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BCB TEST UTILITY EVALUATED BY CASPASE-3 ACTIVITY IN BLASTOCYSTS DEVELOPED FROM BOVINE OOCYTES WITH DIFFERENT G6PDH LEVEL*

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Abstract

This study was conducted in order to determine whether BCB staining performed on immature bovine oocytes to assess G6PDH level has an impact on IVF/IVP technology in bovine embryos, by measuring caspase-3 activity in blastocysts. Higher caspase-3 activity was noted in morphologically healthy embryos originating from BCB+ oocytes, compared to blastocysts obtained from non-stained oocytes. In our opinion, this feature in morphologically healthy embryos does not necessarily reflect poor embryo quality, but in this case may indicate better preparation of the embryo to react to suboptimal *in vitro* environmental conditions and/or be a sign of preparation for differentiation. High caspase-3 activity may thus have both positive and negative meaning. It may be a reflection of apoptosis as a result of *in vitro* culture conditions or a sign of cell differentiation. We recommend that the BCB test be used for all procedures relying on single oocyte manipulations such as cloning or transgenesis. In regard to IVF, the decision to perform BCB staining should be left for individual consideration, as this test has advantages and disadvantages, as described in this paper.

Key words: blastocyst, BCB, caspase-3

Brilliant cresyl blue (BCB) can be used for the selection of competent oocytes of pigs, goats and cattle for IVF or other procedures (Roca et al., 1998; Rodríguez-González et al., 2002; Pujol et al., 2004; Alm et al., 2005; Wongsrikeao et al., 2006; El Shourbagy et al., 2006; Bhojwani et al., 2007; Kątska-Książkiewicz et al., 2007; Spikings et al., 2007). The test determines the activity of glucose-6-phosphate dehydrogenase (G6PDH), an enzyme synthesized in growing oocytes but inactive in oocytes that have finished their growth phase. Therefore, the oocytes that have com-

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pleted the growth phase are blue (BCB+) because the G6PDH activity is too small to reduce the staining, while the growing oocytes remain colourless (BCB^-). It has been demonstrated that BCB staining increases the veracity of oocyte selection and, as a result, improves IVP efficacy (Roca et al., 1998; Rodríguez-González et al., 2002; Pujol et al., 2004; Alm et al., 2005; Wongsrikeao et al., 2006; El Shourbagy et al., 2006; Bhojwani et al., 2007; Kątska-Książkiewicz et al., 2007). However, the results of our previous experiments (Opiela et al., 2008) showed the lack of significant differences in the blastocyst rates developed from BCB+ and control oocytes, and in our opinion, this decreases the validity of the BCB test in IVP technology. Moreover, the results of another experiment seem to indicate that oocytes subjected to BCB staining show a tendency towards apoptosis (Opiela et al., 2008). In immature oocytes, the Bax transcript level in BCB^- oocytes was significantly higher ($P<0.001$) in comparison to non-stained oocytes. Moreover, there was a tendency for higher Bax protein expression in immature BCB+ oocytes, but no relationship was found between the activity of G6PDH and the expression of apoptotic proteins.

Therefore, to better estimate the impact of BCB staining on IVF/IVP technology in bovine development, we expanded our research to include blastocysts. The quality of blastocysts developed from fertilized oocytes subjected to BCB staining and control, not stained oocytes, was estimated by means of caspase-3 activity.

In apoptosis cascade the activation of caspases is regulated by the proteins of Bcl-2 family. Activated caspases cleave proteins that turn the death promoting activators on. In some forms of apoptosis, the extrinsic apoptotic pathway is initiated by activation of the apical caspase-8 following death receptor ligation. In other forms, cellular stress leads to activation of the intrinsic apoptotic pathway initiated by the apical caspase-9 (Abraham and Shaham, 2004), which activates the executioner caspase-3, 6 and -7. Both upstream and downstream caspases as well as other proteases (e.g. calpains) cooperate to regulate apoptosis in a cell-specific manner. The intrinsic pathway is triggered by cytochrome c release from mitochondria (Jousan et al., 2008), which crosses the mitochonrial outer membrane. This is possibly only after the formation of specific channels or coformation of megachannels (mPTP) in outer mitochondrial membrane by proapoptotic proteins such as: bad, bid, bax and bak. These channels are responsible for the release of apoptogenic proteins such as cytochrome c, Smac/DIABLO (second mitochondria-derived activator of caspases/direct IAP binding protein with low pI), AIF (apoptosis inducing factor), Omi/HtrA2, procaspase-9 and -3 and endonuclease G from mitochondrial intermembrane space. Smac/DIABLO is second (after cytochrome c) activator of caspases, functioning through the release of caspases from inhibitory effects of IAPs (inhibitors of apoptotic proteases). Caspases are the executors of cell death. Active caspase-3 is pivotal to activation of DNA-degrading endonucleases, which are responsible for cutting the DNA into oligosomal fragments.

Active caspase-3 was detected on day 8 in *in vitro* produced bovine embryos. This activation concurred with changes in nuclear morphology and preceded DNA degradation detectable by TUNEL (Gjorret et al., 2004). A recent study of Vandaele et al. (2007) detected active caspases in bovine embryos as early as in 2-cell stage. So far, caspase-3 activity has been used as an indicator of embryo quality, and the de-

gree of its activity is a determinant of embryonic fate (Exley et al., 1999; Jurisicova et al., 2003; Mullen and Critser, 2004; Gjørret et al., 2007; Jousan et al., 2008).

Material and methods

Unless otherwise indicated, all plasticware, i.e., culture vessels, dishes and tubes, used in our experiments was obtained from Nunc, Wiesbaden, Germany, and all chemicals and media were purchased from Sigma, Poznań, Poland.

All procedures regarding *in vitro* embryo production follow the protocol developed and optimized by Kątska-Książkiewcz et al. (2002, 2006).

Oocyte retrieval

Cumulus-oocyte complexes (COCs) were retrieved from ovaries from slaughtered cows by isolation and rupture of follicles 2–8 mm in diameter in holding medium (TCM 199 – Earle's salt with 25 mM HEPES containing 10% foetal calf serum). COCs were pooled into fresh manipulation medium and held at 38°C until subjected to BCB staining. Degenerate oocytes and those without cumulus cells were discarded.

BCB test

The BCB test was performed according to Alm et al. (2005) with minor modifications. The compact COCs fulfilling the morphological criteria (evenly granulated cytoplasm and compact cell layers tightly attached to the oocyte) were placed in 26 μM of BCB diluted in Dulbecco's PBS for 60 min at 38°C in a humidified air atmosphere. Following BCB exposure, the COCs were washed twice in a warm solution of PBS with 0.4% BSA. Then COCs were examined under a stereomicroscope and divided into two groups according to their cytoplasm colouration: the oocytes with blue colouration of cytoplasm were designated as BCB+, and those without any colouration were designated as BCB^-. After classification, the COCs were washed in maturation medium and placed in culture. The COCs not subjected to BCB test (control) were washed in maturation medium and placed in culture immediately after morphological selection.

In vitro maturation of oocytes

COCs were matured in modified TCM 199 Earle's salt buffered with sodium bicarbonate, pH 7.4 supplemented with 20% oestrus cow serum (ECS, heat inactivated) and an additional 3 to 5×10^6 granulosa cells/ml. The COCs were cultured for 22 to 23 h at 38.5°C under 5% CO_2 in air at maximum humidity.

Sperm preparation and *in vitro* fertilisation

After IVM, oocytes were fertilized *in vitro* using frozen-thawed spermatozoa of the same bull, containing at least 50% progressively motile spermatozoa. Spermatozoa were separated on a discontinuous Percoll (Pharmacia, Uppsala, Sweden) density gradient (1 ml 45% Percoll over 1 ml 90% Percoll) by centrifugation for

30 min at 300 × g at room temperature, washed in calcium ion-free TALP medium, and pelleted by centrifugation at 100 × g for 10 min. The spermatozoa were counted and diluted to approx. 3 to 5 × 10^7 sperm/ml in the calcium ion-free TALP medium. This suspension was added to the fertilization drops containing 10 μg/ml heparin and a mixture of penicillamine (20 μM), hypotaurine (10 μM) and epinephrine (1 μM) at a concentration of 1–2 × 10^6 spermatozoa/ml of medium.

Mature COCs were washed and partially deprived of expanded cumulus before being transferred in groups of up to 20 into 40 μl of TALP-IVF medium. Gametes were incubated together for 18 to 21 h at 38.5°C under 5% CO_2 in air (Kątska et al., 2002).

***In vitro* embryo culture in co-culture with VERO cells**

After 18 to 21 h of fertilization, the oocytes were washed with a holding medium to remove corona cells and attached spermatozoa. Then, approximately 20 presumptive zygotes were transferred into 40 μl drops of B_2 medium (C.C.D., Paris, France) under mineral oil for 20 to 24 h (40 to 44 h post insemination). Uncleaved ova were discarded, and approximately 20 embryos were placed in co-culture with Vero cells in 40 μl drops of B2 medium supplemented with 2.5% FCS, under mineral oil. Half of the medium in the culture drops was changed at intervals of 48 h. Embryos were maintained in co-culture for 7 to 8 days.

The Vero cells were obtained frozen from ECACC, Salisbury, UK. Cell samples were thawed and washed in HEPES buffered TCM 199 and then suspended in Dulbecco's MEM (DMEM; Gibco BRL, Life Technologies, Paisley, Scotland) supplemented with 10% FCS and 1% antibiotic-antimycotic solution, enriched with 2mM L-glutamine, 0.36 mM pyruvate, and 2 mM nonessential amino acids (Kątska-Książkiewicz et al., 2006). For co-culture with the small group of embryos, cells were seeded at a concentration of 1 × 10^2 in 40 μl medium per drop.

Caspase-3 activity in embryos

Activity of caspase-3 in blastocysts was assessed using a PhiPhiLux G_2D_2 kit (Calbiochem, Darmstadt, Germany). The kit contains a peptide substrate for caspase-3, which is conjugated with two rhodamine fluorophores on each side of the substrate. Upon cleavage by active caspase-3, high intensity red rhodamine fluorescence can be observed, while no fluorescence is emitted by intact substrate.

Embryos were placed in 50 μl RPMI 1640 medium containing 10 μM peptide substrate and 10% FBS for 60 min at 38.5ºC and 5% CO_2 in air at maximal humidity (Men et al., 2003). After incubation, embryos were washed three times in PBS to reduce the background fluorescence, mounted onto glass slides and examined under the fluorescence microscope using a rhodamine filter combination (EX 510-560, BA 590).

The embryos showing red fluorescence of more than 50% of their surface were estimated to have high caspase-3 activity (++). The embryos showing red fluorescence of less than 50% of their surface were estimated to have low caspase-3 activity (+).

Statistical analysis

Differences with a probability value of 0.05 or less were considered significant. Differences in caspase-3 activity were assessed using chi-square test (χ^2) or Fisher test (Graph-Pad software, San Diego, California, USA).

Results

Activity of caspase-3 in blastocysts obtained from IVM/IVF oocytes with different levels of G6PDH activity

A total of 204 blastocysts (88 BCB+; 22 BCB- and 94 control) were cultured in B2+VERO for assessment of caspase-3 activity as described in Tables 1 and 2. The data regarding number of oocytes taken to IVF, number of cleaved oocytes, developed blastocysts and hatched blastocysts was presented in our previous paper (Opiela et al., 2008).

There was a tendency towards increased caspase-3 activity during the progression of blastocyst development (Tables 1 and 2). The highest activity of caspase-3 was assessed in the expanded and hatched blastocysts (70.5%, 100% and 58.2% respectively for BCB+, BCB^- and control). Inverse relationships in the proportion of blastocysts with high caspase-3 activity were observed for the early and middle stages of blastocyst development in B2+VERO (43.8%, 20.0% and 29.4% respectively for BCB+, BCB- and control blastocysts). Approximately 50% of the embryos cultured in B2+VERO showed high caspase-3 activity in the late blastocyst stage, regardless of the origin (Table 2). As shown in Table 2, low activity of caspase-3 (42.0%, 36.4%, and 47.9%) was observed in BCB+, BCB- and control blastocysts, when blastocysts were cultured in B2+VERO. A significant difference ($P<0.05$) between blastocysts with low and high activity of caspase-3 was only observed in the BCB+ group (Table 2).

Table 1. Caspase-3 activity in different blastocyst stages in B2+VERO

Blastocyst stage	Fluorescence intensity *	BCB+ N = 88	BCB^- N = 22	Control N = 94
early-middle	+	9 (56.3%)	4 (80.0%)	12 (70.6%)
	++	7 (43.7%)	1 (20.0%)	5 (29.4%)
late	+	15 (53.6%)	4 (44.4%)	5 (50.0%)
	++	13 (46.4%)	5 (55.6%)	5 (50.0%)
expanding and hatching	+	13 (29.5%)	0 (0.0%)	28 (41.8%)
	++	31 (70.5%)	8 (100%)	39 (58.2%)

*The embryos showing red fluorescence of more than 50% of their surface were estimated to have high caspase-3 activity (++). The embryos showing red fluorescence of less than 50% of their surface were estimated to have low caspase-3 activity (+).

Table 2. Comparison of low and high caspase-3 activity in blastocysts developed from fertilized BCB+, BCB$^-$ and control oocytes

Fluorescence intensity	Blastocysts BCB+ (%)	Blastocysts BCB- (%)	Blastocysts Control (%)
+	37 (42.0)a *	8 (36.4)	45 (47.9)a
++	51 (58.0)a *	14 (63.6)	49 (52.1)a

Blastocysts were cultured in co-culture with VERO in B2 medium, at 5% CO_2 in air.

Letters in the same row do not differ statistically. Asterisks within the same column differ statistically; *$P<0.05$ (test χ^2).

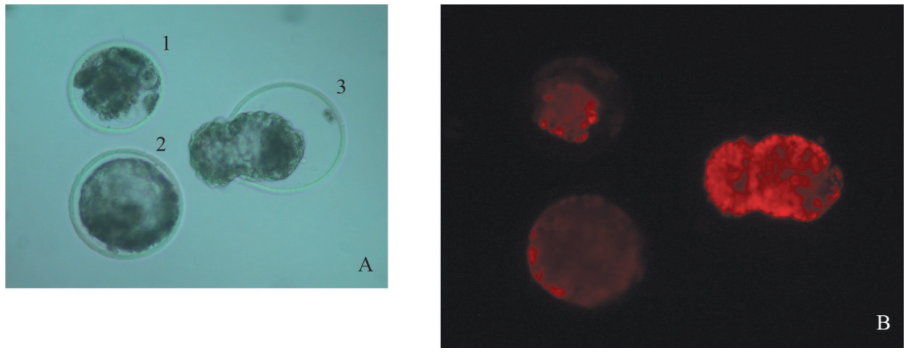

Figure 1. Caspase-3 activity in bovine embryos: degenerating embryo (1); expanding blastocyst (2); hatching blastocyst (3);
A – image in the visible spectrum, B – fluorescence (1) ++ (2) + (3) ++ (100 × magnification)

Discussion

According to Alm et al. (2005), the BCB test does not have a negative effect on oocytes as similar blastocyst rates for stained and non-stained fertilized oocytes were found. The blastocysts from BCB+ oocytes had a similar cell number as control oocytes (Alm et al., 2005). Wongsrikeao et al. (2006) noted an increase in the number of cells in porcine blastocysts from BCB+ oocytes when compared to blastocysts from control oocytes. These are so far the sole reports regarding the quality of blastocysts obtained by fertilization of oocytes subjected to BCB staining. Wongsrikeao et al. (2006) determined the toxicity of BCB staining by estimating the potential for fertilization and embryonic development of porcine oocytes subjected to single and double (before and after IVM) BCB staining. None of the double stained oocytes were able to develop to the blastocyst stage (Wongsrikeao et al., 2006).

In the present work, we used caspase-3 activity as a quality marker for blastocysts produced from oocytes with different levels of G6PDH activity. There was a significantly higher number of blastocysts with high caspase-3 activity that developed from BCB+ oocytes, compared with the numbers of blastocysts obtained from IVM/IVF oocytes with low G6PDH activity, BCB$^-$ and controls. Due to the

low number of blastocysts that developed from BCB- oocytes, the statistical analysis could not be performed. This may imply that the BCB test exerts a slightly negative effect on blastocyst quality. However, because of the lack of a second control group (holding control) in our experiments, it is difficult to determine the reason for the elevated caspase-3 activity: the BCB stain itself or the prolonged time of oocyte incubation before IVM. In light of previous studies (Jurisicova et al., 1998; Byrne et al., 1999; Matwee et al., 2000; Makarevich and Markkula, 2002; Paula-Lopes and Hansen, 2002; Fahrudin et al., 2002; Gjørret et al., 2003; Maddox-Hyttell et al., 2003; Pomar et al., 2005) where high quality embryos had low or even no caspase activity, our results imply that BCB staining of oocytes may have a detrimental effect on blastocyst quality. However, when using apoptosis as a quality marker, it is important to take into account that this process contributes to the survival of an embryo in response to stress or other damaging factors. As such, cell loss does not always lead to embryonic death. Limited apoptosis helps the embryo to maintain homeostasis, while extensive apoptosis may cause embryo death (Paula-Lopes and Hansen, 2002). Moreover, Maddox-Hyttell et al. (2003) showed that blastocysts that appear healthy by stereomicroscopy may have subcellular defects, as was shown by transmission electron microscopy, immunocytochemistry for confocal laser scanning microscopy and fluorescence in situ hybridization. Also Men et al. (2003) detected caspase-3 activity and DNA fragmentation after cryopreservation both in the morphologically healthy oocytes and in oocytes with typical apoptotic morphology. In bovine embryos, at least one apoptotic cell was reported in 90–100% of *in vivo* and *in vitro* produced embryos (Byrne et al., 1999; Matwee et al., 2000; Makarevich and Markkula, 2002; Gjørret et al., 2003). Pomar et al. (2005) showed low levels of apoptotic features only in high quality equine, porcine, ovine, caprine and bovine blastocysts. Blastocysts were graded as high quality when they "exhibited a spherical and symmetrical embryo mass, occupying 70–80% of the periviteline space with cells uniform in size, with the trophoblast, blastocoele and inner cell mass clearly identifiable and no blastomere extrusion" (Pomar et al., 2005). In embryos that did not attain the above criteria, up to 100% of the cells were observed to be apoptotic (Pomar et al., 2005). According to these authors (Pomar et al., 2005), the assessment of apoptosis is not sufficient to appropriately assess the developmental capacity of an embryo.

Contrary to the generally accepted opinion that high caspase activity is a marker of poor embryo quality, there are several papers reporting no correlation between embryo morphology and caspase activity (Martinez et al., 2002; Vandaele et al., 2007; 2008). Much earlier, Martinez et al. (2002) showed that caspase activity in pre-implantation human embryos is not associated with apoptosis. Their conclusions were more recently verified by Vandaele et al. (2007) in bovine embryos. The authors stated that abnormal morphology during embryo development could not be related to higher caspase activity since the proportion of caspase activity did not differ between embryos of good and poor morphology, while symmetry and absence of fragmentation were used as morphological criteria (Vandaele et al., 2007). Interestingly, the latest research from Vandaele et al. (2008) showed that the value of caspase activity as a reliable apoptosis detection method is questionable, since caspase-3 and -7

mRNAs were not differently expressed in staurosporine-treated embryos compared to non-treated embryos (Vandaele et al., 2008). On the other hand, an earlier report by Gjorret et al. (2007) showed that active caspase-3 seems to be involved in both spontaneous and staurosporine-induced apoptosis in bovine pre-implantation embryos. However, sensitivity of the embryo to staurosporine-induced apoptosis varied between embryos at the same stage and varied with the stage of development. However, if the hypothesis that caspase-3 and -7 do not reflect the apoptotic state of the cell (Vandaele et al., 2008) is true, then the caspase that is responsible for the activity that has been observed is unclear.

Caspase activation might be important for cell differentiation, as it was discovered in somatic cells (reviewed in Schwerk and Schulze-Osthoff, 2003; Abraham and Shaham, 2004). Such a pro-survival function of caspases might explain the high caspase-3 activity in healthy looking expanded and hatched blastocysts. If caspases contribute to differentiation, then their activity would be viewed as a positive marker of embryo quality. Indeed, Yamashita et al. (2008) showed that in transgenic and wild-type zebrafish embryos, complete caspase deficiency caused the arrest of early embryogenesis. Moreover, the same authors observed that pro-caspase-3 transgenic fish that exhibited high levels of caspase activity had reduced eye size and degeneration of the retina and photoreceptor cell layers but had normal pigmentation and lens formation. Therefore, caspase-3-mediated pro-apoptotic signalling may regulate retinal differentiation and development while retinal degeneration may result from enhanced apoptotic signalling in response to harmful environmental stimuli. Also heart, eye and notochord development depends on the caspase-3-mediated proapoptotic pathway. Another interesting conclusion drawn from the above observations is that overexpression of caspase-3 may affect only a limited number of apoptotic cells under normal growing conditions and that apoptosis regulation may be maintained by intercellular morphogenetic signalling factors, such as BMP4 and other TGF-β superfamily members. The results described by Yamashita et al. (2008) refer to experiments conducted *in vivo*, while our studies were performed *in vitro*. Therefore, higher caspase-3 activity observed in morphologically healthy embryos that originate from BCB+ oocytes does not necessarily reflect poor embryo quality, but may reflect better preparation to react to suboptimal *in vitro* environmental conditions. The ability to quickly respond to an apoptotic stimulus can definitely be regarded as positive.

It was demonstrated that the intrinsic quality of the oocyte determines the blastocyst yield, while the post fertilization culture system determines the blastocyst quality (Rizos et al., 2002). It is also known that under *in vitro* culture conditions, gene expression in the embryo can be altered (Rizos et al., 2002; Lonergan et al., 2003). Although pre-implantation mammalian embryos show some level of resistance to suboptimal culture conditions, this plasticity is effective only to a certain critical point. It is possible that the caspase-3 activity in analysed blastocysts is affected by suboptimal culture conditions. We observed the tendency of growth with high levels of caspase-3 activity during the progression of embryo development, regardless of the type of oocytes used for fertilization. Our observations are in agreement with previous data where apoptosis was estimated in embryos at different developmental

pre-implantation stages (Byrne et al., 1999; Neuber et al., 2002; Gjørret et al., 2003). The appearance of apoptotic characteristics is accelerated by *in vitro* conditions, but the number of apoptotic cells is insignificant prior to compaction of embryo (Gjørret et al., 2003). The dead cell index (DCI, total number of apoptotic nuclei/total number of nuclei) tends to increase as the *in vitro* culture time increases (Neuber et al., 2002). *In vitro* culture conditions are thought to increase apoptotic incidence, decrease embryonic cell number, decrease implantation rates and increase foetal resorption (Gjørret et al., 2003). According to Gjørret et al. (2003) certain features of apoptosis may be differentially regulated and independently modulated by the mode of the bovine embryo production. This conclusion may also support the idea that prolonged *in vitro* culture on the embryo has harmful effects.

Contrary to the results described above and our observations, Vandaele et al. (2007) reported that the percentage of caspase-positive cells declined in advanced developmental stages. The authors interpreted this as a result of embryo arrest earlier in development. Here, we used a different caspase-3 estimation method, which might explain this discrepancy since it has been shown that the detection of caspase activity differs depending on the method used (Jurisicova et al., 2003). Our results are in agreement with another conclusion of Vandaele et al. (2007) that embryos with slow development (lagging one cell cycle behind) had a higher incidence of apoptosis. Indeed, blastocysts developed from BCB^- oocytes were delayed in achieving the expanded blastocyst stage (data not shown), and all embryos had high caspase-3 activity.

We estimated caspase-3 activity in a total of more than 200 blastocysts cultured in B2+VERO. This is the first time that such an analysis has been performed with such a large study population. Although this assay was qualitative, the large pool of analysed blastocysts minimizes the potential for error in subjective estimation methods. Moreover, we used a simple, two-level estimation method for caspase-3 activity. When more than 50% of the embryo surface showed red fluorescence, the caspase-3 activity was estimated as high (++). When less than 50% of the embryo surface showed red fluorescence, the activity was estimated as low (+). This is a simple test, and most importantly, the embryo is not destroyed by this test if it is carefully handled. Therefore, the embryo can be transferred to a recipient cow. Procedures that are transferred into practice need to be quick, simple, easy to perform and preferably cost effective. The method of caspase-3 activity estimation proposed in this paper fulfils the above criteria and therefore should be further tested by *in vivo* study to confirm that it does not impact proper development and birth of a healthy calf.

To summarize, in regard to IVF, the decision of whether to include BCB staining in the routine laboratory protocol should be left for individual consideration. Based on our previous (Opiela et al., 2008) and present results, the advantages of BCB staining include: selection of oocytes with better developed mitochondria and higher cleavage and blastocyst numbers for BCB+ stained oocytes, compared to BCB^- oocytes. The disadvantages include lack of significant differences between blastocyst numbers from BCB+ and control oocytes, and extension of the overall time needed for oocyte recovery since the BCB test requires extra handling and manipulation. The

increased time requirement will also result in increased cost. In cases where careful morphological selection was conducted before subjecting oocytes to BCB staining, only 30% of COCs remained colourless. This is the most likely cause of the lack of statistical differences between blastocysts developed from BCB+ oocytes and control oocytes. In cases where less careful morphological selection was performed, the differences between BCB+ and control blastocysts were more evident, as we also concluded in another work (Kątska-Książkiewicz et al., 2007). Interpretation of statistically higher caspase-3 activity in blastocysts developed from BCB+ oocytes is unequivocal as it may mean that BCB staining of oocytes could have a detrimental effect on blastocysts. On the other hand, higher caspase-3 activity might be a reflection of better blastocyst quality. This hypothesis warrants further research. However, having in mind that BCB+ oocytes produce statistically more blastocysts, which have more cells than blastocysts originating from BCB^- (Wongsrikeao et al., 2006) and that we found high caspase-3 activity in morphologically healthy embryos, the interpretation that caspase-3 may act as a differentiation factor seems possible.

When do caspases act as a differentiation factor, and when do they act as a cell killer? What is responsible for this phenomenon? These are questions that still need to be addressed.

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Przydatność testu BCB oceniona za pomocą aktywności kaspazy-3 w blastocystach bydlęcych otrzymanych z zapłodnienia oocytów o różnym poziomie G6PDH

STRESZCZENIE

Doświadczenie miało na celu wykazanie, czy barwienie BCB, któremu poddano niedojrzale oocyty bydlęce w celu oceny aktywności G6PDH, ma wpływ na efektywność technologii IVF/IVP u bydła poprzez pomiar aktywności kaspazy-3 w blastocystach. Odnotowano wyższą aktywność kaspazy-3 w morfologicznie prawidłowych blastocystach rozwiniętych z zapłodnienia oocytów BCB+ w porównaniu do blastocyst otrzymanych z zapłodnienia oocytów nie poddanych testowi BCB. Naszym zdaniem, wyższa aktywność kaspazy-3 w morfologicznie prawidłowych blastocystach niekoniecznie jest oznaką ich niskiej jakości, ale może wskazywać na lepsze przygotowanie zarodka do reagowania na nieoptymalne warunki środowiska *in vitro* i/lub być oznaką przygotowania komórek do różnicowania. Wysoka aktywność kaspazy-3 może mieć zatem zarówno pozytywne, jak i negatywne znaczenie. Może być miernikiem zaawansowania apoptozy lub różnicowania komórek. Uważamy, że test BCB powinien znaleźć zastosowanie we wszystkich procedurach bazujących na oocycie jako obiekcie poddawanym manipulacjom, np. w klonowaniu czy transgenezie. W przypadku IVF decyzja powinna być podejmowana indywidualnie, gdyż test posiada zalety i wady opisane w artykule.