Reproduction in Domestic Animals

Reprod Dom Anim **49**, 711–718 (2014); doi: 10.1111/rda.12352
ISSN 0936–6768

MALDI-MS Lipid Profiles of Oocytes Recovered by Ovum Pickup from *Bos indicus* and 1/2 *indicus* × *taurus* with High vs Low Oocyte Yields

KC Silva-Santos[1], CR Ferreira[2], GMG Santos[1], MN Eberlin[2], LS Siloto[1], CO Rosa[1], TN Marcantonio[1] and MM Seneda[1]

[1]*Laboratório de Biotecnologia da Reprodução Animal (ReproA), DCV-CCA-Universidade Estadual de Londrina (UEL), Londrina, Brazil;*
[2]*ThoMSon Mass Spectrometry Laboratory, Institute of Chemistry, University of Campinas, Londrina, Brazil*

Contents

The aim of the present study was to compare the lipid profile in oocytes of *indicus* and 1/2 *indicus* × *taurus* cows with high and low antral follicle count (AFC)/oocyte yields. After an OPU procedure (D0), antral follicles ≥3 mm were counted by ultrasonography (D4, 19, 34, 49, 64), and cows were assigned to groups with either high AFC (≥30 follicles; *indicus*, NH group; 1/2 *indicus* × *taurus*, AH group) or low AFC (≤15 antral follicles; *indicus*, NL group; 1/2 *indicus* × *taurus*, AL group). The lipid profiles of the oocytes were determined by MALDI-MS. For GI, GII and GIII oocytes, the *indicus* samples tend to cluster separately from the 1/2 *indicus* × *taurus* samples. The lipid species $[PC\ (P\text{-}38{:}5) + H]^+$ and/or $[PC\ (P\text{-}36{:}2) + Na]^+$, $[PC\ (38{:}2) + H]^+$, $[PC\ (38{:}5) + Na]^+$ and $[TAG\ (60{:}8) + NH^4]^+$ were more abundant in *indicus* (NH and NL groups) than 1/2 *indicus* × *taurus*. The higher lipid content in the *indicus* oocytes likely reflects differences in the rate of lipid metabolism and may contribute to oocyte competence and embryo development.

Introduction

Bos indicus cows usually produce more oocytes than *Bos taurus* donors (Machado et al. 2006; Pontes et al. 2009; Pontes et al. 2010). Good oocyte yield can be obtained from *B. indicus* and *B. taurus* crosses (Pontes et al. 2010). The adaptability of *indicus-taurus* cross-bred females to tropical climates has encouraged their use in tropical and subtropical areas. There is, however, great variation in oocyte production among individuals (Pontes et al. 2009, 2011). Although it is possible to produce more embryos and pregnancies following OPU/*in vitro* embryo production (IVP) than superovulation/embryo transfer (ET) in *indicus* cows (Pontes et al. 2009), individual variation is observed in the oocyte production of cows with *indicus* and *indicus-taurus* backgrounds. There is, however, little information about the oocyte differences between these cattle types.

Triacylglycerides (TAG) predominate the lipid content of the cytoplasm of mammalian cells, forming lipid droplets (Ferguson and Leese 1999; Hulbert 2003; Aardema et al. 2011; McKeegan and Sturmey 2011). These lipids are crucial for oocytes and embryos because they function as a form of energy storage (Sturmey et al. 2009). In eukaryotic cell membranes, phospholipids (PL) are the most abundant lipids, and phosphatidylcholines (PCs) and sphingomyelins (SMs) influence fluidity, permeability, and thermal phase behaviour (Edidin 2003).

In mammalian gametes, immature oocytes have much lower mitochondrial activity than *in vitro*-matured oocytes (Van Blerkom et al. 2002; Tarazona et al. 2006), and developmental competence does not seem to be influenced by the number of active mitochondria in immature bovine oocytes (Castaneda et al. 2013). In oocytes with low-developmental competence, early activation of mitochondria may be triggered by lipids (Castaneda et al. 2013).

The developmental capacity of oocytes to undergo maturation, fertilization and early cleavage is influenced by both lipid content and mitochondrial activity (McEvoy et al. 2000; Cummins 2004). During maturation, lipase activity remains constant (Cetica et al. 2002), whereas triacylglyceride and cholesterol stores are reduced (Kim et al. 2001; Ferguson and Leese 1999) and mitochondrial activity increases (Tarazona et al. 2006). These trends suggest that lipids are used as a cellular energy source. In addition, lipid–organelle interactions likely occur during oocyte maturation because the mitochondria, lipids and smooth endoplasmic reticulum remain in clusters (Fleming and Saacke 1972; Hyttel et al. 1997).

Among the analytical techniques for lipid analysis of mammalian oocytes and pre-implantation embryos, mass spectrometry (MS) is emerging as a powerful tool. MS has been used to obtain lipid structural information from individual or small pools of oocytes (2–5) from human, bovine, sheep, mouse, canine and feline species as well as from individual pre-implantation embryos obtained from cattle and mice (Ferreira et al. 2010, 2012a,b; Apparicio et al. 2012; Sudano et al. 2012). The most commonly used MS technique for lipid profiling in oocytes and embryos is matrix-assisted desorption/ionization (MALDI; Ferreira et al. 2010). A more direct approach using an ambient desorption/ionization technique (DESI) has also been demonstrated recently (Ferreira et al. 2012b). MALDI-MS has been used under conditions that favour the detection of PC, SM and TAG.

In the present study, MALDI-MS lipid profiles of oocytes from *B. indicus* and 1/2 *indicus* × *taurus* donors with high and low oocyte yields were compared to identify significant lipid structural differences. We predict that lipids have potential as biomarkers for early diagnosis of high-oocyte-yield animals and that lipid analysis may contribute to the elucidation of the molecular mechanisms underlying higher oocyte yields.

Materials and Methods

Animals

Nelore females (*B. indicus*, n = 20) and 1/2 Nelore × Angus females (*indicus*/*taurus*, n = 20) that were 6–8 years old and had a body weight of 450 ± 10 kg and a

body condition score of 3.5 ± 0.5 (scale, 1–5; Lowman et al. 1976) were subjected to follicular aspiration on random days during the oestrous cycle (D0) to promote the ablation of all follicles ≥3 mm and the beginning of a new follicular wave. Animals were maintained in a *Brachiaria brizantha* pasture supplemented with mineral salt *ad libitum*. Some of the animals were selected for lipid profile evaluation based on the antral follicle count (described below).

Antral follicular counting

Ovaries from each animal were monitored with a 7.5-convex intravaginal array transducer (Áquila PRO; Pie medical, Maastricht, The Netherlands) at 15-d intervals after OPU (Days 4, 19, 34, 49, and 64), and antral follicles were counted as described previously (Burns et al. 2005; Ireland et al. 2008). Each ovary was scanned from end to end to identify positions of the CL and antral follicles ≥3 mm, which were drawn on an ovarian map. Each follicle was measured, and the diameter was averaged and recorded next to the appropriate follicle on each ovarian map. The antral follicle count (AFC – total number of antral follicles ≥3 mm in diameter) per pair of ovaries was determined for each animal. After five ultrasound evaluations performed by the same operator, females were assigned to two groups based on the number of antral follicles ≥3 mm in diameter: females with a consistently high (≥30 follicles; *indicus*, n = 3; 1/2 *indicus* × *taurus*, n = 5) or low AFC (≤15 follicles; *indicus* n = 4; 1/2 *indicus* × *taurus*, n = 4) in all ultrasound scans. Animals with intermediate AFC (>15 and <45 follicles) were not studied further.

Follicular aspiration and oocyte recovery

Oocytes were obtained by follicular aspiration from *indicus* and 1/2 *indicus* × *taurus* females with high and low AFC. Briefly, each visible follicle was aspirated using a real-time B-mode ultrasound scanner (Áquila PRO; Pie medical), a 7.5-MHz convex array transducer fitted into the intravaginal device (Pie Medical) and a stainless steel guide. Follicular puncture was performed using a disposable 19-gauge 1/2″ hypodermic needle (Becton Dickinson, Curitiba, PR, Brazil) connected to a 50-ml conical tube (Corning, Acton, MA, USA) via silicon tubing (0.8 m; 2 mm id). Aspiration was performed using a vacuum pump (WTA, Watanabe, Cravinhos, São Paulo, Brazil) with a negative pressure of 75 mm Hg. The collection medium was phosphate buffer solution (PBS-Nutricell, Campinas, SP, Brazil) with 10 000 IU/l sodium heparin (Sigma H-3149; Sigma-Aldrich, St. Louis, MO, USA). Immediately after recovery, the aspirated material was washed and filtered through an Emcon embryo filter (Immuno Systems Inc., Spring Valley, WI, USA) with a phosphate buffer solution (PBS-Nutricell). The cumulus oocyte complexes (COCs) were classified according to the presence of cumulus cells and the oocyte quality using the following criteria: (i) COCs from pre-antral follicles, (ii) grade I (GI), (iii) grade II (GII), (iv) grade III (GIII), (v) denuded, (vi) expanded and (vii) atretic oocytes.

Experimental design

Of the 83 oocytes recovered by OPU, only oocytes classified as GI, GII and GIII (total n = 53; 23 mass spectra) were considered in the lipid profile and statistical analysis for the breed and oocyte yield comparison. The four experimental groups were defined as follows: oocytes from *indicus* cows with high AFC (NH group; n = 31; 10 mass spectra), *indicus* cows with low AFC (NL group; n = 5, 3 mass spectra), 1/2 *indicus* × *taurus* cows with high AFC (AH group; n = 12, 6 mass spectra) and 1/2 *indicus* × *taurus* with low AFC (AL group, n = 5, 4 mass spectra).

Lipid analysis by MALDI-MS

Sample preparation

Each *indicus* or 1/2 *indicus* × *taurus cumulus* oocyte complex with *cumulus* cells and homogeneous cytoplasm was denuded of cells by gentle pipetting in 0.5% hyaluronidase. All oocytes were washed three times in drops of a PBS solution containing 0.1% polyvinyl alcohol and stored in microtubes containing 100 μl of a 50% aqueous methanol solution (ACS/HPLC grade; Burdick and Jackson, Muskegon, MI, USA)/ultrapure water (Millipore, Bedford, MA, USA) at −80°C for transportation and immediate analysis. Sample preparation involved placing each oocyte in a given spot of the target plate under the stereomicroscope. Samples were allowed to dry at room temperature, and their location was recorded to place the laser at the correct location during analysis. Just before analysis, 1 μl of 1.0 M 2,5-dihydroxybenzoic acid (DHB) in methanol was placed in each target spot and allowed to dry at room temperature.

MALDI-MS data acquisition

Oocytes were washed in ultrapure H_2O/methanol 1:1 (v/v) and placed (one to four oocytes of the same quality/spot) in the MALDI target plate. After drying at room temperature, samples were then covered with 1 μl of 2,5-DHB 0.5 M dissolved in pure methanol as the MALDI matrix. The MALDI target plate was then immediately placed in a Q-ToF Premier (Synapt HDMS) mass spectrometer (Waters, Manchester, UK) equipped with a 200 Hz solid-state laser in the m/z 700–1200 range, operated in the reflection and QTOF modes. The instrument operating conditions were a laser energy of 250 a.u. and a sample plate voltage of 20 V. Unmodified (i.e. no extraction procedure) oocytes were directly analysed. All mass spectra were manually collected for approximately 1 min in positive ion mode. Mass spectra were processed with MassLynx 4.1 software (Waters Corp. Milford, MA, USA).

Statistical analysis

A list of the relative ion abundances of the samples organized in columns was constructed using Excel software. The data were uploaded as a .cvs file in the online software MetaboAnalyst (Xia et al. 2009; Xia et al. 2011a; Xia et al. 2012) as a peak intensity table in unpaired columns. Because relative ion abundances

(and not absolute ion counts) were used, no further normalization was performed. The data were submitted to a partial least squares-discriminant analysis (PLS-DA). This analysis is a supervised clustering method in which previous knowledge of the classes is used during the classification process. PLS-DA projects the data into a low-dimensional space that maximizes the separation between the different groups of data in few dimensions (called latent variables), which are ranked by how well they explain the variance of the data. PLS-DA in MetaboAnalyst produces the variable importance measures by weighting a sum of squares of the PLS loadings that takes into account the amount of explained variance of each component (Xia and Wishart 2011b,c). The average values of lipid ion abundance and the standard deviation were calculated using Excel software.

Results

Bovine oocytes recovered by OPU were classified according to their quality in (i) COCs from pre-antral follicles, (ii) GI, (iii) GII, (iv) GIII and (v) expanded oocytes. When only information on oocyte quality – regardless of breed and oocyte yield/antral follicle count – was plotted in PLS-DA analysis, the pre-antral and expanded samples were separated from the other groups (Fig. 1). COCs from the pre-antral follicles presented the lowest abundance of SM [SM (16:0) + Na]$^+$ at *m/z* 725.4; PC [PC (38:2) + H]$^+$ at *m/z* 814.8; plasmalogen [PC (P-38:5) + H]$^+$ and/or [PC (P-36:2) + Na]$^+$ at *m/z* 792.8; and [PC (38:7) + H]$^+$ and/or [PC (36:4) + Na]$^+$ at *m/z* 804.4; and higher abundances of [PC (32:1) + Na]$^+$ at *m/z* 754.4 (Table 1), whereas the expanded oocytes presented higher abundances of *m/z* 725.4, 814.8, 792.8 and 804.4 and lower abundances of *m/z* 754.4. The lipid profiles of oocytes from GI, GII and GIII were similar and could not be distinguished based on their lipid profiles. Figure 2 shows representative MALDI mass spectra. To eliminate interference due to oocyte quality in further PLS-DA analysis of the four experimental groups, only GI, GII and GIII oocytes were considered.

When considering only GI, GII and GIII oocytes, *indicus* samples tended to cluster separately in the PLS-DA plot from 1/2 *indicus* × *taurus* samples, particularly the NH group (Fig. 3), whereas 1/2 *indicus* × *taurus*

Table 1. Lipid species presenting the highest PLS-DA scores in the MALDI-MS lipid profiles of bovine oocytes used in this work

m/z	Attribution [class (number of carbons/unsaturated carbons) + proton/adduct]$^+$
725.4	[SM (16:0) + Na]$^{+a}$
754.4	[PC (32:1) + Na]$^{+a}$
792.4	[PC (P-38:5) + H]$^+$, [PC (P-36:2) + Na]$^{+b}$
804.4	[PC (38:7) + H]$^+$ and/or [PC (36:4) + Na]$^{+a}$
814.4	[PC (38:2) + H]$^{+a}$
830.8	[PC (38:5) + Na]$^{+b}$
990.8	[TAG (60:8) + NH^4]$^{+b}$

SM, sphingomyelin; PC, phosphatidylcholine, TAG, triacylglycerol.
An error of up to 0.3 Da was considered acceptable due to the TOF resolution.
The 'P-' prefix is used for the 1Z-alkenyl ether (Plasmalogen) substituent.
[a]Attribution has been performed based on our previous work using the same methodology of lipid analysis in mammalian oocytes (Ferreira et al. 2010; Sudano et al. 2012).
[b]Attribution has been performed based on LipidMaps (The LIPID MAPS Lipidomics Gateway, http://www.lipidmaps.org/) considering that MALDI in positive ion mode with DHB as an organic matrix is highly selective for SM, PC and TAG detection.

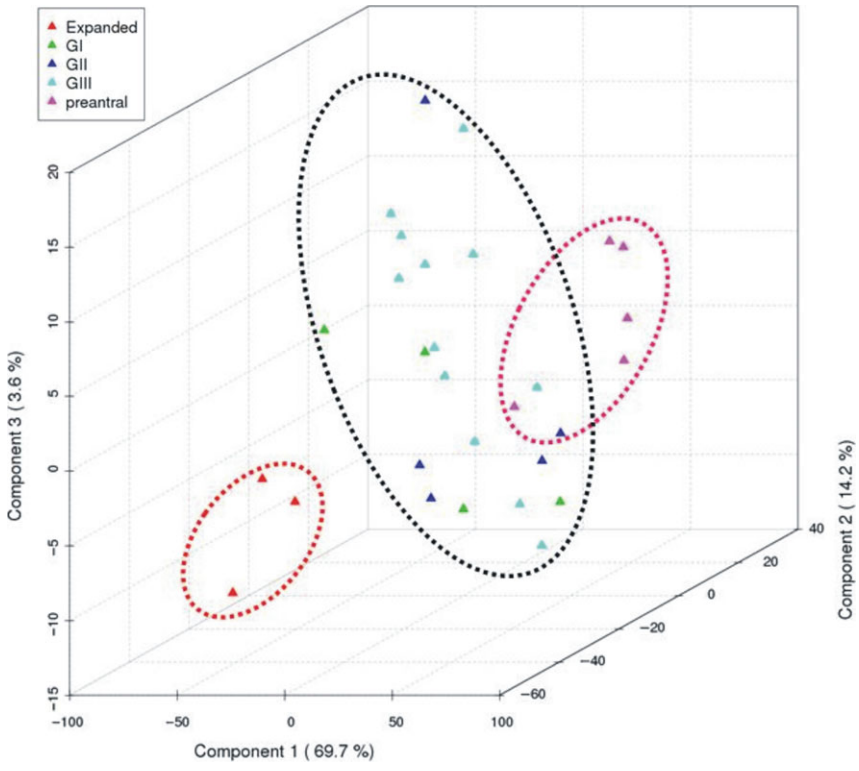

Fig. 1. Tridimensional PLS-DA score plot of the oocytes collected by OPU for use in this study (n = 83, 31 mass spectra). The analysis considered the lipid profile according to oocyte quality independent of breed or oocyte yield. Expanded oocytes (n = 8, 3 spectra) are represented by red triangles; GI oocytes (n = 10, 4 spectra) by green triangles; GII oocytes (n = 12; 5 spectra) by dark blue triangles; GIII oocytes (n = 31, 14 spectra) by light blue triangles; and COCs from pre-antral follicles (n = 23; 5 spectra) by pink triangles. Each component indicates the percentage of the variability of the data that is explained

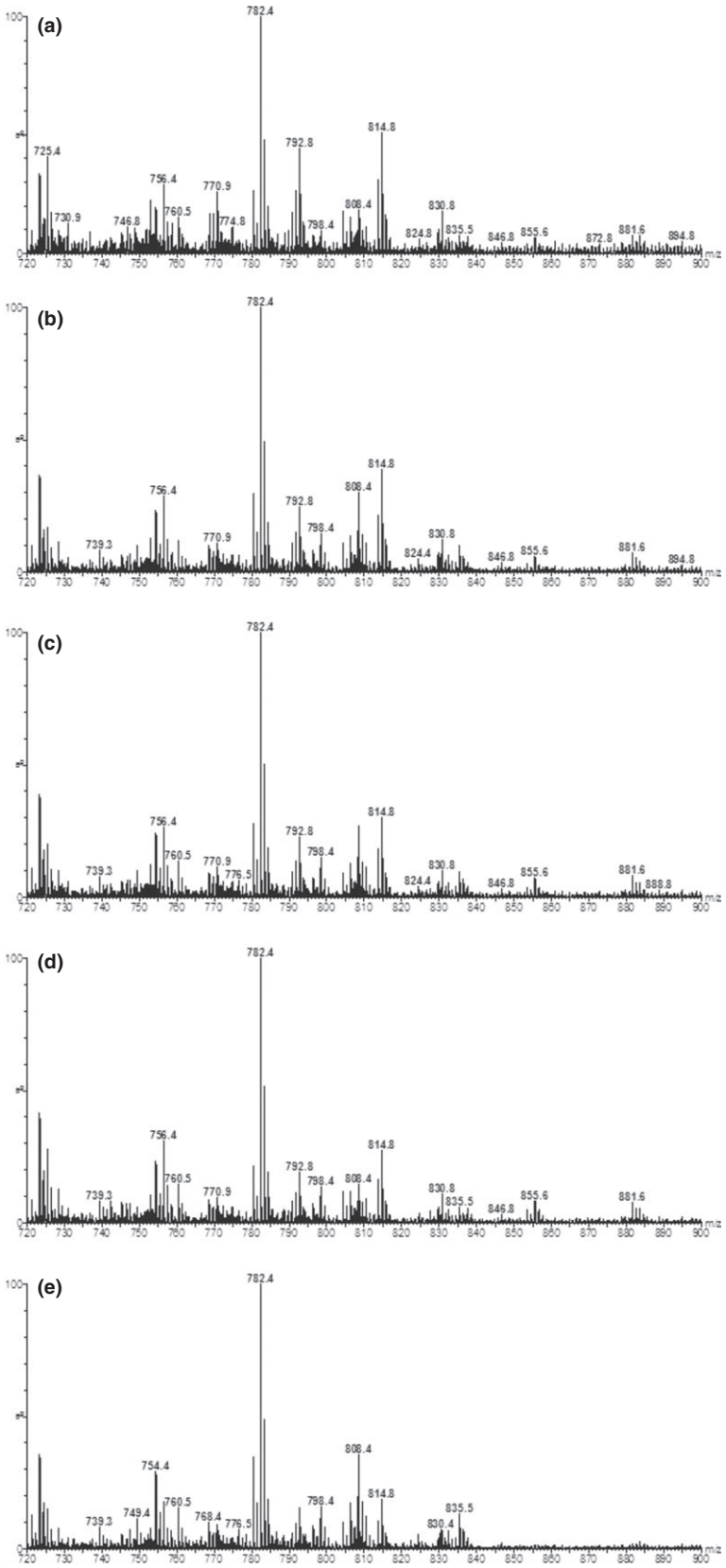

Fig. 2. Representative MALDI-MS of (a) expanded oocytes; (b) GI; (c) GII; (d) GIII oocytes; (e) COCs from pre-antral follicles

oocytes (AH and AL group) were mixed. The most important ions that explain this variability are those at m/z 792.8, 814.8, $[PC\ (38:5) + Na]^+$, at m/z 830.8, and $[TAG\ (60:8) + NH4]^+$ at m/z 990.8, which were more abundant in the NH and NL groups than in the AH and AL groups.

The relevance of the four most abundant ions present in *indicus* oocytes was further confirmed by plotting the

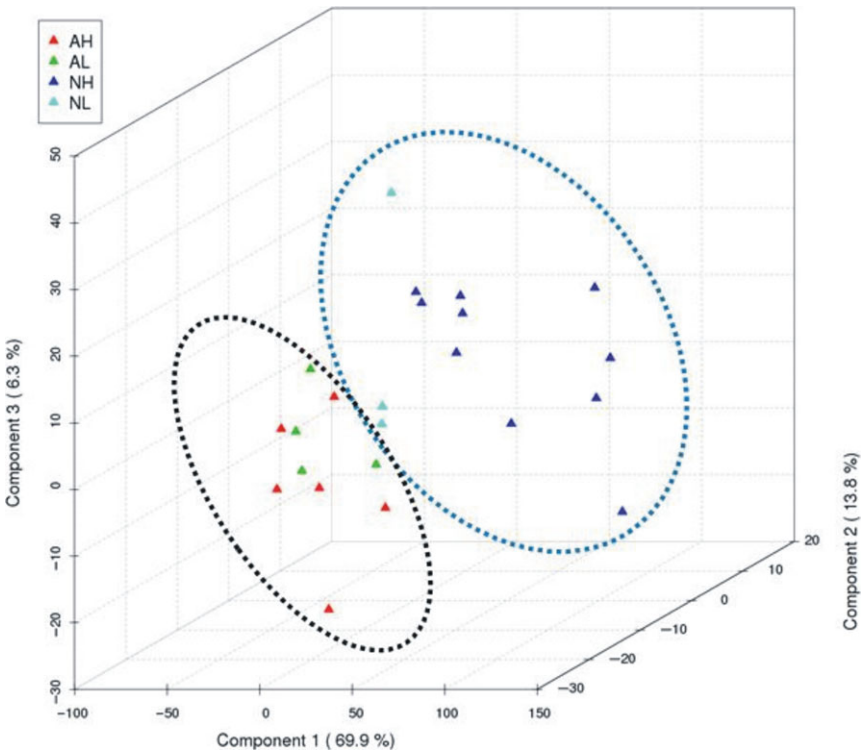

Fig. 3. Tridimensional PLS-DA score plot of representative oocytes from each of the four experimental groups. 1/2 *indicus* × *taurus* cows with high AFC (AH group, n = 12, 6 mass spectra) are represented by red triangles; 1/2 *indicus* × *taurus* with low AFC (AL group, n = 5, 4 mass spectra) by green triangles; *indicus* cows with high AFC (NH group; n = 31; 10 mass spectra) by dark blue triangles; and *indicus* cows with low AFC (NL group; n = 5, 3 mass spectra) by light blue triangles. Each component indicates the percentage of the variability of the data that is explained

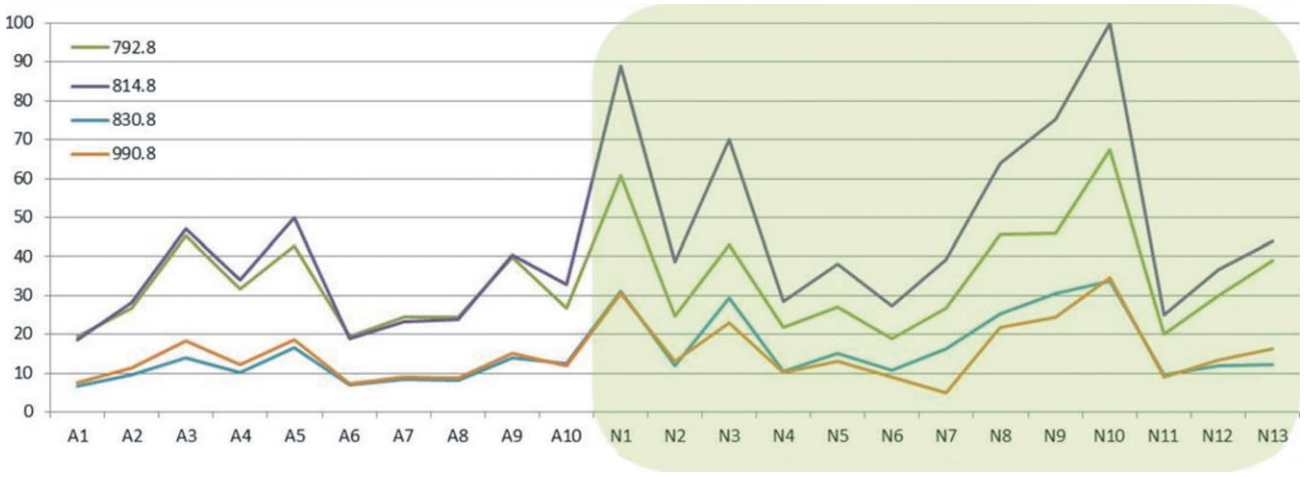

Fig. 4. Line graphic showing the relative ion abundances (*y*-axis) in the lipid mass spectra of each sample of 1/2 *indicus* × *taurus* (AH group samples: A1 to A6; AL group samples: A7 to A10) and *indicus* (NH group: N1 to N10; NL group: N11 to N13)

respective relative ion abundances in individual samples (Fig. 4). Figure 5 shows representative spectra for the four experimental groups.

Discussion

Herein, we report the first comparative MALDI-MS-based study of lipid profiles of immature oocytes recovered by OPU from *B. indicus* and 1/2 *indicus-taurus* females with remarkably high or low antral follicle count/oocyte yield.

Lipids have been studied and may represent reliable biomarkers for oocyte development because they play many significant roles, such as energy storage and cell structure (by tuning the physical properties of the cell; Kim et al. 2001). In the present study, independent of the breed and oocyte yield/antral follicle count, COCs from pre-antral follicles and expanded oocytes tended to be separated from the other groups (Fig. 1) due to substantial differences in their lipid profiles. COCs from expanded oocytes presented high abundance of some ions that were in lower abundance in COCs from pre-antral follicles. Conversely, the ion that was present in low quantity in COCs from expanded oocytes presented higher abundance in COCs from pre-antral follicles (Fig. 2). These changes in lipid molecular design observed in the COCs from pre-antral follicles could be related to metabolic status, reduced cytoplasmic membrane content or membrane specialization in oocytes at growing stages compared with oocytes in the antral follicles, because differences in the lipid profile between COCs from pre-antral or

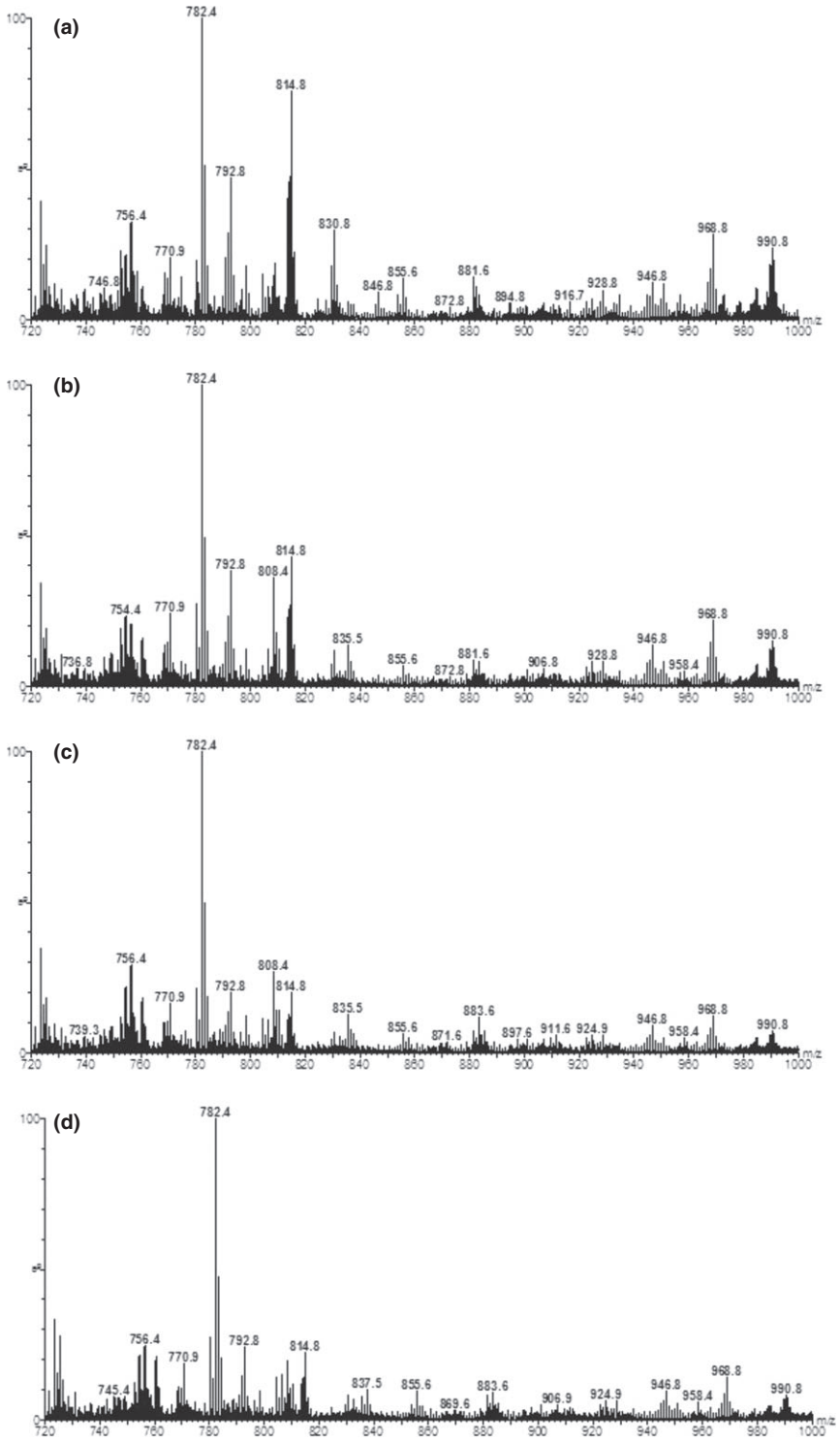

Fig. 5. Representative MALDI-MS of the (a) NH group; (b) NL group; (c) AH group; and (d) AL group

expanded follicles were mainly related to the PC lipid species.

Large amounts of intracellular lipids, represented by lipid droplets, are present in the oocyte cytoplasm (Kim et al. 2001). Lipid droplets store neutral lipids, such as TAG, and are surrounded by a monolayer of phospholipids and associated proteins (Bartz et al. 2007). Indeed, the majority of the intracellular lipids in oocytes are TAG (Homa et al. 1986; McEvoy et al. 2000). A metabolic role of TAG in the *in vitro* maturation of pig and bovine oocytes has been demonstrated (Ferguson and Leese 1999; McEvoy et al. 2000), which indicates that oocytes may use lipids as an energy source, providing ATP for the protein synthesis that is necessary for the continuation of cytoplasmic maturation and meiosis (Kruip et al. 1983). During *in vitro* maturation, the TAG content of pig oocytes decreases (Sturmey and Leese 2003). Similarly, higher amounts of triglyceride have been measured in immature oocytes compared with *in vitro*

matured oocytes (Kim et al. 2001; Ferguson and Leese 1999). Conversely, a study has found no lipid variation in bovine oocytes after *in vitro* maturation (Aardema et al. 2008).

TAG can also be used as a source of energy during early embryo development (Hillman and Flynn 1980; Waterman and Wall 1988; Khandoker and Tsujii 1998; Spindler et al. 1999; Gomez et al. 2001, 2002). It was reported that *in vitro*-produced bovine zygotes were able to cleave in the complete absence of exogenous nutrients (Ferguson and Leese 1999). Indeed, substrate oxidation by five- to eight-cell embryos was continuous (Ferguson and Leese 2006), which suggests the use of lipid internal stores. In the present study, the oocytes of *indicus* females clustered separately from the oocytes of 1/2 *indicus* × *taurus*, particularly the NH group, when only the GI, II and III oocytes were considered, whereas the 1/2 *indicus* × *taurus* oocytes (AH and AL groups) were mixed (Fig. 3). This difference was due mainly to the greater abundance of four ions in the NH and NL groups compared with the AH and AL groups, most of them corresponded to PC lipid species (Fig. 4). These findings indicate different metabolic rates among *indicus* and 1/2 *indicus* × *taurus* oocytes and that *indicus* females with high AFC/oocyte yields have an energy store advantage over 1/2 *indicus* × *taurus* donors with high and low oocyte yields. These PC lipid species may be associated with lipid droplets and other cellular membranes and have an active role in the metabolism of stored lipids during oocyte maturation.

A recent study (Pontes et al. 2011) demonstrated that oocytes recovered by OPU from *indicus* donors with high oocyte production produced significantly more embryos and more pregnancies compared with females with low oocyte production. The greater abundance of the four lipid ions observed in the present study could be related to the differences between *indicus* donors with high and low oocyte yields reported by Pontes et al. (2011). However, great variation in the lipid droplet number in oocytes from the same ovary has been observed (Aardema et al. 2008).

Because PC lipids are involved in the bilayer membrane structure, the present results indicate membrane structural differences between *indicus* and 1/2 *indicus* × *taurus* oocytes from females with high and low AFC/oocyte yields. The physiological significance of the greater abundance of the four lipid species in *indicus* oocytes compared with 1/2 *indicus* × *taurus* oocytes could be related to membrane metabolic rate differences and may contribute to oocyte competence and embryo development. The results also suggest that expanded oocytes may have lipid metabolic changes related to preovulation. This intriguing possibility will be further investigated by MALDI-MS with a larger set of samples.

Acknowledgements

The authors thank the ThoMSon Mass Spectrometry Laboratory, the Coordination for the Improvement of Higher Education Personnel (CAPES), the Post-Graduation Program in Animal Science at the State University of Londrina and the National Council for Scientific and Technological Development (CNPq).

Conflict of interest

Authors declare no conflict of interest.

Author contributions

Katia Cristina Silva-Santos – writing, COCs selection and classification, Christina Ramires Ferreira – mass spectrometry and data analyses, Marcos Nogueira Eberlin – mass spectrometry, Gustavo Martins Gomes dos Santos – ultrasonograph, OPU Letícia Schmidt Siloto – OPU, COCs processing Camila Oliveira Rosa – OPU, COCs processing, Thiago Nogueira Marcantonio – ultrasonograph, OPU Marcelo Marcondes Seneda – supervisor.

References

Aardema H, Knijn HM, Oei CHY, Vos PLAM, Gadella BM, 2008: Lipid droplet dynamics during in vitro maturation of bovine oocytes. Reprod Domest Anim **43**, 76. (Abstract).

Aardema H, Vos PL, Lolicato F, Roelen BA, Knijn HM, Vaandrager AB, Helms JB, Gadella BM, 2011: Oleic acid prevents detrimental effects of saturated fatty acids on bovine oocyte developmental competence. Biol Reprod **85**, 62–69.

Apparicio M, Ferreira CR, Tata A, Santos VG, Alves AE, Mostachio GQ, Pires-Butler EA, Motheo TF, Padilha LC, Pilau EJ, Gozzo FC, Eberlin MN, Lo Turco EG, Luvoni GC, Vicente WR, 2012: Chemical composition of lipids present in cat and dog oocyte by matrix-assisted desorption ionization mass spectrometry (MALDI-MS). Reprod Domest Anim **47**, 113–117.

Bartz R, Li WH, Venables B, Zehmer JK, Roth MR, Welti R, Anderson RG, Liu P, Chapman KD, 2007: Lipidomics reveals that adiposomes store ether lipids and mediate phospholipid traffic. J Lipid Res **48**, 837–847.

Burns DS, Jimenez-Krassel F, Ireland JLH, Knight PG, Ireland JJ, 2005: Numbers of antral follicles during follicular waves in cattle: evidence for high variation among animals, very high repeatability in individuals, and an inverse association with serum follicle-stimulating hormone concentrations. Biol Reprod **73**, 53–62.

Castaneda CA, Kaye P, Pantaleon M, Phillips N, Norman S, Fry R, D'Occhio MJ, 2013: Lipid content, active mitochondria and brilliant cresyl blue staining in bovine oocytes. Theriogenology **79**, 417–422.

Cetica P, Pintos L, Dalvit G, Beconi M, 2002: Activity of key enzymes involved in glucose and triglyceride catabolism during bovine oocyte maturation in vitro. Reproduction **124**, 675–681.

Cummins J, 2004: The role of mitochondria in the establishment of oocyte functional competence. Eur J Obstet Gynecol Reprod Biol **115**, 23–29.

Edidin M, 2003: Lipids on the frontier: a century of cell-membrane bilayers. Nat Rev Mol Cell Biol **4**, 414–418.

Ferguson EM, Leese HJ, 1999: Triglyceride content of bovine oocytes and early embryos. J Reprod Fertil **116**, 373–378.

Ferguson EM, Leese HJ, 2006: A potential role for triglyceride as an energy source during bovine oocyte maturation and early embryo development. Mol Reprod Dev **73**, 1195–1201.

Ferreira CR, Saraiva SA, Catharino RR, Garcia JS, Gozzo FC, Sanvido GB, Santos LF, Lo Turco EG, Pontes JH, Basso AC, Bertolla RP, Sartori R, Guardieiro MM, Perecin F, Meirelles FV, Sangalli JR, Eberlin MN, 2010: Single embryo and oocyte lipid fingerprinting by mass spectrometry. J Lipid Res **51**, 1218–1227.

Ferreira CR, Pirro V, Eberlin LS, Hallett JE, Cooks RG, 2012a: Developmental phases of individual mouse preimplantation embryos characterized by lipid signatures using desorption electrospray ionization mass spectrometry. Anal Bioanal Chem **404**, 2915–2926.

Ferreira CR, Eberlin LS, Hallett JE, Cooks RG, 2012b: Single oocyte and single embryo lipid analysis by desorption electrospray ionization mass spectrometry. J Mass Spectrom **47**, 29–33.

Fleming TP, Saacke RG, 1972: Fine structure of the bovine oocyte from the mature

graafian follicle. J Reprod Fertil **29**, 203–212.
Gomez E, Duque P, Díaz E, Díez C, 2001: Effects of acetoacetate on in vitro development of bovine embryos in medium containing citrate and myo-inositol. Reprod Domest Anim **36**, 189–194.
Gomez E, Duque P, Díaz E, Facal N, Antolín I, Hidalgo C, Díez C, 2002: Effects of acetoacetate and D-beta-hydroxybutyrate on bovine in vitro embryo development in serum free medium. Theriogenology **57**, 1551–1562.
Hillman N, Flynn TJ, 1980: The metabolism of exogenous fatty acids by pre-implantation mouse embryos developing in vitro. J Embryol Exp Morphol **56**, 157–168.
Homa ST, Racowsky C, McGaughey RW, 1986: Lipid analysis of immature pig oocytes. J Reprod Fertil **77**, 425–434.
Hulbert AJ, 2003: Life, death and membrane bilayers. J Exp Biol **206**, 2303–2311.
Hyttel P, Fair T, Callesen H, Greve T, 1997: Oocyte growth, capacitation and final maturation in cattle. Theriogenology **47**, 23–32.
Ireland JLH, Scheetz D, Jimenez-Krassel F, Themmen APN, Ward F, Lonergan P, Smith GW, Perez GI, Evans ACO, Ireland JJ, 2008: Antral follicle count reliably predicts number of morphologically healthy oocytes and follicles in ovaries of young adult cattle. Biol Reprod **79**, 1219–1225.
Khandoker MAMY, Tsujii H, 1998: Metabolism of exogenous fatty acids by preimplantation rabbit embryos. Jpn J Fertil Steril **43**, 195–201.
Kim JY, Kinoshita M, Ohnishi M, Fukui Y, 2001: Lipid and fatty acid analysis of fresh and frozen-thawed immature and in vitro matured bovine oocytes. Reproduction **122**, 131–138.
Kruip TAM, Cran DG, Van Beneden TH, Dieleman SJ, 1983: Structural changes in bovine oocytes during final maturation in vivo. Gamete Res **8**, 29–47.
Lowman BG, Scott NA, Somerville SH, 1976: Condition Scoring of Cattle, Rev. Ed. East Scotland College of Agriculture, Edinburgh, Scotland, pp. 1–31 (Bulletin 6).
Machado SA, Reichenbach HD, Weppert M, Matos LF, Wolf E, Gonçalvez PBD, 2006: The variability of ovum pick-up response and in vitro embryo production from monozygotic twin cows. Theriogenology **65**, 573–583.
McEvoy TG, Coull GD, Broadbent PJ, Hutchinson JS, Speake BK, 2000: Fatty acid composition of lipids in immature cattle, pig and sheep oocytes with intact zona pellucida. J Reprod Fertil **118**, 163–170.
McKeegan PJ, Sturmey RG, 2011: The role of fatty acids in oocyte and early embryo development. Reprod Fertil Dev **24**, 59–67.
Pontes JHF, Nonato-Junior I, Sanches BV, Ereno-Junior JC, Uvo S, Barreiros TRR, Oliveira JA, Hasler JF, Seneda MM, 2009: Comparison of embryo yield and pregnancy rate between in vivo and in vitro methods in the same Nelore (*Bos indicus*) donor cows. Theriogenology **71**, 690–697.
Pontes JH, Silva KC, Basso AC, Rigo AG, Ferreira CR, Santos GM, Sanches BV, Porcionato JP, Vieira PH, Faifer FS, Sterza FA, Schenk JL, Seneda MM, 2010: Large-scale in vitro embryo production and pregnancy rates from *Bos taurus*, *Bos indicus*, and indicus-taurus dairy cows using sexed sperm. Theriogenology **74**, 1349–1355.
Pontes JHF, Melo-Sterza FA, Basso AC, Ferreira CR, Sanches BV, Rubin KCP, Seneda MM, 2011: Ovum pick up, in vitro embryo production, and pregnancy rates from a large-scale commercial program using Nelore cattle (*Bos indicus*) donors. Theriogenology **75**, 1640–1646.
Spindler RE, Renfree MB, Shaw G, Gardner DK, 1999: Reactivating tammar wallaby blastocysts oxidise fatty acids and amino acids. J Reprod Fertil **115**, 79–86.
Sturmey RG, Leese HJ, 2003: Research energy metabolism in pig oocytes and early embryos. Reproduction **126**, 197–204.
Sturmey RG, Reis A, Leese HJ, McEvoy TG, 2009: Role of fatty acids in energy provision during oocyte maturation and early embryo development. Reprod Domest Anim **44**, 50–58.
Sudano MJ, Santos VG, Tata A, Ferreira CR, Paschoal DM, Machado R, Buratini J, Eberlin MN, Landim-Alvarenga FD, 2012: Phosphatidylcholine and sphingomyelin profiles vary in *Bos taurus indicus* and *Bos taurus taurus* in vitro- and in vivo-produced blastocysts. Biol Reprod **87**, 1–11.
Tarazona AM, Rodriguez JI, Restrepo LF, Olivera-Angel M, 2006: Mitochondrial activity, distribution and segregation in bovine oocytes and in embryos produced in vitro. Reprod Domest Anim **41**, 5–11.
Van Blerkom J, Davis P, Mathwig V, Alexander S, 2002: Domains of highpolarized and low-polarized mitochondria may occur in mouse and human oocytes and early embryos. Hum Reprod **17**, 393–406.
Waterman RA, Wall RJ, 1988: Lipid interactions with in vitro development of mammalian zygotes. Gamete Res **21**, 243–254.
Xia J, Psychogios N, Young N, Wishart DS, 2009: MetaboAnalyst: a web server for metabolomic data analysis and interpretation. Nucleic Acids Res **37**, 652–660.
Xia J, Sinelnikov IV, Wishart DS, 2011a: MetATT: a web-based metabolomics tool for analyzing time-series and two-factor datasets. Bioinformatics **2**, 2455–2456.
Xia J, Wishart DS, 2011b: Web-based inference of biological patterns, functions and pathways from metabolomic data using MetaboAnalyst. Nat Protoc **6**, 743–760.
Xia J, Wishart DS, 2011c: Metabolomic data processing, analysis, and interpretation using MetaboAnalyst. In: Baxevanis AD (ed.), Current Protocols in Bioinformatics. Wiley, New York, NY. Chapter 14, Unit 14.10.
Xia J, Mandal R, Sinelnikov IV, Broadhurst D, Wishart DS, 2012: MetaboAnalyst 2.0 – a comprehensive server for metabolomic data analysis. Nucleic Acids Res **40**, 127–133.

Submitted: 16 Dec 2013; Accepted: 14 May 2014

Author's address (for correspondence): Marcelo Marcondes Seneda, Laboratório de Reprodução Animal (ReproA), DCV-CCA-Universidade Estadual de Londrina (UEL), Londrina, PR 86051-990 Brazil. E-mail: mseneda@uel.br