Body composition, dietary carbohydrates and fatty acids determine post-fertilisation development of bovine oocytes *in vitro*

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Abstract

This study assessed the interactive effects of carbohydrate type (fibre vs starch) and fatty acid (FA) supplementation (0% vs 6% calcium soaps of palm oil FA) on the post-fertilisation development of oocytes recovered from low and moderate body condition score (BCS) heifers. A secondary objective was to compare the FA composition of plasma to that of granulosa cells (GCs) and cumulus–oocyte complexes (COCs) from these animals, and to relate these findings to the developmental potential of oocytes. Plasma, GCs and COCs were recovered from 32 heifers on day 5 of a synchronised oestrous cycle for FA analyses. Oocytes were also recovered on days 10 and 15 of the same cycle after short-term ovarian stimulation (FSH + GnRH), and matured, fertilised and cultured to the blastocyst stage *in vitro*. High levels of dietary starch increased ($P < 0.01$) plasma insulin but, together with dietary FA, reduced ($P < 0.05$) blastocyst yields in low, but not in moderate, BCS heifers. Diet-induced alterations to the FA content of plasma were less apparent in GCs and COCs. In summary, although dietary lipids increased the FA content of COCs, the selective uptake of saturated FAs at the expense of mainly polyunsaturated FAs within the follicular compartment ensured that the FA composition of COCs was largely unaffected by diet. However, the concentration of saturated FAs within COCs was inherently high, and so further increases in FA content may have impaired post-fertilisation development. The data establish a robust nutritional framework for more detailed studies into the mechanistic effects of dietary composition on the post-fertilisation developmental potential of oocytes.

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Introduction

Nutritional status is a major factor determining reproductive success in mammals and can act at various levels within the hypothalamus–pituitary–ovarian axis to regulate follicular development and ovulation (Cameron 1996, Schneider *et al.* 2000, Armstrong *et al.* 2003). While there is a considerable body of data describing the effects of nutritional status on the central mechanisms governing gonadotrophin release (Schneider 2004), much less is known about the direct effects of nutrition on the follicle-enclosed oocyte, and the consequences that this might have for post-fertilisation development. Studies with ruminants have, to date, tended to focus on the effects of plane of nutrition (normally expressed as multiples of the energy requirements for body maintenance) in moderately obese animals (e.g. Nolan *et al.* 1998, Armstrong *et al.* 2001). We recently demonstrated that plane of nutrition can interact with the degree of heifer

body fatness (body condition score) to determine the post-fertilisation developmental potential of bovine oocytes (Adamiak *et al.* 2005). The current work extends these studies to investigate the effects of altering the nature and level of carbohydrates and fatty acids in the diet.

Increasing the proportion of starch within the ruminant diet alters the molar proportions of the principal short-chain volatile fatty acids, favouring an increase in propionate production within the rumen and glucose synthesis within the liver (Demigne *et al.* 1988). Consequently, in addition to increasing glucose, high-starch diets increase mean basal and postprandial plasma insulin concentrations in ruminants (Vernon & Peaker 1983). This in turn can lead to improvements in oocyte and embryo quality, although our recent evidence indicates that high planes of nutrition in moderately obese animals lead to hyperinsulinaemia and impaired oocyte quality (Adamiak *et al.* 2005).

The inclusion of rumen bypass fatty acids (such as calcium soaps of palm oil fatty acids) within ruminant diets has been shown to improve fertility, with beneficial effects on whole-animal energy metabolism, and reported actions on the ovarian follicle and corpus luteum (Mattos *et al.* 2000). In contrast, although the fatty acid composition of oocytes across a number of mammalian species has been chronicled (e.g. McEvoy *et al.* 2000), little is known about the uptake of specific fatty acids by the follicle-enclosed oocyte, how this may be altered by maternal nutrition and the consequences that this might have for post-fertilisation development. For example, although the feeding of a high polyunsaturated fatty acid (PUFA) diet to pubertal ewe lambs has been found to increase the number of ovarian follicles and improve oocyte quality (by morphological assessment), the PUFA content (% w/w) of these oocytes was not affected, and post-fertilisation development was not determined (Zeron *et al.* 2002).

The primary objective of the present study, therefore, was to assess the interactive effects of carbohydrate type (fibre vs starch) and fatty acid supplementation in moderately lean (low body condition) and fat (moderate body condition) heifers on the post-fertilisation developmental potential of oocytes recovered by ultrasound guided transvaginal follicular aspiration. A secondary objective was to compare the fatty acid composition of plasma to that of granulosa cells and cumulus–oocyte complexes from these animals, and to relate these findings to the post-fertilisation developmental potential of these oocytes.

Materials and Methods

The experiment described in this paper was reviewed by the Animal Experiments Committee of the Scottish Agricultural College and was conducted under the auspices of, and in accordance with, the requirements of the Home Office Animals (Scientific Procedures) Act 1986.

Animals, dietary treatments and experimental design

Thirty-two beef × dairy heifers of approximately 20 months of age and with an average initial weight (mean ± S.E.M.) of 396.9 ± 4.1 kg were used. All animals were accommodated in individual pens on slatted floors. Immediately prior to the experiment, the body-condition scores (BCS) (six-point scale of Lowman *et al.* 1976, where 0 = lean and 5 = obese) of these animals were determined, and they were allocated accordingly to either a low or moderate-BCS group. Animals within each BCS group were then allocated at random to one of four dietary treatments: high fibre (F) either with or without 6% Megalac (calcium soaps of palm oil fatty acids, 440 g/kg palmitic acid (C16:0), 400 g/kg oleic acid (C18:1, n-9), 95 g/kg linoleic acid (C18:2, n-6), 50 g/kg stearic acid (C18:0) and 15 g/kg myristic acid (C14:0); Volac

International, Royston, UK), or high starch (S) again either with or without 6% Megalac. This resulted in a $2 \times 2 \times 2$ factorial arrangement with animals of low or moderate BCS, offered diets containing predominantly one of two types of carbohydrate (fibre vs starch), either with or without Megalac, replicated four times. Alterations to the source of fermentable carbohydrate and the lipid content of the diets inevitably led to differences in energy density (MJ metabolisable energy (ME)/kg dry matter) (Table 1). Concentrate levels for each of the four dietary treatments, therefore, were adjusted to ensure that each animal received 1000 kJ ME/kg live weight^{0.75}/day, equivalent to approximately twice the maintenance energy requirements for this type of animal (AFRC 1993), offered as two meals (at 0800 and 1600 h). This feeding level accounted for the intake of barley straw, which was fixed at 2 kg fresh material daily. These diets were supplemented (80 g/head per day) with a proprietary mineral and vitamin supplement (Norvite, Insch, UK) containing 18% Ca, 12% Na, 0.2% Cu, 0.02% Co, 0.1% Mn, 0.3% Zn, 0.01% I, 0.003% Se, 400 000 IU/kg vitamin A, 80 000 IU/kg vitamin D₃ and 600 IU/kg vitamin E. Animals were weighed every 2 weeks and feeding levels adjusted accordingly. The experimental diets were introduced 1 week prior to the initial period of oestrous synchronisation (Fig. 1).

Table 1 Composition of concentrates and chemical analysis of feeds.

Principal carbohydrate Megalac (%)	Fibre		Starch	
	0	6	0	6
(a) Ingredient (g/kg)				
Wheat	–	–	450	500
Maize	–	–	200	200
Malt culms	–	62	14	146
Wheat feed	219	250	250	–
Oat feed	200	200	28	18
Sugar beet pulp	489	307	–	–
Rapeseed meal	–	33	–	18
Urea	12	8	8	8
Molasses	80	80	50	50
Megalac	–	60	–	60
	1000	1000	1000	1000
(b) Chemical analysis [†] – concentrate				
Dry matter, g/kg	864	863	857	866
ME, MJ/kg DM	11.3	12.1	13.1	14.2
Crude protein, g/kg DM	139	140	153	149
NDF, g/kg DM	361	380	170	143
Starch, g/kg DM	210	187	512	495
AHEE, g/kg DM	24	67	35	71
(c) Chemical analysis [†] – straw				
Dry matter, g/kg			860	
ME, MJ/kg DM			5.6	
Crude protein, g/kg DM			31	
NDF, g/kg DM			855	
Starch, g/kg DM			11	
AHEE, g/kg DM			18	

[†] DM: dry matter; ME: metabolisable energy; NDF: neutral detergent fibre; AHEE: acid hydrolysed ether extract.

Figure 1 Schematic representation of oestrous synchronisation (controlled internal drug-release dispenser (CIDR) plus prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$)), ovarian stimulation (four doses of FSH administered over 36 h) and follicular aspiration (ovum pick-up (OPU)). A GnRH agonist was administered 6 h before the second and third sessions of OPU, and a $PGF_{2\alpha}$ analogue was administered 48 h after the last of these sessions. See text for details.

Oestrous synchronisation, oocyte recovery and follicular growth

A reference oestrus was established with a 10-day progesterone-releasing device, CIDR (controlled internal drug-release dispenser) (SmithKline Beecham, Tadworth, UK), containing 1.9 g progesterone. A prostaglandin $F_{2\alpha}$ agonist (luprolisone 15 mg; Prosolvin, Intervet, Cambridge, UK) was administered intramuscularly 8 days after CIDR insertion. Heifers were predicted to be in oestrus 2 days after CIDR removal (day 0 = day of reference oestrus) (Fig. 1).

Animals were subjected to ultrasound guided transvaginal follicular aspiration (ovum pick-up (OPU)), as described by Goodhand *et al.* (1999), on day 5 of the subsequent oestrous cycle, and harvested oocytes and granulosa cells were used for fatty acid analysis (described later). The ovarian stimulation programme used to collect oocytes for *in vitro* culture was based on that of Blondin *et al.* (2002) with the following modifications. Four constant doses of follicle-stimulating hormone (FSH) (total amount equivalent to 9 mg NIADDK-oFSH-17; Ovagen, ICP, Auckland, New Zealand) were administered intramuscularly at 12-h intervals over a 36-h period commencing 48 h after follicular aspiration on day 5 (Fig. 1). There followed a 40-h 'coasting' period before OPU, during which no further FSH was administered. A gonadotrophin-releasing hormone (GnRH) analogue (0.012 mg Buserelin; Receptal, Hoechst Animal Health, Milton Keynes, UK) was administered intravenously 6 h before the second and third sessions of OPU. Oocytes from these stimulated ovaries were selected for *in vitro* maturation, fertilisation and culture to the blastocyst stage. The rationale for adopting this protocol was to create developmentally competent oocytes representative of those within preovulatory follicles. It was derived from the concept that the ability of the oocyte to develop after fertilisation is acquired before the onset of meiosis (Sirard 2001) and, in the context of the current study, follicular aspiration. Central to this concept is the withdrawal of FSH for

36–48 h before oocyte retrieval, so inducing early atresia in follicles, followed by luteinising hormone (LH) (or GnRH) treatment several hours before aspiration, a treatment which attempts to mimic the periovulatory endocrine milieu.

At the beginning of each OPU session, heifers were sedated by 28 mg xylazine hydrochloride (Rompun; Bayer, Leverkusen, Germany) administered i.m. before epidural anaesthesia (150 mg lignocaine hydrochloride) (Locovetic; Bimeda, Anglesey, UK) between the first and second coccygeal vertebrae. A 5 MHz curvilinear transducer (Aloka SSD-500 V; BCF, Livingston, UK) was inserted vaginally, and all visible follicles over 2 mm diameter were categorised by diameter before aspiration. All visible follicles were then aspirated with a single lumen needle (20 gauge) at a vacuum pressure of 70 mm Hg into conical tubes containing 5 ml warm PBS supplemented with 0.1% w/v fatty acid-free BSA and antibiotics.

Blood samples were collected before the early morning meal on days 0 (oestrus), 5, 8, 10 (OPU 2), 13, 15 (OPU 3), 18, 19 and 20 (oestrus) by jugular venipuncture into EDTA-treated tubes on ice. Plasma was harvested from these samples within 30 min of collection and stored at -20°C until analysis.

In vitro embryo production (IVP)

Full details of these procedures were given previously (Sinclair *et al.* 2000, Adamiak *et al.* 2005), and only a brief summary is provided here. All reagents and media used in the experiment were obtained from Sigma unless otherwise stated. Animal identity was retained throughout. Tubes containing aspirants were transported in a polystyrene box (at 37°C) to the laboratory, and aspirants were filtered through a sterile, 70 μm nylon gauze (BD Falcon, Bedford, MA, USA) rinsed with PBS supplemented with 0.2% w/v fatty acid-free BSA and antibiotics. Cumulus–oocyte complexes (COCs) were graded on a four-point scale by the number of compact cumulus cell layers and granulation of

the oocyte cytoplasm, as described by Goodhand *et al.* (1999). Selected (grades 1–3) COCs were matured in 50 μ l drops of maturation medium (bicarbonate-buffered TCM199 with Earle's salts and supplemented with 0.4% w/v fatty acid-free BSA, 10 μ g/ml FSH, 10 μ g/ml LH and antibiotics) with granulosa cell (GC) monolayers (≤ 20 oocytes/drop), overlaid with mineral oil. These monolayers were prepared as described previously (Adamiak *et al.* 2005). Oocytes were matured for 24 h at 38.8°C in a humidified atmosphere of 5% CO₂ in air.

Frozen semen from a single bull was thawed at 37°C and layered (85 μ l) under 1 ml modified, calcium-free Tyrode's albumin lactate pyruvate medium (TALP) in conical tubes for a swim-up procedure, as described previously (Adamiak *et al.* 2005). Motile spermatozoa were counted and added in a maximum of 4 μ l capacitation media to a 46 μ l drop of fertilisation media containing matured oocytes (up to 20 per drop), to give a final concentration of 1×10^6 motile spermatozoa/ml fertilisation media. Fertilisation medium was modified TALP supplemented with (in nmol/ml) 0.2 penicillamine, 0.1 hypotaurine and 0.02 epinephrine, with 0.6% w/v fatty acid-free BSA, 30 μ g/ml heparin and antibiotics. The oocytes and spermatozoa were incubated in 50 μ l drops under mineral oil for 22 h at 38.8°C in a humidified atmosphere of 5% CO₂ in air. Putative zygotes were cultured in groups of up to 20 zygotes per 30 μ l drops of synthetic oviductal fluid medium (Tervit *et al.* 1972), containing (in μ mol/ml) 25 NaHCO₃, 108 NaCl, 7.2 KCl, 1.7 CaCl₂, 1.2 KH₂PO₄, 0.5 MgCl₂, 1.5 glucose, 3.3 sodium pyruvate, 33 sodium lactate and 1 L-glutamine, supplemented with 0.3% w/v fatty acid-free BSA, 1% v/v non-essential amino acids, and 2% v/v essential amino acids and antibiotics. Putative zygotes were incubated in a humidified atmosphere of 5% CO₂, 5% O₂, and 90% N₂ at 38.8°C, and droplets were renewed every 48 h. On day 2 of development (day 0 = *in vitro* fertilisation), the cleavage rate was recorded, and cleaved zygotes were then cultured in 30 μ l microdrops until day 8.

Embryo development and TUNEL analysis

Blastocyst yield and developmental stage were recorded on day 8 after *in vitro* fertilisation. Blastocysts were then washed and fixed in 4% w/v paraformaldehyde and stored in a cold room for up to 1 week in preparation for cell counts and TUNEL analysis (ApopTag staining kit, Intergen, Purchase, NY, USA). Briefly, after washing in PBS with 0.1% w/v polyvinylpyrrolidone (PVP), embryos were permeabilised in 1% w/v saponin for 1 h at room temperature. The embryos were then washed in 20 μ l drops of PBS with 0.1% w/v PVP and 0.1% v/v Tween 20 (PPT), incubated for 10 min in equilibration buffer, transferred to 20 μ l drops of working strength TdT enzyme and incubated for 1 h in a humidified chamber at 37°C. The reaction was terminated, the embryos washed again in PPT and transferred to 20 μ l drops of working strength

antidigoxigenin conjugate, and incubated in a darkened, humidified chamber for 1 h at room temperature. After washing in PPT, the embryos were transferred to 20 μ l drops of Hoechst 33324 (10 μ g/ml in 2.3% w/v sodium citrate) and incubated for 15 min at room temperature. Finally, the embryos were mounted on silanised slides, overlaid with 30 μ l Citifluor solid mounting medium (Agar Scientific, Stansted, UK), and visualised by fluorescence microscopy. Cell number and the proportion of TUNEL-positive cells were promptly recorded.

Lipid analysis

Cumulus–oocyte complexes from two animals per treatment were pooled to ensure a minimum of five COCs per replicate. This also permitted the grading of COCs within each pair according to the amount of surrounding cumulus cells. Poor-quality COCs (grades 3–4) with few cumulus cells were analysed separately from good-quality COCs (grades 1–2), which had several layers of cumulus cells (Goodhand *et al.* 1999). The COCs were washed several times in SOF (containing 0.4% w/v fatty acid free-BSA) before transfer in 5 μ l media into a test tube containing 25 ml chloroform:methanol (2:1 v:v solution) and 137.5 mg internal standard (pentadecanoic acid, C15:0). Granulosa cells from each animal were washed twice in PBS/BSA by centrifugation before lipid extraction. Total lipid was extracted as described by Christie (1982). The esterified lipid fractions were subjected to transmethylation by refluxing with methanol:toluene:sulphuric acid (20:10:1 v:v) (Christie *et al.* 1970), extracted into a final volume of 50 μ l hexane and assayed in duplicate. The fatty acid methyl esters were injected via a CP8200 autosampler (Varian Analytical Instruments, Walton-on-Thames, UK), into a 30 mm, 0.25 mm internal diameter, 0.25 μ m film thickness, HP-225 capillary column (Hewlett Packard, Stockport, UK). Injection volume was 2 μ l by the spitless method. Peak areas were determined with a Varian Star Chromatography Workstation (Version 5.5; Varian Analytical Instruments), identified with reference to a known standard (37-Component Mix; Supelco, Poole, UK) and quantified by the internal standard method.

Due to the scarcity of biological material, it was impracticable to set up appropriate quality control for the COCs. However, the recovery of triglyceride (trionadecanoin, 19:0) and phospholipid (phosphatidylcholine, diheptadecanoyl, 17:0) was monitored throughout. Recoveries (16 observations) were $96.8 \pm 2.36\%$ and $97.5 \pm 2.32\%$ for triglyceride and phospholipid respectively, which did not differ ($P > 0.05$) from 100%. Furthermore, there was no significant difference (paired *t*-test) between recoveries of triglyceride and phospholipid.

Hormone analysis

Plasma insulin concentrations were determined by ¹²⁵I-labelled insulin double-antibody RIA by the method of

Starr *et al.* (1979). The assay was modified to use porcine insulin (I-3505, 24 IU/mg) obtained from Sigma and guinea-pig antiporcine insulin, normal guinea-pig serum and sheep anti-guinea-pig IgG donated by the Scottish Antibody Production Unit, Law Hospital, Carlisle, UK. The ^{125}I -labelled porcine insulin solution was prepared by the chloramine T method (Greenwood *et al.* 1963). The minimum detection limit of the assay (defined at ED_{85}) was 2.6 $\mu\text{IU/ml}$. The interassay coefficients of variation for low, medium and high controls were 10.0%, 7.9% and 10.3% respectively, and corresponding intra-assay coefficients of variation were 6.7%, 7.9% and 7.1%.

Plasma insulin-like growth factor (IGF)-I concentrations were determined by a ^{125}I -labelled IGF-I double-antibody RIA based on the method of Armstrong *et al.* (1990). Serum IGF-I was first separated from its binding proteins by acid gel HPLC by the method of Owens *et al.* (1990). The RIA used recombinant human IGF-I, anti-human IGF-I polyclonal antiserum (rabbit) both obtained from GroPep (Adelaide, Australia), normal rabbit serum and donkey anti-rabbit IgG obtained from Diagnostics Scotland (Law Hospital). The ^{125}I -labelled IGF-I solution was prepared by the chloramine T method (Greenwood *et al.* 1963). All samples analysed for IGF-I concentrations were processed in a single assay with a detection limit of 12.5 ng/ml where the coefficients of variation for low- and high-quality controls were 11.7 and 8.4% respectively.

Plasma leptin concentrations were determined in duplicate by the method of Blache *et al.* (2000). The primary antibody (emu anti-recombinant ovine/bovine leptin), normal emu serum and sheep antiemu serum were both provided by Dr Blache (University of Western Australia). Ovine leptin (supplied by Dr Keisler, University of Missouri, USA) was iodinated in-house as described above. The detection limit for leptin concentration was 0.2 ng/ml, and the interassay coefficients of variation for low, medium and high controls were 13.0%, 9.7% and 10.5% respectively. Corresponding intra-assay coefficients of variation were 12.8%, 9.9% and 10.3% respectively.

Plasma progesterone was analysed without prior extraction by a ^{125}I -progesterone double-antibody RIA (McNeilly & Fraser 1987). The assay was modified and validated to enable the use of a rabbit anti-progesterone first antibody, donkey antirabbit IgG and normal rabbit serum, which were donated by the Scottish Antibody Production Unit, Carlisle, UK. The sensitivity of the assay at ED_{80} was 0.62 ng/ml. The interassay coefficients of variation for low, medium and high controls were 13.0%, 9.7% and 10.5% respectively, and corresponding intra-assay coefficients of variation were 12.8%, 9.9% and 10.3% respectively.

Data analysis

Changes in plasma hormone concentrations were analysed by repeated measures ANOVA within GenStat 6 (Genstat 2002), where BCS (low vs moderate), carbohydrate source (fibre vs starch), fatty acid inclusion (0% vs 6%)

and interactions between these factors formed the between-animal stratum; sample day, BCS, carbohydrate source and fatty acid inclusion formed the within animal $\times$ day stratum. Similarly, the fatty acid composition of plasma, granulosa cells and COCs were analysed by repeated measures ANOVA. In the case of COCs, however, COC grade (poor vs good) replaced sample day in the model. Data presented are predicted means with either appropriate standard errors of differences or standard errors of means. Oocyte recovery and *in vitro* embryo production data (i.e. oocytes cleaved and day-8 blastocyst yields) were analysed by generalised linear models assuming binomial errors with logit link functions within the same statistical package. Terms fitted to the model were animal, BCS, carbohydrate source, fatty acid inclusion and OPU session. Comparisons between means were conducted by analysis of deviance. Data are presented as predicted means from these models.

Results

Live weight and body condition

Mean ($\pm$ S.E.M.) live weight and BCS were lower ($P < 0.001$) at the beginning of the experimental period for heifers allocated to the low- than the moderate-BCS group (382.2 ± 4.86 vs 411.7 ± 4.19 kg; and 2.5 ± 0.09 vs 3.0 ± 0.06 units). However, neither BCS nor dietary composition (i.e. carbohydrate source and fatty acid supplementation) affected the extent of live weight and BCS gained during the experimental period. Heifers gained, on average, 1.29 ± 0.07 kg/day and 0.31 ± 0.12 units BCS over the 40-day period.

Endocrinology

Plasma progesterone concentrations peaked on day 15 of the oestrous cycle and, for low-BCS heifers, were greater ($P < 0.01$) when Megalac was included in the diet (14.4 vs 8.6 ng/ml; S.E.D. = 1.56). There was an interaction between carbohydrate source and Megalac, indicating that the inclusion of rumen bypass fatty acids increased peak plasma progesterone concentrations in low-BCS heifers offered the high-fibre, but not the high-starch, diet (Table 2a).

Metabolic hormone concentrations were determined over a 3-day period leading up to oestrus (day 20) (Fig. 1). On the day of oestrus, mean concentrations of plasma insulin were greater ($P < 0.01$) for heifers in moderate than low BCS (17.06 vs 12.84; S.E.D. = 1.49). Plasma insulin concentrations averaged over this 3-day period were greater ($P < 0.01$) for low-BCS heifers on the high-starch than on the high-fibre diet (16.7 vs 10.8 $\mu\text{IU/ml}$; S.E.D. = 1.47) (Table 2). Similarly, plasma insulin concentrations were greater ($P < 0.01$) for moderate-BCS heifers on the high-starch than high-fibre diet but, in these animals, only on the day of oestrus (20.4 vs 13.8 $\mu\text{IU/ml}$ for

Table 2 Peak plasma progesterone concentrations (coinciding with day 15 (third OPU) of the oestrous cycle), and plasma insulin, IGF-I and leptin concentrations averaged over the last 3 days leading up to the second induced oestrous (day 20).

Principal carbohydrate (C)	Fibre		Starch		S.E.D.	Significance of effect		
	0	6	0	6		C	FA	CxFA
Megalac (%) (FA)								
(a) Low BCS								
No. of heifers	4	4	4	4				
Peak progesterone (ng/ml)	6.7 ^a	16.0 ^b	10.5 ^{ab}	12.8 ^b	2.20		<0.01	<0.05
Insulin (μ IU/ml)	11.2 ^a	10.5 ^a	16.1 ^b	17.2 ^b	1.47	<0.01		
IGF-I (ng/ml)	232.7	225.5	275.0	234.2	35.08			
Leptin (ng/ml)	2.4	2.1	1.8	2.7	0.44			<0.06
(b) Moderate BCS								
No. of heifers	4	4	4	4				
Peak progesterone (ng/ml)	14.5	9.5	13.1	13.9	2.98			
Insulin (μ IU/ml)	14.3	13.6	18.2	15.9	2.60			
IGF-I (ng/ml)	232.5	221.2	266.8	244.6	21.88			
Leptin (ng/ml)	4.4	5.4	4.9	3.7	0.93			

Means within a row with different superscripts are significantly different ($P < 0.05$).

the high-starch and high-fibre diets respectively; S.E.D. = 2.6). There was an indication that plasma IGF-I concentrations at oestrus were also greater ($P = 0.05$) for moderate-BCS heifers offered the high-starch than high-fibre diet (261.0 vs 226.0 ng/ml for the high-starch and high-fibre diets respectively; S.E.D. = 15.84), but this observation was not consistent for low-BCS heifers. Plasma leptin concentrations were greater ($P < 0.001$) for heifers in moderate than low BCS (4.6 vs 2.2 ng/ml; S.E.D. = 0.37), but were not significantly influenced by dietary composition.

Oocyte and embryo development *in vitro*

The number of follicles aspirated was not affected by experimental treatment but was greater ($P < 0.001$) for each of the two FSH/GnRH stimulated cycles of OPU per

animal than for the non-stimulated cycle (25.9 ± 1.87 vs 15.4 ± 1.15 follicles). However, the number of oocytes recovered did not differ between stimulated (12.1 ± 0.92) and non-stimulated (11.6 ± 1.00) cycles. In spite of this, the percentage of grades 1-2 oocytes recovered was greater ($P < 0.001$) for stimulated than non-stimulated ovaries ($62.4 \pm 2.77\%$ vs $42.5 \pm 2.93\%$). Furthermore, compared with COCs from non-stimulated heifers after slaughter at the end of the current study (data not presented), ovarian stimulation significantly ($P < 0.05$) enhanced both cleavage rates and blastocyst yields *in vitro* (74% vs 64% and 32% vs 18% for cleavage rates and blastocysts of cleaved oocytes respectively).

There was a suggestion that cleavage rate (oocytes cleaved of matured oocytes) was greater for oocytes recovered from low- than moderate-BCS heifers (75.7 ± 2.1 vs $68.0 \pm 2.8\%$; $P = 0.05$) and from heifers offered the

Table 3 Embryo development following maturation, fertilisation and culture *in vitro*.

Principal carbohydrate (C)	Fibre		Starch	
	0	6	0	6
Megalac (%) (FA)				
(a) Low BCS				
No. of heifers	4	4	4	4
Oocytes matured (<i>n</i>)	11.2 $\pm$ 3.6	14.4 $\pm$ 4.1	15.2 $\pm$ 4.2	10.1 $\pm$ 3.5
Oocytes cleaved (<i>n</i>)	8.6 $\pm$ 2.6	11.5 $\pm$ 3.0	10.9 $\pm$ 3.0	7.5 $\pm$ 2.5
Cleaved of matured (%)	76.7 $\pm$ 4.8	80.0 $\pm$ 4.0	71.1 $\pm$ 4.4	74.1 $\pm$ 5.2
Blastocysts (<i>n</i>)	4.6 $\pm$ 1.4	3.9 $\pm$ 1.3	3.5 $\pm$ 1.2	1.6 $\pm$ 0.8
Blastocyst yield (%)	53.6 ^a $\pm$ 7.9	33.7 ^b $\pm$ 6.5	32.2 ^b $\pm$ 6.6	21.7 ^b $\pm$ 7.0
Cell number	111.3 ^a $\pm$ 5.3	131.7 ^b $\pm$ 5.7	154.2 ^c $\pm$ 6.2	123.2 ^{ab} $\pm$ 6.4
TUNEL-positive cells (%)	4.6 $\pm$ 1.2	4.8 $\pm$ 1.1	3.5 $\pm$ 0.9	3.1 $\pm$ 1.1
(b) Moderate BCS				
No. of heifers	4	4	4	4
Oocytes matured (<i>n</i>)	11.9 $\pm$ 3.7	13.1 $\pm$ 3.9	10.2 $\pm$ 3.5	9.5 $\pm$ 3.3
Oocytes cleaved (<i>n</i>)	7.6 $\pm$ 2.5	10.2 $\pm$ 2.9	6.6 $\pm$ 2.3	6.1 $\pm$ 2.2
Cleaved of matured (%)	64.2 $\pm$ 5.3	78.1 $\pm$ 4.3	64.6 $\pm$ 5.7	64.5 $\pm$ 5.9
Blastocysts (<i>n</i>)	1.0 $\pm$ 0.6	3.5 $\pm$ 1.2	2.0 $\pm$ 0.9	2.1 $\pm$ 0.9
Blastocyst yield (%)	13.1 $\pm$ 5.7	34.2 $\pm$ 6.9	30.2 $\pm$ 8.3	34.7 $\pm$ 9.0
Cell number	105.1 ^{ac} $\pm$ 5.9	134.5 ^b $\pm$ 5.8	119.4 ^c $\pm$ 6.3	104.7 ^a $\pm$ 5.1
TUNEL-positive cells (%)	4.2 $\pm$ 1.4	3.0 $\pm$ 0.9	3.3 $\pm$ 1.2	5.0 $\pm$ 1.3

Means within a row with different superscripts are significantly different ($P < 0.05$).

high-fibre than the high-starch diets ($74.9 \pm 2.3\%$ vs $68.9 \pm 2.6\%$; $P < 0.1$) (Table 3). Heifer BCS, however, did not affect blastocyst yields (i.e. blastocysts of cleaved oocytes), but there was an interaction ($P < 0.01$) between BCS and dietary Megalac, and the suggestion of interaction ($P = 0.05$) between BCS and carbohydrate source, indicating that the inclusion of either Megalac or high levels of starch in the diet reduced blastocyst yields from oocytes recovered from low-BCS, but not moderate-BCS, heifers (Table 3). Both high-starch diets and the inclusion of Megalac in the diet reduced ($P < 0.05$) blastocyst yields in low-BCS heifers. In contrast, neither dietary constituent significantly influenced blastocyst yields in moderate-BCS heifers, although, numerically, blastocyst yields were lower (13.1%) for oocytes derived from heifers offered the high-fibre, zero Megalac diet than for oocytes derived from heifers offered the other dietary treatments (range = 30.2–34.7%).

The total number of blastomeres per embryo was greater ($P < 0.001$) for oocytes derived from low- than moderate-BCS heifers (129.7 ± 2.96 vs 116.2 ± 2.90). There were interactions ($P < 0.001$) between BCS and carbohydrate source, and carbohydrate source and Megalac on blastomere number, indicating, in the first instance, that the highest blastomere numbers were for embryos derived from low-BCS heifers offered high-starch diets and moderate-BCS heifers offered high-fibre diets and, in the second instance, for embryos derived from heifers offered the high-fibre diet with Megalac and heifers offered the high-starch diet without Megalac (Table 3). In contrast, the percentage of TUNEL-positive cells averaged $4.2 \pm 0.45\%$ and did not differ between BCS groups nor between the dietary treatments (Table 3).

Fatty acid analysis

The total fatty acid content of plasma averaged 1120 ± 97 ng/ml and was not affected by heifer BCS. However, the concentration of unsaturated fatty acids was greater ($P < 0.05$) for heifers with moderate than low BCS (604 vs 517 ng/ml; S.E.D. = 38). This was associated with a reduction ($P < 0.05$) in monounsaturated fatty acids (211 vs 243 ng/ml; S.E.D. = 14) and an increase ($P < 0.001$) in PUFAs (393 vs 272 ng/ml; S.E.D. = 28). The former was due mainly to a reduction ($P < 0.05$) in oleic acid (C18:1, n-9) (181 vs 207 ng/ml; S.E.D. = 11), while the latter was due mainly to an increase ($P < 0.001$) in linoleic acid (C18:2, n-6) concentrations; however, the concentration of arachidonic acid (C20:4, n-6) was also greater ($P < 0.001$) in the fatter animals (23 vs 9 ng/ml; S.E.D. = 2).

The fatty acid content of good-quality (grades 1–2) COCs with several layers of cumulus cells was greater ($P < 0.01$) than that of poor-quality COCs (grades 3–4) with few cumulus cells (79.2 vs 67.8 ng/COC; S.E.D. = 3.23). There were no other qualitative differences between these two categories of COC. In contrast to plasma, the fatty acid content of COCs was lower

($P < 0.01$) for those recovered from moderate- than low-BCS heifers (69.18 vs 78.47 ng/COC; S.E.D. = 2.37). This was due in part to a reduction in the content of saturated fatty acids, of which the reduction in stearic acid (C18:0) was greatest (24.36 vs 30.00 ng/COC; S.E.D. = 1.73; $P < 0.05$), but also to a reduction ($P < 0.01$) in the content of unsaturated fatty acids (7.74 vs 11.57 ng/COC; S.E.D. = 0.92), of which reductions in linoleic acid ($P < 0.01$) and arachidonic acid ($P < 0.05$) were greatest (0.40 vs 0.95 ng/COC; S.E.D. = 0.13; and 0.02 vs 0.30 ng/COC; S.E.D. = 0.09 for linoleic and arachidonic acid respectively).

In spite of the differences in plasma and COC fatty acid composition between low- and moderate-BCS heifers, there were no interactions between the dietary treatments (i.e. fibre vs starch and 0% vs 6% Megalac) and BCS, so that the fatty acid data for both low- and moderate-BCS heifers were pooled (Table 4). Both carbohydrate source and Megalac influenced the fatty acid composition of plasma. Relative to the starch-based diets, the high-fibre diets increased ($P < 0.001$) total (1289 vs 950 ng/ml) and therefore all constituent fatty acid concentrations. The proportions of fatty acids also changed in response to diet; plasma from cattle fed the starch-based diets contained proportionately more saturated (mainly palmitic acid) and monounsaturated fatty acid (mainly oleic acid) and less PUFA (mainly linoleic acid) than plasma from the fibre-based diets. Similarly, relative to the non-supplemented diets, the inclusion of 6% Megalac increased ($P < 0.001$) total (1611 vs 628 ng/ml), saturated (782 vs 336 ng/ml) and all individual fatty acid classes. Proportionately, the main effect of Megalac supplementation was to decrease the amount of saturated fatty acids (mainly stearic acid). Furthermore, the inclusion of Megalac in the diet reduced ($P < 0.001$) the ratio of saturated to unsaturated fatty acids (0.97 vs 1.21). There was also an interaction ($P < 0.001$) between carbohydrate source and Megalac inclusion in the diet in the ratio of PUFA:monounsaturated fatty acids (1.90 , 1.56 , 1.03 and 1.41 for fibre and starch diets either without or with 6% Megalac respectively; S.E.D. = 0.14).

Differences in the fatty acid content of COCs were less marked than for plasma (Table 4). The inclusion of Megalac in the diets increased ($P < 0.05$) the total fatty acid content of COCs (77.12 vs 70.53 ng/COC; S.E.D. = 2.37) but did not affect the content of saturated fatty acids. Interactions ($P < 0.05$) between carbohydrate source and Megalac inclusion indicated that Megalac in the diet increased the concentration of unsaturated (specifically monounsaturated) fatty acids in COCs from heifers offered the high-starch diet, but not the high-fibre diet. The concentrations of PUFAs within the COCs were unaffected by dietary treatments.

The fatty acid composition (% total fatty acids) of granulosa cells and COCs differed markedly from that of plasma (Table 5). The percentage of saturated fatty acids was greater ($P < 0.001$) for COCs than for granulosa cells and plasma, while the percentage of unsaturated fatty acids

Table 4 Effect of carbohydrate source and fatty acid supplement on the fatty acid composition of plasma and cumulus–oocyte complexes.

Principal carbohydrate	Plasma					Cumulus–oocyte complexes (COC)				
	Fibre		Starch		S.E.D.	Fibre		Starch		S.E.D.
	0	6	0	6		0	6	0	6	
Megalac (%)										
No. of observations	8	8	8	8		4	4	4	4	
	ng/ml					ng/COC				
Fatty acids										
Total	754 ^a	1824 ^b	501 ^c	1399 ^d	79	70.2 ^x	73.7 ^{xy}	70.9 ^x	80.56 ^y	3.35
Saturated	392 ^a	868 ^b	281 ^c	696 ^d	35	61.3	62.8	61.2	72.7	4.48
Unsaturated	362 ^a	956 ^b	220 ^c	702 ^d	17	8.4 ^x	11.8 ^y	10.0 ^{xy}	8.5 ^x	1.30
Monounsaturated	127 ^a	378 ^b	110 ^a	295 ^c	19	6.8 ^x	9.9 ^y	8.3 ^{xy}	7.8 ^x	0.88
Polyunsaturated	235 ^a	578 ^b	111 ^c	406 ^d	39	1.6	1.9	1.7	0.7	0.95
n-6 series	222 ^a	543 ^b	103 ^c	377 ^d	36	1.3	1.3	1.4	0.5	0.41
n-3 series	13 ^a	35 ^b	7 ^a	28 ^b	4	0.3	0.6	0.3	0.2	0.25
Palmitic 16:0	154 ^a	397 ^b	121 ^c	325 ^d	14	29.6 ^x	29.8 ^x	27.8 ^x	34.1 ^y	1.71
Stearic 18:0	224 ^a	449 ^b	151 ^c	352 ^d	24	25.1	26.8	26.2	30.6	2.44
Oleic 18:1, n-9	109 ^a	326 ^b	93 ^a	248 ^d	16	4.2	5.8	5.7	3.9	1.17
Linoleic 18:2, n-6	188 ^a	457 ^b	81 ^c	318 ^d	2	0.8	0.8	0.6	0.4	0.19
Arachidonic 20:4, n-6	13 ^a	25 ^b	8 ^a	19 ^{ab}	3	0.1 ^x	0.2 ^{xy}	0.4 ^y	<0.1 ^x	0.12
α-Linolenic 18:3, n-3	5 ^a	15 ^b	2 ^a	11 ^b	2	0.1	0.4	0.1	0.1	0.19
Eicosapentaenoic 20:5, n-3	6 ^a	15 ^b	5 ^a	11 ^b	2	0.1	0.2	0.2	<0.1	0.09
Docosahexaenoic 22:6, n-3	1 ^a	3 ^b	1 ^a	2 ^{ab}	0.5	<0.1	<0.1	<0.1	<0.1	0.02

Means within rows with a different superscript are significantly ($P < 0.05$) different.

was lower. The percentage of monounsaturated fatty acids was greater in granulosa cells than in either plasma or COCs, and the percentage of PUFAs was less in follicular cells than in plasma.

Discussion

One of the major findings from this study was that the effects of altering dietary composition (both carbohydrate type and the level of fatty acids) on oocyte quality depend on the initial BCS of the animal. This is an important finding, for it confirms and possibly explains our previous observation, in a similar group of animals, that the effects of level of feeding on oocyte quality depend on animal body composition (Adamiak *et al.* 2005). In addition, in contrast to high-fibre diets, the effects of feeding high levels of starch or fatty acids in the present study were detrimental to post-fertilisation development *in vitro*, but only in low-BCS heifers. Similarly, the effects of diet composition on plasma hormone concentrations were observed only in the leaner group of animals. Furthermore, the effects of diet composition on oocyte quality occurred in

the absence of any significant alteration to the fatty acid profile of COCs, although dietary lipid did increase the fatty acid content of COCs. Indeed, a second major finding from this study was the selective uptake of saturated fatty acids at the expense of mainly PUFAs within the follicular compartment.

Endocrinology

The substitution of starch for fibre in the diet increased plasma insulin in low-BCS heifers (from ~11 to 17 μ U/ml), equivalent to the increase in plasma insulin (from ~11 to 22 μ U/ml) observed for low-BCS heifers fed a comparable starch-based diet at maintenance (M) and twice maintenance (2 M) in the study of Adamiak *et al.* (2005). However, whereas the increase in plasma insulin concentrations with 2 M feeding in that study was associated with increased blastocyst yields, the increase in plasma insulin associated with feeding starch-based diets in the present study was associated with reduced blastocyst yields (Table 3). Consequently, the detrimental effects of starch relative to fibre-based diets on oocyte quality may

Table 5 Fatty acid composition (% w/w total fatty acids) of plasma, granulosa cells and cumulus–oocyte complexes.

Fatty acids (% of total)	Plasma	Granulosa cells	Cumulus–oocyte complexes	S.E.D.	Significance of effect
Saturated	51.6 ^a	55.7 ^a	86.3 ^b	1.92	$P < 0.001$
Unsaturated	48.5 ^a	43.2 ^a	12.8 ^b	1.93	$P < 0.001$
Monounsaturated	20.3 ^a	32.6 ^b	11.6 ^c	1.15	$P < 0.001$
Polyunsaturated	28.2 ^a	11.6 ^b	2.4 ^c	1.80	$P < 0.001$
n-6 series	26.4 ^a	10.7 ^b	1.7 ^c	1.43	$P < 0.001$
n-3 series	1.8 ^a	0.9 ^b	0.7 ^b	0.21	$P < 0.001$
Ratio n-6:n-3	16.6 ^a	10.9 ^b	4.1 ^c	1.80	$P < 0.001$

be independent of the effects of insulin. Both increasing the level of feeding and substituting starch for fibre in the diet increase plasma insulin in low-BCS animals but, unlike the former, the latter dietary modification is associated with impaired embryo development.

Neither plasma IGF-I nor plasma leptin concentrations were significantly affected by dietary composition. However, plasma leptin concentrations were greater in moderate- than low-BCS heifers, an observation consistent with previous observations in this type of animal (Delavaud *et al.* 2002, Adamiak *et al.* 2005). Although it is difficult at present to reach a consensus on the effects of leptin on oocyte growth and maturation (Swain *et al.* 2004, Craig *et al.* 2005), it seems that, at lower physiological concentrations, leptin may have a positive effect on oocyte maturation and post-fertilisation development, so that elevated concentrations of this cytokine may have attenuated, in some way, the negative effects of feeding high levels of starch and fat on oocyte quality in moderate-BCS heifers in the present study.

The increase in plasma progesterone concentrations in low-BCS heifers offered Megalac (Table 2) is consistent with previous experiments with ruminants (Mattos *et al.* 2000), and may have arisen as a consequence of either the enhanced steroidogenic activity of the corpus lutea in heifers fed a palmitic acid-rich lipid source (Kuran *et al.* 1999), or a reduced rate of liver metabolism and clearance of progesterone from peripheral circulation (Hawkins *et al.* 1995).

Principal carbohydrate source

There has been only one previous attempt to assess the effects of altering the supply of dietary starch and digestible fibre on embryo quality in heifers (Yaakub *et al.* 1999, Wrenzycki *et al.* 2000). These authors reported a decrease in the number of 'transferable embryos' (based on morphological assessment) after ovarian stimulation and artificial insemination that was associated with an increase in the metabolism of [2-¹⁴C]pyruvate in embryos derived from heifers offered the high-starch diet. This in turn was negatively correlated with transcript expression for the antioxidant enzyme Cu/Zn-SOD. These observations broadly agree with those presented in the current study, where the effects of dietary treatments were limited to the period of oocyte growth prior to the resumption of meiosis. The present study extends these observations and confirms our earlier findings (Adamiak *et al.* 2005) that the effects of diet depend on animal BCS, so that high-starch diets are detrimental to oocyte quality only in moderately thin animals. Unlike the study of Yaakub *et al.* (1999), dietary treatments in the current study were not extended beyond the period of fertilisation to include preimplantation development. However, using heifer serum from the current study to supplement SOF media during zygote culture *in vitro*, we also observed our starch-based diet to increase [2-¹⁴C]pyruvate metabolism in day-8 blastocysts,

and this was associated with an increase in the proportion of TUNEL-positive cells (Adamiak *et al.* 2004). Consequently, one mechanism by which the feeding of high-starch diets may reduce post-fertilisation development, both in the current study and in that of Yaakub *et al.* (1999), may be through the production of free oxygen radicals within both the follicular compartment and the oviduct. Certainly, transcripts for a number of antioxidant enzymes are expressed to varying degrees in immature and meiotically mature oocytes (Lonergan *et al.* 2003). However, if this is the mechanism underlying the reduction in blastocyst yields when feeding high-starch diets to low-BCS heifers, it does not explain, in the current study, the increase in blastomere number (with no change in the percentage TUNEL-positive cells) in surviving embryos (Table 3), nor does it explain the apparent lack of an effect of high-starch diets on post-fertilisation development of oocytes from moderate-BCS heifers. At present, we are unable to advance a credible hypothesis to explain this effect of BCS, although it may involve leptin and differences in nutrient partitioning within animals that differ in body composition.

Dietary lipid and fatty acids

Proximate analysis of the experimental diets confirmed the greater lipid (acid hydrolysed ether extract (AHEE)) content of the Megalac-supplemented diets (Table 1). However, fatty acid composition of the diets was not determined in the current study; rather, the content and composition of fatty acids within plasma were used as a reference to assess the effects of diet composition on fatty acid uptake and metabolism within the follicular compartment.

Lipids are transported in plasma in the form of lipoproteins. For non-lactating cattle, approximately 90% of this lipoprotein fraction is in the form of high-density lipoprotein (HDL), which consists of approximately 54% cholesterol esters, 25% cholesterol and 17% phospholipids (Christie 1981). It is highly probably, therefore, that much of the fatty acids taken up by the follicular compartment in the present study was derived from HDL, although this was not determined. Further, the paucity of biological material (i.e. the relative small numbers of COCs that could be recovered per animal) in the current study meant that no distinction could be made between polar and neutral fatty acids in these cells. Working with much greater numbers of oocytes, previous authors (e.g. McEvoy *et al.* 2000, Kim *et al.* 2001) reported the distribution (% w/w) of lipid classes for bovine oocytes to be approximately 50% triglyceride, 29% phospholipid, 20% cholesterol and 10% free fatty acids. The proportion of saturated, monounsaturated and polyunsaturated fatty acids within each of these classes was remarkably consistent in the ruminant oocyte. It is not known whether these proportions can be altered *in vivo* by dietary composition, although it is worth noting that changing the composition of *in vitro*

culture media (inclusion of serum rather than albumin) changed the relative amounts of fatty acids in neutral and polar lipids of bovine blastocysts (Reis *et al.* 2003).

An important finding of the present study was the apparent selective uptake of specific classes of fatty acids within the ovarian follicle that ensured the composition of the COC was low in unsaturated, particularly polyunsaturated, fatty acids (Table 5); similar observations have been made for sheep granulosa cells and oocytes by Reis *et al.* (2002). Importantly, unlike plasma, the profile of PUFAs in COCs in the present study was not affected to any significant degree by dietary composition. The fatty acid profile of COCs certainly bore little relation to that of plasma (Table 4), although this could be due in part to differences in the distribution of fatty acids within different fractions (i.e. cholesterol esters, phospholipids and triglycerides), as these are known to differ between tissues and plasma (Christie 1981).

Compared with other reports of the fatty acid composition of bovine mural-granulosa cells and oocytes (Kim *et al.* 2001, Zeron *et al.* 2001), the content of stearic acid (C18:0) was greater, whereas the contents of mono- and polyunsaturated fatty acids were somewhat less for COCs in the present study. This is almost certainly a reflection of the nature and composition of the experimental diets in the present study, although other factors such as genotype and season may have contributed (Zeron *et al.* 2001). Nevertheless, all reports agree that the most abundant fatty acids in ruminant oocytes are palmitic (C16:0), stearic (C18:0), oleic (C18:1) and linoleic (C18:2) acid.

Given the preferential uptake of saturated fatty acids by the COC within the follicular compartment and the fact that the composition of the lipid supplement used in this study comprised mostly these fatty acids, it is, perhaps, not surprising that the fatty acid profile of COCs was largely unaffected by dietary composition. Nevertheless, the inclusion of rumen bypass lipid in the diet of low-BCS heifers increased the total fatty acid content of COCs and reduced post-fertilisation development. Jorritsma *et al.* (2003) observed the concentration of non-esterified fatty acids (NEFA) in follicular fluid to increase when animals were placed in negative energy balance. The fatty acid profile of plasma NEFA is rich in saturated and monounsaturated fatty acids (21% palmitic, 32% stearic and 37% oleic; Pethick & Dunshea 1993). Although this profile differs slightly in follicular fluid (23%, 13% and 33% for palmitic, stearic and oleic acids respectively), Leroy *et al.* (2004) found that the addition of both palmitic and stearic acids, at corresponding physiological concentrations, during *in vitro* maturation reduced subsequent cleavage and post-fertilisation development of bovine oocytes.

Finally, there was a significant reduction in the content of n-6 PUFAs within the COCs of moderate- compared with low-BCS heifers. It is noteworthy that the cleavage rate of oocytes from moderate-BCS heifers was also less than that for low-BCS heifers (68% vs 76% respectively), and that this was associated with significantly

lower concentrations of linoleic and arachidonic acid within the COC. These fatty acids are precursors for prostanoid synthesis (Abayasekara & Wathes 1999) and, under the influence of cyclooxygenase-2 (COX-2), are metabolised to prostaglandin E₂ among other 2-series prostaglandins. COX-2 is expressed in bovine cumulus cells and, together with local prostanoid syntheses, is required for cumulus expansion and successful oocyte maturation (Calder *et al.* 2001, Nuttinck *et al.* 2002). At present, however, the functional significance of these observations remains to be confirmed, with no direct evidence of substrate-mediated alterations to these metabolic pathways affecting oocyte maturation and post-fertilisation development.

In conclusion, this study confirms and extends our previous findings that the effects of diet on oocyte quality depend on animal body composition. Diet-mediated alterations in plasma concentrations of key reproductive and metabolic hormones, in addition to oocyte quality, were detectable only in low-BCS animals. The underlying mechanisms for such effects remain elusive, although the inclusion of high levels of starch and fatty acids (mainly saturated fatty acids) in the diet of relatively lean oocyte donors reduces post-fertilisation development. For the range of diets offered in the present study, the follicular compartment was largely effective in ensuring oocytes of uniform fatty acid composition. The concentration of saturated fatty acids within COCs, however, is inherently high and it is possible that further increases in the content of these fatty acids, brought about by dietary lipid supplementation, impairs post-fertilisation development, perhaps by compromising membrane fluidity and function in ways that are not fully understood.

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