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Lipid Content in Pig Blastocysts Cultured in the Presence or Absence of Protein and Vitamin E or Phenazine Ethosulfate*

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In the present study, total lipid content and content of triglycerides, phospholipids and cholesterol were determined in pig blastocysts cultured in medium without protein, supplemented with bovine serum albumin (BSA), with fetal calf serum (FCS), vitamin E or phenazine ethosulfate (PES). In comparison to blastocysts cultured in NCSU-23 with BSA, we observed a decrease of the total lipid content in PES-treated embryos. Triglyceride content in FCS-, vitamin E- and PES-treated embryos as well as in blastocysts cultured without protein was 81.9%, 70.2%, 57.2% and 74.8% of that found in the blastocysts cultured in NCSU-23 with BSA, respectively. Nevertheless the content of phospholipids remained unchanged. This decrease of triglyceride content in the porcine blastocyst after *in vitro* culture may be explained by altered lipid metabolism in embryos.

Key words: Pig embryo, lipid, vitamin E, PES, FCS, BSA.

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Pig embryos have a very low survival rate after cryopreservation due to their extreme, species-specific sensitivity to cooling below 15°C (DOBRINSKY 2002). This chilling susceptibility appears to depend on the developmental stage of the embryo (DOBRINSKY 2002), so the expanded blastocyst exhibits higher cryotolerance than the less advanced stages, but it is low in comparison to other mammalian species (GAJDA & SMORAG 2000). On the other hand porcine embryos have a very high total lipid content which decreases after morulae compaction (MCEVOY *et al.* 2000; ROMEK *et al.* 2009). Therefore, susceptibility to cryopreservation correlates with lipid content because physical changes of lipids in freezing temperatures cause cellular cryodisruption (PEREIRA & MARQUES 2008) and embryos with a high lipid level are more susceptible to oxidative damage (MCEVOY *et al.* 2001) and to injury of cytoplasmic lipid droplets (VAJTA & NAGY 2006). In fact, improvement of pig embryo survival after freezing can be achieved by mechanical removal of cytoplasmic lipid droplets (NAGASHIMA *et al.* 1994; YONEDA *et al.* 2004). Another possible strategy may be developing a porcine embryo culture system to obtain porcine blastocysts with reduced level of lipids.

North Carolina State University (NCSU)-23 medium is known to be one of the most successful for porcine embryo culture (RATH *et al.* 1995). This medium is usually supplemented with bovine serum albumin (BSA) or fetal calf serum (FCS). In some previous reports, the culture of early porcine embryos with FCS was detrimental to blastocyst development *in vitro,* significantly reduced the formation of hatched blastocysts (DOBRINSKY *et al.* 1996; GAJDA *et al.* 2008) and caused accumulation of lipids in bovine embryos (BARCELO-FIMBRES & SEIDEL 2007). Alternatively, it was shown that NCSU-23 medium supplemented with BSA

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significantly improved porcine embryo development and the quality of the resulting blastocyst (GAJDA *et al.* 2008). Beneficial effects of supplementing the culture medium with vitamin E (α-tocopherol), the predominant lipid-soluble antioxidant, were also described (GAJDA *et al.* 2008; TSUJII *et al.* 2002). This antioxidant increases the survival rates and quality of porcine blastocysts by protecting cells from oxygen radical damage and suppresses peroxidation of membrane lipids. In addition, it has a significant impact on glucose incorporation and metabolism at the blastocyst stage (TSUJII *et al.* 2002). It was demonstrated previously that bovine embryos cultured in a medium with phenazine ethosulphate (PES) (DE LA TORRE-SANCHEZ *et al.* 2006; BARCELO-FIMBRES & SEIDEL 2007) accumulated less lipids and had an apparently more normal distribution of lipid deposits due to the stimulation of the pentose phosphate pathway rate by PES (PALASZ *et al.* 2008). Bovine embryos cultured with PES also showed an increased ability to survive cryopreservation (SEIDEL 2006). Finally, it is possible to improve the quality of cultured bovine (DE LA TORRE-SANCHEZ *et al.* 2006; PALASZ *et al.* 2006) and porcine embryos (GAJDA *et al.* 2008) using different chemicals to regulate their metabolism.

It was previously pointed out that Nile red (NR) could be applied to measure the total lipid content in single mammalian oocytes and embryos (LEROY *et al.* 2005; GENICOT *et al.* 2005). Furthermore, NR is a solvatochromic probe with fluorescent emission spectrum sensitive to the polarity of its environment (MUKHERJEE *et al.* 2007; KĘPCZYŃSKI *et al.* 2008). In embryos, especially in the intracellular lipid droplets, such an environment consists of different types of lipids, mainly triglycerides, phospholipids and cholesterol (MCEVOY *et al.* 2000) which exhibit different degrees of polarity. Thus, NR staining followed by decomposition of the emitted spectrum yields quantitative information about the content of the main types of lipids in embryos.

Taking into account that BSA, FCS, PES and vitamin E may regulate the rate of different processes in the embryo during *in vitro* culture, an important question arises whether supplementation of the culture medium with or without proteins (FCS or BSA), metabolic regulators or antioxidants will decrease the total lipid content and the content of the main types of lipids, i.e. triglycerides, phospholipids and cholesterol in cultured porcine blastocysts.

Material and Methods

All chemicals were supplied by Sigma-Aldrich Co. (St. Louis, MO, USA) unless otherwise indicated. Experimental procedures used in this study were approved by the Local Ethics Committee at Jagiellonian University, Kraków, Poland.

Embryo recovery and culture

Porcine embryos were collected from mature, six-month-old gilts (Polish Landrace) with body weight ranging from 90 to 110 kg. The gilts were superovulated by intramuscular injection of 1500 IU of PMSG (Serogonadotropin, Biowet, Poland), followed 96 h later by 1000 IU of hCG, (Biogonadyl, Biowet, Poland) i.m., and inseminated twice: 12 and 24 h after hCG treatment. The embryos were recovered by flushing oviducts on day 1 after insemination with phosphate buffered saline (PBS) at about 30°C. Embryos at the zygote stage were then cultured in NCSU-23 medium supplemented with 4 mg/ml bovine serum albumin (BSA group) or 10% fetal calf serum (FCS group) or 0.05 μM phenazine ethosulfate (PES group) or 25 μM vitamin E (VE group) or without protein (control group -C). The culture was performed at 39°C, with 5% CO_2 in air, for 96 to 120 h up to the expanded blastocyst stage.

Blastocyst processing

Nile Red (NR, Molecular Probes, Inc., Eugene, OR, USA) was used to label lipid droplets. All processing stages were performed at room temperature. Blastocysts were washed in PBS, incubated with 0.1% (w/v) pronase to remove zona pellucida, washed in PBS again, fixed with 4% paraformaldehyde in PBS for 3 h, washed three times for 15 min in PBS and stained with 10 μg/ml NR in PBS for 3 h in dark. Subsequently embryos were rinsed twice in PBS to remove excess dye, immersed in small PBS droplets placed on glass coverslips and sealed with a second glass coverslip. To prevent embryo-destroying we formed vaseline ring around PBS droplets. In the each group ten blastocysts were evaluated.

Image acquisition

Blastocysts were analysed on a LSM 510 META confocal system and a Zeiss Axiovert 200M inverted microscope (Carl Zeiss GmbH, Jena, Germany) equipped with a Plan- Apochromat 20× (0.8 NA) lens. We used a 514 nm Argon/2 laser line for NR excitation. The whole volume of each blastocyst was sampled by a set of adjacent optical sections taken at intervals of 5 μm ("z-stack"). Each optical section in the z-stacks consisted of 22 images taken at different emission wavelengths ranging from 518 to 754 nm at intervals of 10.4 nm

("lambda stack"). This analysis, named the Lambda Mode scanning procedure, allows acquiring NR emission spectra emitted from optical sections of blastocysts. For each optical section, the emission spectrum emitted from the region of interest (ROI) containing whole blastocyst cross-sections was measured using LSM 5 software (Carl Zeiss GmbH, Jena, Germany). Then background emission spectra, emitted from ROI located outside of the embryos cross-sections were subtracted. In the same way we acquired emission spectra of the three standards: triglyceride mixtures (cat. no. 17811), phospholipid mixture for HPLC (cat. no. P3817) and Liquick Cor-CHOL mini: 2-standard (Cormay Co., Lublin, Poland, cat. no. 2-212) containing cholesterol. These standards were stained with 10 μg/ml NR solution for 3 h at room temperature in the dark and then analysed using confocal microscopy.

Data analysis

We evaluated four parameters for each individual embryo in all groups: the total content of lipids – TF, as well as the content of triglycerides – TF(tri), phospholipids – TF(ph) and cholesterol – TF(chol). To evaluate the total content of lipids for each individual blastocyst we measured the amount of fluorescence (ID) emitted from each optical section in the z-stack as the area under the emission spectra. Sum of ID calculated for all optical sections of each individual blastocyst equals the total amount of fluorescence emitted from the blastocyst and is proportional to the total content of lipids (GENICOT *et al.* 2005). To compensate the effect of losing emitted fluorescence in the z-stack when imaging further into deeper regions of embryos (where excitation and detection efficiency decreases), we employed the Interpolate function of LSM 5 software, Version 4.0 (Carl Zeiss GmbH, Jena, Germany). For statistical analysis we used normalised data |TF|:

$$(1) \qquad |TF| = \frac{TF}{\overline{TF(BSA)}}$$

where $\overline{TF(BSA)}$ is mean TF calculated for BSA group. The total lipid content of blastocysts was compared using a one-way ANOVA with a *post hoc* Tukey test.

In order to evaluate the total content of triglycerides, phospholipids and cholesterol in the individual blastocysts from different groups, we calculated the mean emission spectrum for each blastocyst from the spectra of all optical sections in the embryo and then this mean spectrum was normalised by the area under them. Using principal component analysis (PCA) (TEIXEIRA *et al.* 2009), we obtained the number of principal components, i.e. number of principal or component spectra in the mean, experimental spectra of blastocysts. The next step was decomposition of the experimental emission spectra into principal spectra using a multivariate curve resolution-alternating least squares (MCR-ALS) (TAULER 1995). This method allows receiving the pure spectrum of each principal component – $S_P(j)$ and its multiplying factor $MF^i(j)$, i.e. the pure concentration profiles proportional to the percentage content of the different types of lipid in the *i*–th blastocyst, according to the following equation:

$$(2) \quad S^{i}_{\exp} = \sum_{j} MF^{i}(j) \bullet Sp(j) + E$$

where $S_P(j)$ and $MF^i(j)$ are the principal spectrum and multiplying factor of component *j* retrieved in *i*–th blastocyst and E is an error matrix. The best fit was expressed as percent variance (PVC) captured by the PCA model. All calculations were performed in a two-step procedure using Matlab 7.6 software (The MathWorks, Inc., Natick, MA). Values of $MF^i(j)$ were analysed with three-dimensional ANOVA and a *post hoc* Tukey test using Statistica version 5.0 software (StatSoft Inc., Tulsa, OK, USA). To identify principal components we compared each of the three-component spectrum with the spectrum measured for appropriate standard of triglyceride mixtures, phospholipid mixture or cholesterol. The coefficient of determination R^2 is a measure of the deviation of the component spectrum from the experimental data of the lipid standards and is calculated by the formula (KURGANOV *et al.* 2001):

$$(3) \quad R^2 = 1 - \frac{\sum_{i=1}^{n} (y_i^s - y_i^d)^2}{\sum_{i=1}^{n} (y_i^s - \bar{y}^s)^2} \qquad \text{where: } \bar{y}^s = \sum_{i=1}^{n} y_i^s$$

y_i^s is the value of the emission spectrum of the standard measured at *i*-th wavelengths, y_i^d is the value of the component spectrum at *i*-th wavelengths, n – number of measured fluorescence intensities at different emission wavelengths in spectra. If R = 1 then the component spectrum exactly corresponds to the emission spectra of the standard. To evaluate the total content of triglycerides, phospholipids and cholesterol we used equations:

$$(4) \quad TF(tri) = TF \cdot MF(tri),\ TF(ph) = TF \cdot MF(ph) \text{ and } TF(chol) = TF \cdot MF(chol)$$

because the content of triglycerides TF(tri), phospholipids TF(ph) and cholesterol TF(chol) is a product of the total lipid content TF and appropriate multiplying factor of the component spec-

trum $S_P(j)$. To simplify the later discussion we calculated relative values of TF(tri), TF(ph) and TF(chol). They were normalised by the mean value of TF obtained for BSA group $\overline{TF(BSA)}$ (and marked as |TF(tri)|, |TF(ph)| and |TF(chol)|:

$$(3)\quad |TF(tri)| = \frac{TF(tri)}{\overline{TF(BSA)}},\ |TF(ph)| = \frac{TF(ph)}{\overline{TF(BSA)}}$$

$$\text{and } |TF(chol)| = \frac{TF(chol)}{\overline{TF(BSA)}}.$$

One-dimensional ANOVA and a *post hoc* Tukey test were used to compare the content of triglycerides TF(tri), phospholipids TF(ph) and cholesterol TF(chol) calculated for different groups.

Table 1

In vitro survival of porcine zygotes cultured in NCSU-23 medium supplemented with BSA (BSA group), FCS (FCS group), without protein (control group – C), BSA and PES (PES group) or BSA and vitamin E (VE group)

	No. of zygotes cultured/replicates	No. of expanded blastocysts (%)
BSA	73/9	59 (80.8)
FCS	71/9	20 (28.2)
C	77/9	17 (22.1)
PES	98/7	74 (75.5)
VE	75/6	60 (80.6)

Results

Results of the *in vitro* survival of porcine zygotes cultured in NCSU-23 medium supplemented with BSA, FCS, BSA and PES, BSA and vitamin E or protein free NCSU23 are summarized in Table 1. Fluorescence spectra emitted from blastocysts stained with NR showed a maximum emission at 588 nm and ranged from 518 nm to 754 nm. Therefore, morphological analysis of the blastocysts was carried out using images of the optical sections taken at 588 nm wavelength. For all analysed groups the morphology of the cultured blastocysts was the same and no developmental abnormalities were observed (Fig. 1). The Nile red fluorescence signal was mainly restricted to the elliptical-shaped lipid droplets distributed in the cytoplasm, whereas the cytoplasm and blastocyst cavity remained unstained.

Each mean emission spectrum taken for all individual blastocysts always consists of the three component spectra $S_P(1)$, $S_P(2)$ and $S_P(3)$ (Fig. 2). Multiplying factors of the component spectra: MF(1), MF(2) and MF(3) differed between the blastocysts and between the groups of embryos under study, but $S_P(1)$, $S_P(2)$ and $S_P(3)$ did not change. Maxima of $S_P(1)$, $S_P(2)$ and $S_P(3)$ were found at 588 nm, 631 nm and 641 nm respectively. Percent variance (PVC) of the PCA model was very high (PVC= 99.93%).

Normalized emission spectra |tri|, |ph| and |chol|, taken for three lipid standards and stained with NR, i.e. triglyceride mixtures, phospholipids mixture and cholesterol, respectively, are shown in Fig. 3. Their maxima occurred at 588 nm for |tri|, 631 nm for |ph| and 641 nm for |chol|. They have not only the same maxima but also the same spec-

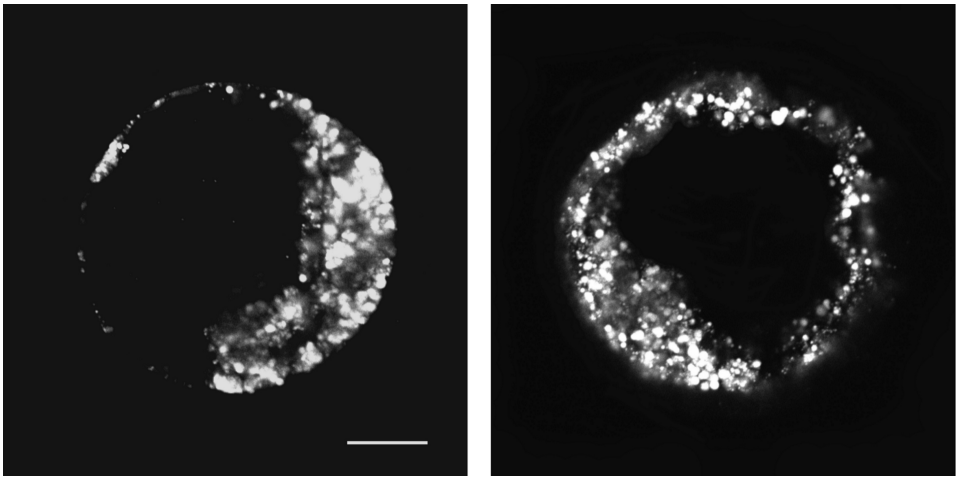

Fig. 1. Confocal images of the cultured blastocysts in NCSU-23 medium (left panel – BSA group) and in NCSU-23 medium supplemented with BSA and 0.05 μM PES (right panel – PES group). Embryos were stained with NR and images were taken at 588 nm wavelength. Bar = 50 μm.

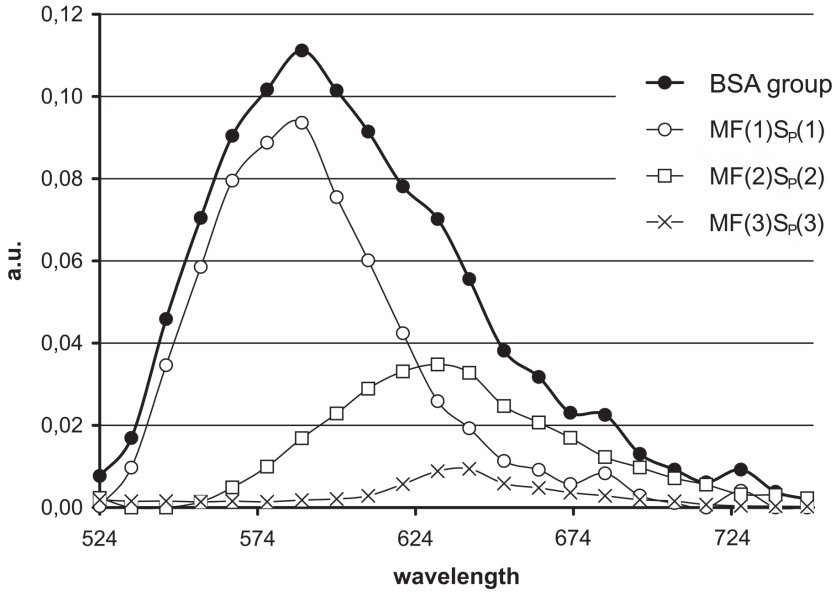

Fig. 2. Emission fluorescence spectrum of the porcine late-blastocyst stained with NR and cultured in NCSU-23 medium with BSA. It was deconvoluted into the three component spectra $S_P(1)$, $S_P(2)$ and $S_P(3)$. Parameters MF(1), MF(2) and MF(3) are multiplying factors of the appropriate component spectra in the experimental spectrum of blastocysts from the BSA group.

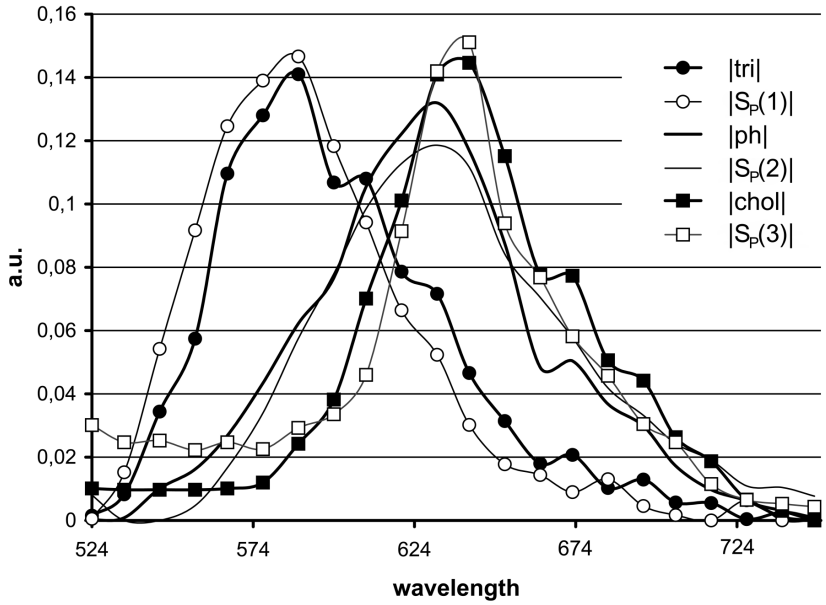

Fig. 3. Normalized component spectra $|S_P(1)|$, $|S_P(2)|$ and $|S_P(3)|$ fit tight with the appropriate normalized emission spectra of triglycerides – |tri|, phospholipids – |ph| and cholesterol – |chol|, respectively. Spectra |tri|, |ph| and |chol| were taken for standard stained with NR.

trum profiles as appropriate component spectra $S_P(1)$, $S_P(2)$ and $S_P(3)$ (Fig. 3). The quality of approximation is very high because the coefficient of determination R^2 equals 0.922 for comparison |s1| with |s(tri)|, 0.956 for |s2| with |s(ph)|, and 0.927 for |s(chol)| with |s3|. Therefore, previously deconvoluted component spectra are responses of triglycerides, phospholipids and cholesterol stained with NR and contained in the lipid droplets of the studied blastocysts, whereas MF(1) = MF(tri), MF(2) = MF(ph) and MF(3) = MF(chol) are multiplying factors (proportional to the percentage content) of appropriate types of lipids in samples.

Mean values of |TF(tri)|, |TF(ph)| and |TF(chol)| are shown in Table 2. Moreover, total lipid content |TF|, which is a ratio of mean lipid content of the experimental group to the mean value calculated for the BSA group of blastocysts, is shown in Ta-

Table 2

Normalized content of triglycerides |TF(tri)|, phospholipids |TF(ph)| and cholesterol |TF(chol)| as well as normalized total lipid content |TF| estimated for different groups of blastocysts

	|TF(tri)| ± SD	|TF(ph)| ± SD	|TF(chol)| ± SD	|TF| ± SD
BSA	$0.636^{1-4} \pm 0.061$	0.284 ± 0.051	$0.071^{1-4} \pm 0.018$	$1^{1} \pm 0.106$
FCS	$0.521^{1,5-7} \pm 0.037$	0.262 ± 0.061	$0.128^{1} \pm 0.040$	0.914 ± 0.090
C	$0.476^{2,5,8} \pm 0.017$	0.269 ± 0.020	$0.137^{2} \pm 0.041$	0.881 ± 0.121
PES	$0.364^{3,6,8,9} \pm 0.018$	0.228 ± 0.040	$0.150^{3} \pm 0.035$	$0.740^{1} \pm 0.125$
VE	$0.446^{4,7,9} \pm 0.014$	0.261 ± 0.035	$0.129^{4} \pm 0.029$	0.828 ± 0.100
F	21.59	0.75	14.67	3.95

BSA – group of blastocysts cultured in NCSU-23 medium with 4 mg/mL BSA, C – group of blastocysts cultured in NCSU-23 medium without BSA or FCS, FCS – group of blastocysts cultured in NCSU-23 medium with 4 mg/mL BSA and with: 10% FCS (FCS group), 0.05 μM PES (PES group) or with 25 μM vitamin E (VE group). ANOVA: F is the value of the F-distribution, f1 = 4 and f2 = 45 are degrees of freedom among and within groups, confidence level P = 0.05, SD – standard deviation. The same superscript next to appropriate mean values in each column indicates statistically significant differences between different blastocyst groups under study. Notation 1-4 or 5-7 at superscript corresponds to 1,2,3,4 or to 5,6,7 respectively.

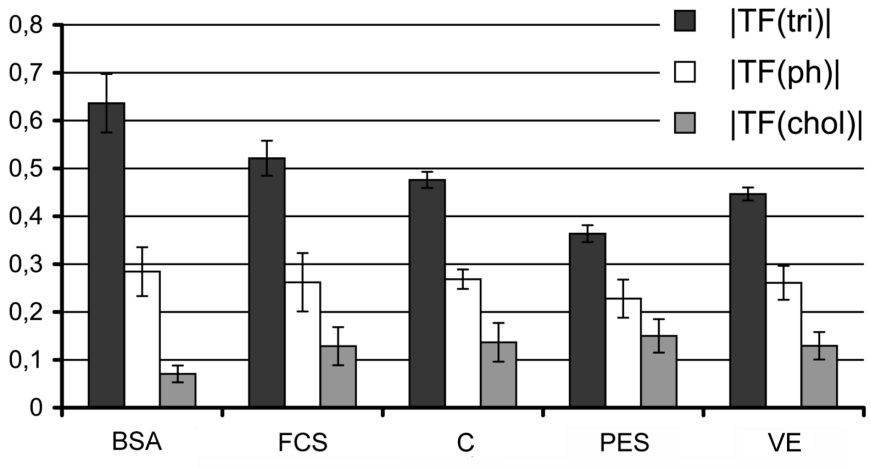

Fig. 4. Normalized values of the triglycerides – |TF(tri)|, phospholipids – |TF(ph)| and cholesterol – |TF(chol)| content for blastocysts cultured in: NCSU-23 medium with BSA (BSA group), without protein (C) and cultured in NCSU-23 medium with BSA and supplemented with: FCS (FCS group), PES (PES group) and vitamin E (VE). Parameters TF(tri), TF(ph) and TF(chol) were normalized by the mean value of the total lipid content estimated for BSA group – $\overline{TF(BSA)}$.

ble 2. The group of blastocysts cultured with addition of 0.05 μM PES (PES group) had lower value of |TF| than that of the BSA group, but no other differences were found for total lipid content.

We found numerous statistically significant differences in content of triglycerides |TF(tri)| between blastocysts of different groups with high values of the F-distribution (Table 2 & Fig. 4). Parameter |TF(tri)| differs between all groups with one exception: no statistically significant difference was found between groups C and VE. With respect to the phospholipid content |TF(ph)|, there are no significant differences between groups (Table 2 & Fig. 4). Finally, the value of |TF(chol)| is significantly lower for the BSA group as compared to other groups.

Discussion

The lipid droplets of porcine oocytes and embryos contain mixtures of several lipid types, mainly triglycerides and phospholipids (MCEVOY *et al.* 2000). They show different degrees of polar-

ity, therefore NR emission spectrum of stained blastocysts is a convolution of several principal spectra (MUKHERJEE *et al.* 2007; KĘPCZYŃSKI *et al.* 2008). The decomposition technique PCA/MCR-ALS suggested a three-component model. In consequence, we detected three types of lipids in lipid droplets of the analysed blastocysts. Their component spectra strictly corresponded to the fluorescence spectra measured for triglyceride, phospholipid and cholesterol standards. These findings are in good agreement with a qualitative, histochemical analysis of pig embryos (unpublished data). Moreover, multiplying factors of component spectra MF(tri), MF(ph) and MF(chol), calculated via the MCR-ALS method for each mean experimental spectrum are proportional to the percentage content of the three identified types of lipid in each individual blastocyst (TAULER 1995). It is important to mention that in the absence of a standard with a strictly defined amount of analysed lipid types placed in the same environment as the embryo cytoplasm, only the relative amount of lipids present in lipid droplets could be estimated by spectra decomposition and multiplying factor evaluation. Consequently, we analysed the content of triglycerides, phospholipids and cholesterol as well as the total lipid content for each studied blastocyst as a ratio to the mean total lipid content found for the BSA group. This scheme allows comparing the total lipid content and content of three classes of lipids in droplets between the different groups of analysed blastocysts.

Our results suggest that the culture system may modify triglyceride content in the porcine blastocysts (Fig. 4) but only PES supplementation leads to a statistically significant decrease in the total lipid content (Table 2). We observed a reduced amount of triglycerides in embryos cultured in medium supplemented with FCS, vitamin E and cultured in medium without protein in comparison to those cultured in this medium with BSA. It was previously shown that triglycerides are important sources of energy for early porcine embryos (TSUJII *et al.* 2001). Free fatty acids derived from triglycerides are metabolized through β-oxidation process in the mitochondrial matrix. Therefore, higher rate of embryo aerobic metabolism is a possible explanation of reduced triglyceride content in the analysed blastocysts. Surprisingly, FCS supplementation to culture medium did not result in lipid accumulation, especially triglycerides in porcine embryos, in contrast to bovine embryos (BARCELO-FIMBRES & SEIDEL 2007; REIS *et al.* 2003). However, fetal calf serum has antioxidant properties, contains growth factors and numerous proteins which are responsible for transport of hormones, free fatty acids and CO_2. Therefore FCS may stimulate aerobic metabolism as previously suggested (BARCELO-FIMBRES & SEIDEL 2007). Consequently we observed a small but statistically significant decrease of triglyceride content in FCS treated blastocysts. A similar explanation can be put forward for the lower triglyceride content in the blastocysts cultured in the presence of vitamin E. As was previously shown, vitamin E stimulates incorporation and oxidation of glucose at the blastocyst stage of mouse embryos (TSUJII *et al.* 2002). This supports the hypothesis that β-oxidation rates may be higher in the presence of vitamin E due to the fact that when free fatty acids are oxidized, small amounts of carbohydrates are required to provide oxaloacetate to prime the TCA cycle. In contrast, lipid accumulation may be the result of impaired β-oxidation due to abnormal mitochondrial activity (DORLAND *et al.* 1994) damaged by free radicals (TSUJII *et al.* 2002). Blastocysts cultured in medium without protein have lower triglyceride levels than those observed in the BSA group. The survival rate of such embryos is low because amino acids are beneficial for pig embryonic development (SWAIN *et al.* 2002). Amino acids are additional energy sources during preimplantation in pig development, however, Krebs cycle metabolism is higher for *in vivo* derived embryos than for *in vitro* cultured embryos (SWAIN *et al.* 2002). The authors speculate that the lipid metabolism rate may be higher in the absence of such additional energy sources.

Phenazine ethosulfate, a pharmacological regulator of embryo metabolism, was previously used for *in vitro* production of bovine (BARCELO-FIMBRES & SEIDEL 2007; DE LA TORRE-SANCHEZ *et al.* 2006) and porcine embryos (GAJDA *et al.* 2008). The positive effect of PES on porcine embryo development was reflected in higher rates of cleaved embryos, morulae and blastocysts as well as low incidence of apoptosis in PES cultured embryos compared to those cultured without PES (GAJDA *et al.* 2008). Phenazine ethosulfate accepts H^+ from NADPH, by converting $NADPH^+$ to $NADP^+$, stimulates glucose metabolism *via* the pentose phosphate pathway and leads to decreased lipid production (DE LA TORRE-SANCHEZ *et al.* 2006; BARCELO-FIMBRES & SEIDEL 2007). Accordingly, we observed reduced amounts of total lipid content and triglycerides in the PES-treated porcine blastocysts (GAJDA *et al.* 2008), although PES supplementation did not affect phospholipid and cholesterol content (Fig. 4).

We detected a significantly lower level of cholesterol only in the BSA group. Cholesterol is an important component of the molecular monolayer enclosing lipid droplets. Lower cholesterol content suggests that lipid droplets of blastocysts cultured in NCSU-23 medium with BSA have larger sizes and thus reduced area of the molecular mono-

layer per unit volume of droplets. This effect was observed previously for bovine cultured embryos (BARCELO-FIMBRES & SEIDEL 2007).

This is the first study in which triglyceride, phospholipid and cholesterol content has been examined in porcine blastocysts cultured in media of different composition. We inferred that porcine blastocysts cultured in NCSU-23 medium supplemented with FCS or BSA and PES or BSA and vitamin E have lower levels of triglycerides in comparison to those cultured in NCSU-23 with BSA only. In addition, embryos cultured in medium without protein also have lower triglyceride content compared to those cultured in medium supplemented with BSA. This may be explained by increased aerobic metabolism. Fetal calf serum supplementation of culture medium does not lead to lipid accumulation in porcine embryos at the blastocyst stage in contrast to bovine embryos.

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