 mg/kg Cu, 120 mg/kg I, 50 mg/kg Se, and 20 mg/kg Co.
[b]Calculated using the NRC (2001) values.

Table 2 Ingredients and chemical composition of the experimental milking cow's diets.

	Treatments		
	SFA	FLX	FO
Ingredients (% of DM)			
Corn grain (ground)	10.6	10.5	10.5
Barley grain (rolled)	10.7	10.6	10.6
Wheat grain (rolled)	1.3	1.3	1.3
Soybean meal	6.1	6.0	6.0
Sunflower meal	6.6	6.6	6.6
Rapeseed meal	2.4	2.4	2.4
Cottonseed	1.7	1.7	1.7
Wheat bran	10.7	10.6	10.6
Wheat silage	16.6	16.5	16.5
Corn silage	16.0	15.9	15.9
Clover hay	2.2	2.2	2.2
Oats hay	7.9	7.9	7.9
Soybean molasses	2.0	1.9	1.9
Urea	0.4	0.4	0.4
Limestone	0.6	0.6	0.6
Calcium bicarbonate	0.7	0.7	0.7
Salt	1.3	1.3	1.3
Encapsulated saturated FA	2.2	–	–
Encapsulated flaxseed oil	–	2.9	–
Encapsulated fish oil	–	–	2.9
Vitamins and minerals[a]	0.1	0.1	0.1
Chemical composition			
NE_L[b] (MJ/kg DM)	7.08	7.03	7.03
Crude protein	16.3	16.2	16.2
NDF	36.0	36.0	36.0
Forage NDF	23.0	22.8	22.8
Ether extract	4.7	4.7	4.8
Ca	0.8	0.8	0.8
P	0.3	0.3	0.3

[a]Contained 20 000 000 IU vitamin A/kg, 2 000 000 IU vitamin D/kg, 15 000 IU/kg vitamin E, 6000 mg/kg Mn, 6000 mg/kg Zn, 2000 mg/kg Fe, 1500 mg/kg Cu, 120 mg/kg I, 50 mg/kg Se, and 20 mg/kg Co.
[b]Calculated using the NRC (2001) values.

(Pie Medical, Maastricht, The Netherlands) connected to a 7.5 MHz vaginal sector transducer equipped with a needle guide and connected to a suction pump (MP86; Biometra, Goettingen, Germany) set at a flow rate of 25–30 ml/min. The needles used were 18 gauge and were changed between follicles. After collection, the FF was centrifuged for 15 min at 3000 ***g***, the sediment, which contained the granulosa cells, was separated from the fluids, and both fractions were frozen at −32 °C pending analysis.

OPU procedure and oocyte collection

Commencing at 60 days in lactation, transvaginal follicular aspiration was applied twice weekly for 10 weeks to five cows per treatment, according to Zachut *et al.* (2010). The cows were sedated with an i.m. injection of 1 ml Sedaxylan (xylazine at 20 mg/ml; Eurovet Animal Health, Bladel, The Netherlands) and were given a local anesthetic of 5 ml 2% lidocaine HCl (esracain 2%, 200 mg/10 ml, Rafa Laboratories, Jerusalem, Israel) injected epidurally between the last sacral and first caudal vertebrae. Follicles were aspirated with the aid of an ultrasound scanner (Pie Medical) connected to a 7.5 MHz vaginal sector transducer equipped with a needle guide and connected to a suction pump (Craft Suction Unit pump, Rocket Medical plc, Watford, England). For each cow in each session, follicular contents from all visible 3–7 mm follicles were collected into a single 50 ml tube that contained HEPES-TL solution medium (Sigma–Aldrich Israel Ltd., Rehovot, Israel) supplemented with antibiotics and 0.008% heparin (Sigma–Aldrich Israel Ltd.) in 0.4% BSA (Sigma–Aldrich Israel Ltd.). The tubes with aspirated follicular contents were transferred to an adjacent laboratory; recovered cumulus–oocyte complexes (COCs) were counted and examined morphologically. Oocytes were classified into four categories, according to the number of layers of cumulus surrounding the oocytes and their cytoplasmic consistency, as described elsewhere (de Loos *et al.* 1989): grade I – spherical, symmetrical, intact oocytes of uniform size, color and texture, and entirely surrounded by three to five compact layers of cumulus cells; grade II – incomplete cumulus layer and oocyte partially denuded; grade III – cumulus expanded, oocyte denuded, and partially degenerated cumulus; and grade IV – totally degenerated cumulus and oocyte.

In two sessions, oocytes of grades I and II were collected for FA profile analysis. As the individual oocyte lipid mass was well below the sensitivity of the analytical method, COCs were analyzed in groups of ∼30 oocytes in two replicates per treatment.

Oocyte maturation

The procedure of oocyte maturation and fertilization was performed according to Zachut *et al.* (2010) with some modifications, and all solutions were prepared according to Parrish *et al.* (1986). Briefly, after the oocytes had been recovered and graded, all COCs were washed with oocyte wash buffer (HP-T buffer) and transferred separately for each treatment group into 2 ml sterile conical tubes containing prewarmed maturation medium. All tubes were kept in an electrically warmed container (Minitube, Tiefenbach, Germany) at 37 °C for about 30 min until arrival at the IVF laboratory. Contents of the tubes were transferred to four-well culture multidishes (Nunc, Roskilde, Denmark), with ∼20 oocytes per well in 500 µl TCM-199 maturation medium (Sigma–Aldrich Israel Ltd.) and incubated for 22 h at 38.5 °C, under humidified air containing 5% CO_2.

Table 3 Fatty acid profile of the supplements (g/100 g fatty acids).

	Supplements		
FA (%)	SFA	FLX	FO
C14:0	1.05	0.08	2.24
C15:0	0	0	0.12
C16:0	64.18	9.23	15.52
C16:1	–	–	2.49
C16:2	–	–	0.19
C17:0	–	–	0.27
C18:0	34.77	52.96	53.26
C18:1	–	8.10	12.65
C18:2n-6	–	6.07	3.15
C18:3n-3	–	23.42	3.32
C20:0	–	0.14	0.32
C20:3	–	–	0.58
C20:5n-3	–	–	2.50
C22:1	–	–	1.52
C22:6n-3	–	–	1.86

Table 4 Fatty acid profile of the whole experimental diets during the *post partum* period (g/100 g fatty acids).

	Treatments		
FA (%)	CTL	FLX	FO
C12:0	0.09	0.04	0.06
C14:0	0.81	0.29	1.22
C14:1	0	0	0.03
C15:0	0.07	0.08	0.13
C16:0	38.99	13.97	15.84
C16:1	0.20	0.22	1.36
C17:0	0.14	0.18	0.28
C17:1	0.03	0.10	0.26
C18:0	21.39	22.71	27.13
C18:1	11.04	15.68	16.55
C18:2n-6	21.45	25.15	21.76
C18:3n-3	2.11	14.87	2.67
C20:0	0.5	0.57	0.61
C20:1	0.25	0.36	1.07
C20:2	0.04	0.09	0.19
C20:3	0.08	0.21	0.17
C20:4	0.03	0.08	1.08
C20:5n-3	0.05	0.09	1.49
C21:0	0.04	0.10	0.14
C22:0	0.28	0.44	0.41
C22:1	0.10	0.27	0.29
C22:2	0.13	0.16	0.13
C22:3	0.06	0.19	0.15
C22:5n-3	0.02	0.02	0.64
C22:6n-3	0.02	0.03	1.46
C23:0	0.17	0.03	0.05
C24:0	0.31	0.39	0.34
C24:1	0.05	0.03	0.15

IVF

After maturation, the COCs were washed once in HEPES-TALP, mechanically denuded of cumulus cells with a pipette, and placed in their respective groups in four-well plates containing, in each well, 500 µl IVF-TALP with 0.0005% heparin. Semen from a single bull was added to each well to a final concentration of 1×10^6 spermatozoa/ml, after which, 20 µl of a solution of 0.5 mM penicillamine (Sigma–Aldrich Israel), 0.25 mM hypotaurine (Sigma–Aldrich Israel), and 25 µM epinephrine (Sigma–Aldrich Israel) in 0.9% (w/v) NaCl were added to each well. Sperm and oocytes were co-incubated for 18 h at 38.5 °C under a humidified atmosphere of 5% CO_2, 5% O_2, and 90% N_2. Putative zygotes were washed once in IVC-TALP and were then placed into 50 µl culture drops of *in vitro* cleave at five to ten oocytes per drop. The oocytes were cultured at 38.5 °C under a humidified atmosphere of 5% O_2, 5% CO_2, and 90% N_2 for another 22 h. The proportion of oocytes that cleaved and the proportions of embryos at two-, three-, and four-cell stages were recorded 40 h after insemination.

In vitro *culture*

Following the recording of zygote cleavage at 40 h after insemination, i.e., day 2 after fertilization, the embryos were cultured under the same conditions as before. At day 5 after fertilization, 5 µl heat-inactivated FCS (Gibco) were added to each culture drop. At days 7–8, the embryos were checked for blastocyst formation and development stage.

Hormone analysis

Concentrations of progesterone (P_4) and estradiol (E_2) in the FF were determined with RIA (Diagnostic Products, Los Angeles, CA, USA) and that of androstenedione (A_4) in the FF was determined with RIA (Diagnostic Systems Laboratories, Webster, TX, USA). Before the determinations of P_4, E_2, and A_4 concentration, the FF samples were diluted 100, 500, or 30 times respectively in order to fit the detection ranges; the minimum detectable amounts were 0.2, 20, and 0.1 ng/ml for P_4, E_2, and A_4 respectively. The intra- and interassay coefficients of variation for the P_4, E_2, and A_4 assays were 8.8 and 8.3%, 3.91 and 3.7%, and 5.9 and 4.3% respectively. Follicles were regarded as E_2-active when the E_2:P_4 ratio was >1 (Ireland & Roche 1982), and these follicles were then subjected to further analysis.

FA composition of plasma, milk fat, and ovarian compartments

FAs in plasma, FF, granulosa cells, and COCs were extracted as described by Moallem *et al.* (1999). Briefly, the samples were saponified in a mixture of 60% KOH and ethanol, extracted with petroleum ether, and methylated with 5% sulfuric acid in methanol. FA methyl esters were analyzed with a model 7890N gas chromatograph (Agilent Technologies, Santa Clara, CA, USA) equipped with a DB-23 capillary column (60 m × 0.25 mm, 0.25 mm; Agilent Technologies) and a flame-iodizing detector. The initial temperature of the column was set at 130 °C, increased at 6.5 °C/min to 170 °C, and then at 2.75 °C/min to 215 °C, at which it was held for 18 min. Then, the temperature was increased to 230 °C at 40 °C/min for the remainder of the analysis. The carrier gas was hydrogen, flowing at a linear velocity of 1.6 m/min; injection volume was 1 µl.

Statistical analysis

Continuous variables (milk and milk solids) were analyzed as repeated measurements using the Proc Mixed procedure, version 9.2 (SAS 2002). The milk and milk solids of the first 120 days of previous lactation were used as co-variables.

The model used was:

$$Y_{ijkl} = \mu + T_i + L_j + C(T \times L)_{ijk} + \mathrm{DIM}_{ijkl} + E_{ijklm}$$

where μ=overall mean; T_i=treatment effect, $_i$=1–3; L_j=parity, $_j$=2 or >2; $C(T\times L)_{ijk}$=cow$_k$ nested in treatment$_i$ and cow nested in parity$_j$; DIM_{ijkl}=day in milk as continuous variable; E_{ijklm}=random residual. The hormone concentrations in FF were analyzed with the general linear models (GLM) procedure, version 9.2 (SAS 2002).

The number of follicles, oocyte numbers and grading, and oocyte recovery rate were calculated for each cow and analyzed with the Proc Mixed procedure, version 9.2 (SAS 2002). The model included the effects of treatment, cow (nested in treatment), session, and treatment × session interaction. For matured oocytes and cleavage rate, sessions were considered as random effects and data were analyzed with the Proc Mixed procedure. The model included treatment, session, and treatment × session interaction.

The FA profiles were analyzed with the GLM procedure (SAS 2002). Least squares means and adjusted S.E.M. are presented in the tables. The level of $P<0.05$ was accepted as statistically significant unless otherwise stated, and tendencies were reported at $0.05<P<0.10$.

Results

Dry matter intake, milk yield, and milk fat FA composition

During the *prepartum* period, no differences between dietary groups were observed in dry matter intake, but PP the intake was 6.1% higher in SFA than in FO (25.0 and 23.6 kg/day respectively; S.E.M.=0.30, $P<0.009$). The total n-3 FA consumed during the PP period was 26.0, 71, and 171 g/day for the SFA, FLX, and FO groups respectively.

No differences were observed between groups, in milk yields. The percentage of milk fat was higher in SFA than in FLX (3.80 and 3.59% respectively; S.E.M.=0.07, $P<0.03$), and the protein percentage was higher in SFA than in FO (3.14 and 3.01% respectively; S.E.M.=0.04, $P<0.03$). The yields of milk fat, protein, and lactose and the amounts of fat-corrected milk (4%) were similar between dietary groups (data not shown).

No differences among groups were observed, in concentrations of nonesterified FA in plasma, either pre- or PP, nor in *prepartum* glucose concentrations (data not shown); however, PP glucose concentrations in plasma were higher in FLX and FO than in SFA cows (62.3, 62.6, and 55.0 mg/l respectively; $P<0.005$).

The FA profile of milk fat changed following FA supplementation and the proportion of ALA was three times as high in FLX as in SFA and FO cows (0.93, 0.31, and 0.35% respectively; $P<0.001$). The proportions of EPA and 22:5n-3 (docosapentaenoic acid n-3 (DPAn-3)) in milk fat were 3.2 times as high in FO as in each of the other groups, and DHA content was 14 and 20 times as high in FO as in SFA and FLX cows respectively ($P<0.001$).

Composition of FA in plasma

As expected, the FA composition of plasma was affected by dietary treatments (Table 5). The proportion of ALA in plasma was 2.9 and 2.5 times as high in the FLX as in the SFA and FO groups respectively ($P<0.001$).

Table 5 Least squares means of fatty acid profile in plasma (% of total fatty acids).

	Treatments[a]				
FA (%)	SFA	FLX	FO	S.E.M.	***P* value**
C14:0	1.19*	0.85†	0.81†	0.08	0.002
C16:0	16.98	15.81	15.46	0.55	NS
C16:1	0.73*	0.63†	0.48‡	0.02	0.001
C16:3	1.37	1.07	1.13	0.11	NS
C18:0	0.58	0.72	0.53	0.09	NS
C18:1n-9	7.44	7.04	6.51	0.59	NS
C18:1n-7	0.78	0.58	1.56	0.41	NS
C18:2n-6	49.99	48.23	52.07	1.09	NS
C18:3n-6	1.11*	0.67†	0.41†	0.1	0.006
C18:3n-3	2.45‡	7.1*	2.9†	0.07	0.001
C18:4	0.14	0.18	0.15	0.04	NS
C20:1n-9	0.18	0.16	0.21	0.04	NS
C20:3	1.81*	1.21†	0.78‡	0.08	0.001
C20:4n-6	1.68*	1.19†	1.02†	0.09	0.001
C20:4n-3	0.08	0.10	0.03	0.06	NS
C20:5n-3	0.23†	0.47†	1.43*	0.11	0.001
C22:5n-3	0.09‡	0.17†	0.24*	0.04	0.006
C22:5n-6	0.04	0.03	0.01	0.01	NS
C22:6n-3	0.04†	0.06†	0.43*	0.02	0.001
C22:6n-6	0.04	0.11	0.14	0.03	NS
C24:1n-9	0.35	0.51	0.41	0.09	NS
n-3[b]	2.75‡	7.78*	5.18†	0.13	0.001
n-6[c]	52.79*,†	50.31†	53.71*	1.07	0.03
Saturated[d]	31.20*	30.10*,†	29.33†	0.66	0.05
MUFA[e]	9.55	9.09	9.53	0.85	NS
PUFA[f]	55.67†	57.77*,†	58.90*	1.18	0.05
n-6/n-3	19.08*	7.31‡	11.31†	0.58	0.001

*,†,‡Within rows, means with different superscripts are statistically different.
[a]Treatments – cows were supplemented with encapsulated fats: SFA – saturated fat at 300 and 560 g/day per cow *pre-* and *post partum* (PP) respectively; FLX – 300 and 700 g/day per cow *prepartum* and PP respectively with fat providing ALA from flaxseed oil at 56.1 and 131.0 g/day per cow respectively; and FO – 300 and 700 g/day per cow *prepartum* and PP respectively, with fat from fish oil providing EPA and DHA at 5.8 and 4.3 g/day per cow respectively *prepartum* and 13.5 and 10.0 g/day per cow respectively *post partum*. [b]Total n-3 FAs. [c]Total n-6 FAs. [d]Total saturated FAs. [e]Total monounsaturated FAs. [f]Total polyunsaturated FAs.

The proportion of EPA was 6.2 and 3.0 times as high in the FO as in the SFA and FLX groups respectively ($P<0.001$). The percentage of DPAn-3 in FO cows was 2.5 and 1.5 times as high as those in SFA and FLX respectively ($P<0.006$). The percentage of C20:4n-6 (AA) was higher in SFA animals than in the other two groups. The proportion of DHA in FO cows was 11 and seven times as high as in SFA and FLX cows respectively ($P<0.001$). Also, the total proportion of n-3 FAs in plasma was highest in FLX cows, intermediate in FO, and lowest in SFA cows ($P<0.001$). Thus, the n-6:n-3 ratio in plasma varied, being highest in SFA and lowest in FLX cows, with intermediate values in the FO group ($P<0.001$).

Composition of FA in FFs

The effects of dietary treatments on the composition of FF obtained from E_2-active follicles are presented in Table 6. The proportion of ALA in FLX cows was 2.6 and three times as high as in SFA and FO cows respectively ($P<0.001$), whereas the proportion of EPA in FO cows was 2.5 times as high as that in both other groups ($P<0.03$). The proportion of DHA in FO cows was 9.3 times as high as in the other two groups ($P<0.001$). The total proportion of n-3 FAs in FLX cows was 2.2 and 1.7 times as high as in SFA and FO groups respectively ($P<0.001$). Although the proportions of total SFA, MUFA, and PUFA were similar between groups, the n-6:n-3 ratio in FF differed among the groups and was higher in SFA than in the other two groups ($P<0.001$).

Composition of FA in granulosa cells obtained from preovulatory follicles

The FA composition in granulosa cells obtained from preovulatory follicles was also affected by dietary treatments (Table 7): C18:2n-6 was the predominant FA in granulosa cells, and its proportion tended to be higher in SFA than in FO cows ($P<0.06$). The percentage of ALA in FLX cows' granulosa cells was 2.5 and 2.9 times as high as in those of SFA and FO cows respectively ($P<0.01$), and the proportion of AA in those of SFA animals was 1.6 and 2.2 times as high as those in FLX and FO cows respectively ($P<0.001$).

The proportion of EPA in granulosa cells was about twice as high in FO cows as in the SFA and FLX groups

Table 6 Least squares means of fatty acid profile in follicular fluid obtained from preovulatory follicles (% of total fatty acids).

	Treatments[a]				
FA (%)	SFA	FLX	FO	**S.E.M.**	***P*<**
n	8	9	8		
C14:0	1.32	1.07	1.85	0.24	NS
C16:0	18.02	17.98	22.19	1.47	NS
C16:1	1.03	1.14	1.19	1.16	NS
C16:2	0.75	0.83	0.43	0.16	NS
C16:3	0.86	0.67	0.69	0.10	NS
C18:0	12.43	13.52	14.77	1.18	NS
C18:1n-9	10.90	8.47	11.21	0.87	NS
C18:1n-7	0.48	0.31	0.21	0.10	NS
C18:2n-6	45.38	45.48	40.99	2.38	NS
C18:3n-6	0.84*	0.46†	0.27†	0.08	0.01
C18:3n-3	2.20†	5.69*	1.90†	0.37	0.001
C18:4n-3	0.11	0.20	0.15	0.04	NS
C20:1n-9	0.98	0.68	0.72	0.16	NS
C20:3	1.73*	0.99†	0.69†	0.08	0.001
C20:4n-6	1.90*	1.30†	1.13†	0.11	0.004
C20:4n-3	0.13	0.10	0.10	0.04	NS
C20:5n-3	0.29†	0.32†	0.76*	0.12	0.03
C22:5n-3	0.10	0.13	0.26	0.04	NS
C22:6n-3	0.03†	0.03†	0.28*	0.03	0.001
C24:1n-9	0.49	0.56	0.38	0.11	NS
n-3[b]	2.78†	6.10*	3.53†	0.35	0.001
n-6[c]	48.12	47.25	42.41	2.45	NS
Saturated[d]	31.77	32.58	38.80	2.75	NS
MUFA[e]	13.88	11.16	13.30	0.93	NS
PUFA[f]	53.29	54.39	48.32	2.86	NS
n-6/n-3	17.37*	7.64‡	12.52†	0.75	0.001

*,†,‡ Within rows, means with different superscript letters are statistically different.
[a]Treatments – cows were supplemented with encapsulated fats: SFA – saturated fat at 300 and 560 g/day per cow *pre-* and *post partum* (PP) respectively; FLX – 300 and 700 g/day per cow *prepartum* and PP respectively with fat providing ALA from flaxseed oil at 56.1 and 131.0 g/day per cow respectively; and FO – 300 and 700 g/day per cow *prepartum* and PP respectively with fat from fish oil providing EPA and DHA at 5.8 and 4.3 g/day per cow respectively *prepartum* and 13.5 and 10.0 g/day per cow respectively *post partum*. [b]Total n-3 FAs. [c]Total n-6 FAs. [d]Total saturated FAs. [e]Total monounsaturated FAs. [f]Total polyunsaturated FAs.

Table 7 Least squares means of fatty acid profile in granulosa cells obtained from preovulatory follicles (% of total fatty acids).

	Treatments[a]				
FA (%)	SFA	FLX	FO	S.E.M.	*P*<
C14:0	0.81†	1.51*,†	1.74*	0.31	0.04
C16:0	19.57†	23.05*,†	25.33*	1.22	0.004
C16:1	1.56	2.23	2.17	0.30	NS
C16:2	1.18	1.76	1.31	0.22	NS
C16:3	0.79	0.91	0.75	0.09	NS
C18:0	14.67*,†	13.62†	16.99*	0.94	0.02
C18:1n-9	14.64	15.83	15.17	1.76	NS
C18:1n-7	1.17	1.73	1.25	0.03	NS
C18:2n-6	38.06	31.93	29.85	2.94	NS
C18:3n-6	0.62*	0.27†	0.11‡	0.04	0.02
C18:3n-3	1.65†	4.10*	1.41†	0.19	0.01
C18:4n-3	0.21	0.38	0.32	0.07	NS
C20:1n-9	0.34	0.65	0.99	0.25	NS
C20:3	1.40*	0.68†	0.49†	0.05	0.03
C20:4n-6	2.54*	1.60†	1.17†	0.17	0.001
C20:4n-3	0.34*	0.20†	0.15†	0.05	0.05
C20:5n-3	0.23†	0.19†	0.47*	0.07	0.02
C22:5n-3	0.20†	0.24*,†	0.40*	0.06	0.03
C22:6n-3	0.01	0.01	0	0.01	NS
n-3[b]	2.64†	4.71*	2.99†	0.24	0.001
n-6[c]	41.22*	33.80*,†	31.13†	2.92	0.03
Saturated[d]	35.04†	38.18†	44.07*	1.65	0.02
MUFA[e]	17.71	20.43	19.58	1.91	NS
PUFA[f]	47.23*	43.48*,†	38.25†	2.88	0.03
n-6/n-3	16.05*	7.55†	10.92†	1.06	0.002

*,†,‡Within rows, means with different superscript letters are statistically different.
[a]Treatments – cows were supplemented with encapsulated fats: SFA – saturated fat at 300 and 560 g/day per cow *pre-* and *post partum* (PP) respectively; FLX – 300 and 700 g/day per cow *prepartum* and PP respectively with fat providing ALA from flaxseed oil at 56.1 and 131.0 g/day per cow respectively; and FO – 300 and 700 g/day per cow *prepartum* and PP respectively with fat from fish oil providing EPA and DHA at 5.8 and 4.3 g/day per cow respectively *prepartum* and 13.5 and 10.0 g/day per cow respectively *post partum*. [b]Total n-3 FAs. [c]Total n-6 FAs. [d]Total saturated FAs. [e]Total monounsaturated FAs. [f]Total polyunsaturated FAs.

($P<0.02$), and the content of DPAn-3 in FO cows' granulosa cells was twice as high as that in those of SFA cows ($P<0.03$). The total percentage of n-3 FAs in granulosa cells was higher in FLX cows than in the other groups ($P<0.001$); the total proportion of n-6 FA in SFA cows tended to be higher than that in FLX ones ($P<0.09$) and was higher than that in FO animals ($P<0.02$). The total n-6:n-3 ratio in granulosa cells was higher in SFA than in either of the other groups ($P<0.05$).

FA composition of COCs

The FA composition of COCs is presented in Table 8. The proportion of C18:0 FA in COCs was ~7% lower in FO cows than in the other groups ($P<0.01$). The percentage of ALA in COCs of FLX cows was 3.2 and 2.4 times as high as in those of SFA and FO cows respectively ($P<0.005$). DPAn-3 and DHA were not detected in COCs of SFA and FLX cows, whereas the percentages of these FAs in FO COCs were 1.11 and 1.17% respectively ($P<0.2$). The total proportion of n-3 FAs tended to be higher in COCs of FO cows than in those of the other groups ($P<0.1$), and the n-6:n-3 ratio was numerically lower in FO cows' COCs than in those of the other dietary groups ($P<0.3$).

Concentrations of hormones in preovulatory follicles

No differences between dietary groups were observed in concentrations or contents of P_4, A_4, or E_2 in FF of E_2-active follicles. Also, the diameters and volumes of the follicles, as well as E_2:P_4 ratios, were similar among all the groups (data not shown).

Number of oocytes obtained by OPU, cleavage rate, and development to blastocysts

In total, 915 oocytes were aspirated in 20 OPU sessions (Table 9). The number of observed follicles (3–7 mm in diameter) that were collected from the FLX and FO cows during OPU procedures was higher than that from the SFA cows ($P<0.05$; Fig. 1), and the number of oocytes recovered per cow was higher in the FLX than in the SFA group ($P<0.02$). The number of oocytes per cow that were chosen for IVM and IVF in each session was also higher in FLX cows than in SFA ones ($P<0.04$). As shown in Table 9, the cleavage rate of oocytes was higher in FLX and FO cows than in SFA ones ($P<0.01$), and the number of cleaved oocytes per session was also higher in the FLX and FO groups than in the SFA group ($P<0.03$). The percentage of

Table 8 Least squares means of fatty acid profile in cumulus–oocyte complex (COC; % of total fatty acids).

	Treatments[a]					
FA (%)	SFA	FLX	FO	FLX	S.E.M.	*P*<
C14:0	4.79	4.58	5.49	4.58	0.35	NS
C16:0	27.46	29.68	29.99	29.68	3.21	NS
C16:1	3.96*	2.27†	3.2*,†	2.27†	0.26	0.01
C16:2	0.99	1.84	2.03	1.84	0.56	NS
C16:3	0.88	1.08	1.29	1.08	0.20	NS
C18:0	30.38*	32.32*	24.39†	32.32*	0.93	0.02
C18:1n-9	17.71	15.68	17.99	15.68	2.13	NS
C18:1n-7	1.11	1.10	1.57	1.10	0.28	NS
C18:2n-6	6.39	6.43	6.00	6.43	1.46	NS
C18:3n-3	0.29†	0.93*	0.39†	0.93*	0.06	0.005
C18:4n-3	0.4	0.5	0.37	0.5	0.2	NS
C20:1n-9	3.33*	0.79†	2.59*,†	0.79†	0.47	0.03
C20:3	0.42	0.51	0.21	0.51	0.17	NS
C20:4n-6	0.34	1.24	0.66	1.24	0.45	NS
C20:5n-3	1.51	1.01	1.5	1.01	0.43	NS
C22:5n-3	0	0	1.17	0	0.55	NS
C22:6n-3	0	0	1.11	0	0.52	NS
n-3[b]	2.21	2.44	4.54	2.44	0.68	0.1
n-6[c]	6.74	7.67	6.66	7.67	1.88	NS
Saturated[d]	62.64	66.59	59.88	66.59	2.80	NS
MUFA[e]	26.11	19.84	25.36	19.84	2.50	NS
PUFA[f]	11.25	13.55	14.74	13.55	2.36	NS
n-6/n-3	3.04	3.14	1.61	3.14	0.81	NS

*,† Within rows, means with different superscript letters are statistically different.
[a]Treatments – cows were supplemented with encapsulated fats: SFA – saturated fat at 300 and 560 g/day per cow *pre*- and *post partum* (PP) respectively; FLX – 300 and 700 g/day per cow *prepartum* and PP respectively with fat providing ALA from flaxseed oil at 56.1 and 131.0 g/day per cow respectively; and FO – 300 and 700 g/day per cow *prepartum* and PP respectively with fat from fish oil providing EPA and DHA at 5.8 and 4.3 g/day per cow respectively *prepartum* and 13.5 and 10.0 g/day per cow respectively *post partum*. [b]Total n-3 FAs. [c]Total n-6 FAs. [d]Total saturated FAs. [e]Total monounsaturated FAs. [f]Total polyunsaturated FAs.

blastocysts per fertilized oocyte tended to be higher in FLX and FO than in SFA ($P<0.1$).

Discussion

The main finding of this study is that dietary FLX was as effective as FO in improving folliculogenesis and IVF performance in dairy cows, even though differing incorporation of n-3 FAs into plasma and ovary components was demonstrated.

FA profile in plasma and ovarian compartments

In several previous studies, a variety of flaxseed sources enhanced the proportion of ALA in blood (Petit *et al.* 2004, Gonthier *et al.* 2005, Zachut *et al.* 2010). The results of this study support our previous findings

Table 9 Follicle and oocyte numbers, maturation, and cleavage rates of oocytes collected by ovum pickup (OPU).

	Treatments[a]				
	SFA	FLX	FO	S.E.M.	*P*<
Follicle number/cow per session	8.0†	10.7*	9.6*	0.55	0.05
Total number of oocytes aspirated	248	361	306		
Oocytes recovered/cow per session	3.3†	4.8*	3.9*	0.3	0.06
No. of oocytes/cow per session chosen for IVM+IVF	2.88†	3.84*	3.5*,†	1.6	0.05
Cleavage rate (%)	35†	52*	48*	0.03	0.01
No. of cleaved oocytes/session	5.1†	9.2*	8.3*	1.1	0.03
No. of cleaved oocytes/cow per session	1.06†	1.97*	1.66*	0.21	0.04
No. of blastocysts/session	1.80	2.28	2.14	0.4	0.4
Rate of blastocysts from oocytes for IVF (%)	8.8	15.2	13.4	0.02	0.1

*,† Within rows, means with different superscript letters are statistically different.
[a]Treatments – cows were supplemented with encapsulated fats: SFA – saturated fat at 300 and 560 g/day per cow *pre*- and *post partum* (PP) respectively; FLX – 300 and 700 g/day per cow *prepartum* and PP respectively with fat providing ALA from flaxseed oil at 56.1 and 131.0 g/day per cow respectively; and FO – 300 and 700 g/day per cow *prepartum* and PP respectively with fat from fish oil providing EPA and DHA at 5.8 and 4.3 g/day per cow respectively *prepartum* and 13.5 and 10.0 g/day per cow respectively *post partum*.

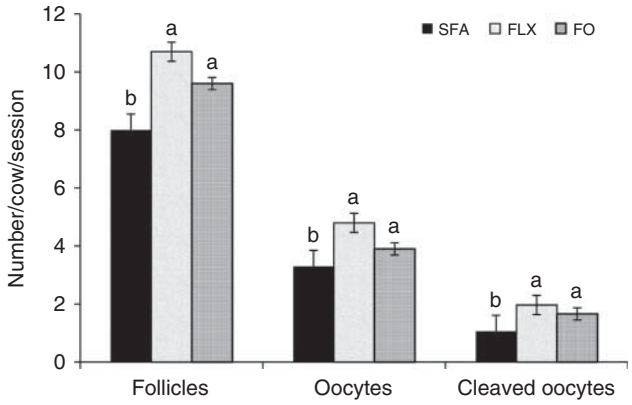

Figure 1 Ovum pickup and IVF performance of cows supplemented with encapsulated fats: SFA – saturated fat at 300 and 560 g/day per cow *pre-* and *post partum* (PP) respectively; FLX – 300 and 700 g/day per cow *prepartum* and PP respectively with fat providing ALA from flaxseed oil at 56.1 and 131.0 g/day per cow respectively; and FO – 300 and 700 g/day per cow *prepartum* and PP respectively with fat from fish oil providing EPA and DHA at 5.8 and 4.3 g/day per cow respectively *prepartum* and 13.5 and 10.0 g/day per cow respectively *post partum*. Different superscript letters are statistically different. The results are from 20 OPU and 18 IVF consecutive sessions (five cows per group twice weekly).

that large amounts of dietary ALA were effectively transferred into the plasma and that the incorporation rate was proportional to the amount consumed (Zachut *et al*. 2011). There was also slightly more plasma ALA in the FO group than in the SFA one, most likely because of the small amount of ALA provided by the FO supplement. This small proportion of ALA in FO was also reported by Childs *et al*. (2008), but without increased plasma ALA in beef heifers fed FO. In this study, the proportions of the longer n-3 FAs – EPA and DPAn-3 – were higher in the FLX than in the SFA cows, which indicates elongation of ALA into longer n-3 FAs. The EPA and DPAn-3 are intermediate products in the elongation and desaturation that occur during the synthesis of DHA from ALA in mammals (Wang *et al*. 2005). Petit *et al*. (2004) found that ALA supplementation increased the proportion of EPA but not of other longer n-3 FAs. Also, the proportion of DHA was dramatically increased in plasma of the FO cows, which indicates successful transfer of this important FA from the diet into the blood.

In general, the profile of FAs in FF was very similar to that in plasma, as was also reported in other studies (Zeron *et al*. 2002, Zachut *et al*. 2011). This indicates that there is relatively free passage of plasma components into the FF, which is a crucial condition for altering ovarian activity and oocyte environment by absorption of blood factors exclusively from dietary supplementation.

Dietary FAs altered the granulosa FA profile, as was also found by Zachut *et al*. (2010) in cows fed microencapsulated FLX and by Wonnacott *et al*. (2010) in ewes fed a mix of flaxseed and FO. By contrast, Fouladi-Nashta *et al*. (2009*b*) found changes in plasma FA composition in response to dietary PUFAs, without any effect on the FA composition in granulosa cells. The development of primordial follicles into larger pre-antral and then into ovulatory follicles takes ~4–6 months in cattle (Evans *et al*. 2012), and the subsequent growth from ~0.3 to 3–5 mm in diameter takes more than 30 days (Mossa *et al*. 2012). Fouladi-Nashta *et al*. (2009*b*) continued the dietary FA supplementation for only a short time of 25 days compared with the 80–100 days in this study, which may account for the difference between the responses obtained in these studies. In this study, the n-6:n-3 ratio in the granulosa cells was changed in a similar manner to that in the plasma and FF; moreover, the ratios were similar to that observed in the plasma and FF, in contrast to the findings of Adamiak *et al*. (2006) and Fouladi-Nashta *et al*. (2009*b*), who reported lower n-6:n-3 ratios in granulosa cells than in plasma.

The FA profile of COCs was also altered by dietary n-3 FA in a manner similar to those of FF and granulosa cells. The proportion of ALA in oocytes was relatively low, although it was greater in the FLX COCs. However, although less EPA and DHA were incorporated into plasma and FF, the integration of these FAs into COCs was distinctly greater than that of ALA so that, consequently, the total n-3 FA content was about twice as great in FO cows as in either of the other groups. This might indicate selective uptake of different n-3 FAs into COCs, in which the longer n-3 FAs entering the oocytes more readily than the shorter n-3 FAs, such as ALA. A similar pattern of selective uptake of n-3 FAs was also demonstrated in maternal–fetal transfer through the placenta of these FAs, in which DHA was readily transferred from dam to calf blood, whereas ALA was barely transferred (Moallem & Zachut 2012).

There are several differences between FA composition in oocytes and that in FF and granulosa cells: the former contains a higher proportion of saturated FAs at the expense of PUFAs and a considerably lower n-6:n-3 ratio in oocytes than in all other ovarian compartments. In this study, the saturated FAs in plasma and FF formed ~30% of the total FAs, and this proportion increased to 35–45% in granulosa cells and to 60–67% in COCs. Reis *et al*. (2002) examined the FA profiles of ovine granulosa cells, oocytes, morula, and early blastocysts, and observed differences between granulosa cells and various developmental stages of embryos in saturated FA, MUFA, and n-6 FA composition. The differences in distribution of FA classes between FF, granulosa cells, and COCs indicate on selective uptake of specific classes of FAs in ovarian compartments, as also suggested by Adamiak *et al*. (2006). Furthermore, as mentioned earlier in this study, there was an indication of selective uptake of specific n-3 FAs. The FA composition has an important role in maintaining the structure of the cell membrane, which influences the functionality of the cell (Fouladi-Nashta *et al*. 2009*b*), and this was also demonstrated in oocytes by Zeron *et al*. (2002). It may be

concluded that the selective uptake of FA into the oocytes reflects the sensitivity of the oocytes to changes in membrane FA composition – which may lead to alterations in membrane viscosity that are associated with functional characteristics.

Preovulatory follicle characteristics

In this study, no differences between treatments were observed in preovulatory follicle characteristics, as was also found by Zachut *et al.* (2011). In another study, Ambrose *et al.* (2006) found that the diameter of ovulatory follicles was increased in cows fed flaxseed, which was not found in this study, by Mendoza *et al.* (2011) in grazing cows fed FO, or by Bilby *et al.* (2006) in lactating dairy cows. Furthermore, in this study, as in Zachut *et al.* (2011), no differences were observed between groups in steroid hormone concentrations. However, in another study, enhanced P_4 concentrations were observed in ewes fed a mixture of flaxseed and FO (Wonnacott *et al.* 2010).

Follicles and oocytes collected

The number of small follicles appropriate for OPU was higher in both n-3 treatments than in the SFA treatment, which matches the findings of Zachut *et al.* (2010) in cows fed microencapsulated FLX and of Zeron *et al.* (2002) in ewes fed FO. A recent study by Mossa *et al.* (2012) demonstrated an association between follicle count during the first wave of follicular growth of the estrous cycle and reproductive performance in cattle, which was also found in humans by Baerwald *et al.* (2003). In other studies, Heravi Moussavi *et al.* (2007) found that cows that were fed FO increased the number of medium follicles (5–10 mm in diameter), but Petit *et al.* (2004) observed no effect of dietary flaxseed on follicle growth. There is inconsistency among findings on the effects of dietary PUFAs on folliculogenesis, and this may be attributable to differences between studies in the amounts of supplements, stage of lactation, and the metabolic status of the cows. In this study, the number of oocytes recovered was also higher in both n-3 groups than in the SFA group. Collectively, it can be concluded that dietary n-3 FAs increase folliculogenesis in ovaries, which may have positive effects on fertility performance, as was suggested by Evans *et al.* (2012) and Mossa *et al.* (2012).

In this study, the cleavage rate was higher in both n-3 treatments than in the SFA treatment, as was also found by Zachut *et al.* (2010) in cows fed microencapsulated FLX. However, Bilby *et al.* (2006) observed no differences in oocyte quality and cleavage rate between cows that were fed FLX at 140 g/day or linoleic acid (LA) at 71 g/day under heat stress conditions. The differences in the results between these studies may be attributed to season, i.e., summer vs winter; also, Zeron *et al.* (2001) showed that unsaturated FA contents in oocytes and granulosa cells were lower in summer than in winter. In another study, dairy cows were fed several unsaturated FAs and no effect on oocyte quality or cleavage rate was observed (Fouladi-Nashta *et al.* 2009*b*); however, in that study, the cows were fed for a short time of 25 days, and no alterations were found in the FA profile of granulosa cells, which may account for the unresponsiveness to FA supplementation. Omega-3 FAs may affect maturation and development of oocytes directly through alteration in membrane FA composition (Bender *et al.* 2010), or indirectly by influencing the concentrations of hormones and metabolites in the FF surrounding the oocytes (Fouladi-Nashta *et al.* 2009*a*).

Although the rate of development to blastocysts was relatively low in this study, it tended to be higher in cows fed either of the n-3 FA sources than in those fed SFA. This low rate of development to blastocysts from OPU was also found by Bilby *et al.* (2006), who suggested that oocytes collected from slaughterhouse ovaries underwent a postmortem effect during a few hours, through which the COCs became less tightly connected to the follicle wall and were, therefore, collected with a more complete morphology than OPU oocytes that were placed in maturation media within 20 min, as in this study.

In conclusion, dietary ALA from FLX and EPA and DHA from FO were incorporated differently into plasma, milk, FF, granulosa cells, and COCs. In general, in the FLX cows, ALA was increased in all tissues, and in the FO cows, the proportions of EPA, DPAn-3, and DHA were enhanced. However, although considerable differences were observed between n-3 groups in FA composition in the ovarian compartments, no differences were observed in the beneficial effects of n-3 FAs on folliculogenesis, oocyte quality, and IVF performance. The present results of the study suggest that supplementing cows with FLX, which is the most widely available botanical source of n-3 FAs and is preferable to FO in ruminant nutrition, represents a satisfactory approach to achieve improvement in folliculogenesis and oocyte fertilization.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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