Intracellular glutathione content, developmental competence and expression of apoptosis-related genes associated with G6PDH-activity in goat oocyte

Amir Hossein Abazari-Kia · Abdollah Mohammadi-Sangcheshmeh ·
Maryam Dehghani-Mohammadabadi · Fatemeh Jamshidi-Adegani ·
Arash Veshkini · Mahdi Zhandi · Mehmet Ulas Cinar · Mohammad Salehi

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Abstract

Purpose To associate glucose-6-phosphate dehydrogenase (G6PDH) activity in goat oocytes with intracellular glutathione (GSH) content, meiotic competence, developmental potential, and relative abundance of *Bax* and *Bcl-2* genes transcripts.

Methods Goat oocytes were exposed to brilliant cresyl blue (BCB) staining test and categorized into BCB⁺ (blue-cytoplasm), and BCB⁻ (colorless-cytoplasm) groups. A group of oocytes were not exposed to BCB test and was considered as a control group. After maturation in vitro, a group of oocytes were used for determination of nuclear status and intracellular GSH content while another group was subjected to parthenogenetic activation followed by in vitro embryo culture.

Results We found that BCB⁺ oocytes not only yielded higher rate of maturation, but also showed an increased level of

intracellular GSH content than BCB⁻ and control oocytes. Furthermore, BCB⁺ oocytes produced more blastocysts than BCB⁻ and control oocytes. Our data revealed that the expression of anti-apoptotic (*Bcl-2*) and pro-apoptotic (*Bax*) genes were interacted with G6PDH-activity in mature oocyte, their surrounding cumulus cells, and blastocyst-stage embryos.

Conclusions The results of this study demonstrate that selection of goat oocytes based on G6PDH-activity through the BCB test improves their developmental competence, increases intracellular GSH content, and affects the expression of the apoptosis-related genes.

Keywords BCB · Goat oocyte · Glutathione · Apoptosis-related gene · Development

Capsule G6PDH-activity in goat oocytes was found to be associated with glutathione content, developmental competence, and expression of the apoptosis-related genes.

A. H. Abazari-Kia · M. Dehghani-Mohammadabadi ·
F. Jamshidi-Adegani
Department of Transgenic Animal Science, Stem Cell Technology
Research Center, Tehran, Iran

A. Mohammadi-Sangcheshmeh · A. Veshkini
Department of Animal and Poultry Science, College of Aburairhan,
University of Tehran, Pakdasht, Tehran, Iran

M. Zhandi
Department of Animal Science, College of Agriculture and Natural
Resources, University of Tehran, Karaj, Iran

M. U. Cinar
Department of Animal Science, Faculty of Agriculture,
Erciyes University, Kayseri, Turkey

M. Salehi (✉)
Department of Biotechnology, Faculty of Medicine, Shahid Beheshti
University of Medical Sciences, Tehran, Iran
e-mail: msalehi78@gmail.com

Introduction

It is widely accepted that oocyte quality is a key factor for optimizing the efficiency of reproductive techniques in animal and human assisted reproductive technologies (ARTs) [1–3]. Visual assessment of the compactness of the cumulus investment as well as the homogeneity of the ooplasm is routinely used for selecting the more competent oocytes in ART programs [3]. Although such morphological characteristics are practically used in determining the quality of oocytes, their overall accuracy for assessing developmental potential are poor and therefore the predictive value of these criteria are more likely controversial [4–7].

Recently several studies [8–12] including our own [13–16], tried to introduce and highlight the usefulness of a test called brilliant cresyl blue (BCB) staining test for screening the most competent oocytes. The test is based on glucose-6-phosphate dehydrogenase (G6PDH) activity during oocyte development. As we well know, pentose phosphate pathway is

an alternative way for oxidation of glucose and is regulated by G6PDH, the activity of which is dependent on NADPH/NADP ratio. This pathway can branch off from several early stages of glycolysis, diverting glucose from energy production towards the synthesis of NADPH and ribose-5-phosphate. NADPH is also important in anabolic processes, such as fatty acid synthesis [17]. As stated above, the BCB test is depend on the capability of G6PDH to convert the dye from blue to a colorless state. Due to sufficient concentrations of the enzyme in oocytes undergoing growth, the dye is reduced to colorless, while fully-grown oocytes remain blue due to the low amount of cytoplasmic G6PDH-activity [4].

Using BCB test, meiotic competence, fertilization ability, potential of embryonic development, and relation of pro- and anti-apoptotic genes has been associated with G6PDH-activity in different animal models [8, 9, 14, 16, 18]. In most of these studies, the better performance of oocytes with low G6PDH-activity has been related to their better cytoplasmic maturation.

Cytoplasmic glutathione (L- γ -glutamyl-L-cysteinyl-glycine; GSH) is the major non protein sulphhydryl compound in mammalian gametes; it serves as a reservoir for cysteine and plays an important role in protecting mammalian gametes from apoptosis induced by reactive oxygen species [19–22]. On the other hand, in several reports, GSH has been introduced as an important indicator of cytoplasmic maturation [20, 23, 24]. If this is true, one could speculate that oocytes with varied G6PDH-activity could possess different subcellular properties and varied molecular characteristics. We also thought that differences in G6PDH-activity of the oocytes might be reflected in intracellular GSH content and expression of the apoptosis-related genes.

Therefore, with the aim of shedding more light on the interactions between G6PDH-activity and some indicator of oocyte cytoplasmic maturity, a prospective study was conducted using BCB staining test as the primary selection parameter for oocyte competence. To accomplish that we associate the G6PDH-activity with intracellular GSH content, meiotic competence and developmental potential after parthenogenetic activation. In addition, we investigate the relationship between G6PDH-activity of immature oocytes with relative abundance of *Bax* and *Bcl-2* genes transcripts in mature oocytes, their surrounding cumulus cells, and in blastocysts-stage embryos in a series of subsequent experiments. Answering these questions might ultimately lead to expanded use of BCB screening method in prediction of human oocyte fertilization rates.

Materials and methods

Unless otherwise specified, all chemicals and media were obtained from Sigma-Aldrich chemical Co. (St. Louis, Mo,

USA) and Gibco (Grand Island, NY, USA) respectively. All plastic dishes and tubes were obtained from Nunc (Roskilde, Denmark).

Oocyte collection

Goat ovaries were obtained from a local slaughterhouse and transported to the laboratory in physiological saline at 30–35 °C within 2 h after collection. Cumulus–Oocyte Complexes (COCs) were retrieved from follicles with diameter from 2 to 6 mm by aspiration method. Then, all aspirated COCs were washed three times in Hepes-TCM199 medium supplemented with 10 % fetal bovine serum (FBS). COCs with a homogenous cytoplasm and more than three layers of cumulus cells were selected under stereomicroscope and used for experiments [25].

BCB staining

The COCs were exposed to 26 mM of BCB dye diluted in modified phosphate buffer saline for 90 min at 38.5 °C in humidified air atmosphere [16]. Following BCB staining, COCs were washed 3 times in modified phosphate buffer saline (mPBS). After washing, COCs were observed under stereomicroscope and classified into two groups based on their cytoplasm coloration: COCs with blue color in cytoplasm considered as BCB⁺ group and COCs with colorless cytoplasm detected as BCB[−] group. A group of COCs were randomly assigned as a control group and were transferred to maturation medium without being exposed to the BCB dye.

In vitro maturation

The COCs were washed three times in maturation medium consisting of bicarbonate-buffered TCM-199 with 2 mM L-glutamine supplemented with 10 % FBS, 5.5 mg/ml sodium pyruvate, 25 μ g/ml gentamycin sulphate, 5.0 μ g/ml LH, 0.5 μ g/ml FSH, and 1 μ g/ml estradiol. In vitro maturation (IVM) was performed by culturing approximately 10 COCs for 24 h in 50 μ L maturation medium droplet under mineral oil at 39 °C, 5 % CO₂ in a humidified atmosphere.

Measurement of intracellular GSH content

Intracellular GSH content was measured according to the previously described method [26]. In brief, immediately after IVM, COCs were denuded and incubated in tyrodes medium plus 5 mg/ml poly vinyl alcohol containing 10 μ M Cell Tracker blue for 30 min. The oocytes were subsequently washed in mPBS, placed into 10 μ L droplets, observed under an epifluorescence microscope (Nikon, Tokyo, Japan) with UV filters, and then all fluorescent images were recorded as graphic files. The fluorescence intensities of oocytes were

analyzed by ImageJ software (<http://rsb.info.nih.gov/ij>) and normalized to untreated control oocytes.

Nuclear chromatin evaluation

After maturation in vitro, a group of COCs from each BCB⁺, BCB⁻ and control group were freed from surrounding cumulus cells, stained with 2.5 mg/mL Hoechst 33258 in 3:1 (v/v) glycerol/PBS, and kept until observation. Then oocytes were evaluated in relation to their meiotic stage under an epifluorescence microscope. Oocytes were classified as being in germinal vesicle (GV), germinal vesicle breakdown (GVBD), metaphase-I (MI), and metaphase-II (MII) stages of the maturation process (Fig. 1), as previously described [14, 16].

Oocyte activation and in vitro culture

Three pools of biological replicates, each containing ten denuded mature oocytes were used for total RNA isolation. The cumulus cells of the mature oocytes after separation were also pooled in three biological replications for total RNA extraction. For extraction of RNA from embryos, three pools of biological replicates, each containing five blastocysts were

used. For RT-PCR analysis, total cellular RNA was extracted using TRI-reagent. Synthesis of cDNA was carried out with M-MuLV reverse transcriptase and random hexamer as primer, according to the manufacturer's instructions. Reverse transcription reaction was performed using a standard procedure with Taq DNA Polymerase with denaturation at 94 °C for 15 s, annealing at 55–60 °C for 30 s according to the melting temperature of each primer, and extending at 72 °C for 45 s. The number of cycles varied between 30 and 40, depending on the abundance of particular mRNA. The primers and product lengths are listed in Table 1.

Real-Time PCR reactions of 25 µl were conducted in Rotor Gene 6000: 12.5 µl 2X SYBR Premix Ex Taq, 0.4 µm of final concentration for each primer, 2 µl template and distilled water to reach the volume of 25 µl. Real-time PCR was performed in two steps with the following thermal setting: 3 min at 95 °C for initial enzyme activation followed by 40 amplification cycles (each 5 s at 95 °C, 20 s at 60 °C with fluorescence detection) and a final step of melting curve analysis. All the samples were analyzed in duplicate, and the average value of the duplicate was used for quantification. The data were normalized to $\beta 2m$ and $2^{-\Delta\Delta Ct}$ methodology was used for relative quantification [27]. Expression of *YWHAZ* transcript was used as an internal housekeeping gene.

Fig. 1 Goat oocytes stained with Hoechst 33258 and evaluated under epifluorescence microscope, demonstrating oocytes in GV (a), GVBD (b), MI (c), and MII (d) stages

Table 1 Details of primers used for gene expression analysis

Gene name	Primer sequences	Annealing temperature (°C)	Product size (bp)
Bax	F:5'-GCATCCACCAA GAAGCTGAG-3'	61	130
	R:5'-CCGCCACTCGG AAAAAGAC-3'		
Bcl-2	F:5'-ATGTGTGTGGA GAGCGTCA-3'	60	182
	R:5'-AGAGACAGCC AGGAGAAATC-3'		
YWHAZ	F:5'-GAAGAGTCCTACAA AGACAGCACGC-3'	60	115
	R:5'-AATTTTCCCCTCC TTCTCCTGC-3'		

PCR polymerase chain reaction, F forward, R reverse

RNA isolation and quantitative real-time PCR analysis

Twenty four hours after IVM, COCs were vortexed for 3 min in mPBS supplemented with 0.1 % bovine testicular hyaluronidase in 1.5-mL centrifuge tubes to disperse the cumulus cells. For chemical activation, denuded oocytes exposed to 5 μ M ionomycin for 1 min followed by 5 min incubation in H-TCM supplemented with 30 mg/ml bovine serum albumin. Then oocytes were cultured in 2 mM 6-dimethyl aminopurine in CR1aa medium for 2 h. After activation, group of 6 to 8 oocytes were cultured in 20 μ l droplets of CR1aa medium for 8 days. Cleavage and blastocyst rates were assessed on days 3 and 8 post-activation, respectively.

Experimental design

Experiment I: Interaction of G6PDH-activity with intracellular GSH content after IVM

In order to determine the association between G6PDH-activity in goat oocyte and intracellular content of GSH after maturation in vitro, immediately after IVM, cumulus cells were removed and intracellular GSH content were assayed for each group of BCB⁺, BCB⁻, and control oocytes.

Experiment II: Effect of G6PDH-activity on meiotic competence

The association between G6PDH-activity of the oocytes and meiotic competence was evaluated after maturation in vitro. Nuclear status was observed following IVM for each group of BCB⁺, BCB⁻, and control oocytes after fixing and staining.

Experiment III: Effect of G6PDH-activity on in vitro development after parthenogenetic activation

The experiment was performed to investigate effect of G6PDH-activity on developmental competence after parthenogenetic activation. For this, matured COCs obtained from different groups were activated chemically and subsequent in vitro developmental capacity in terms of cleavage and development to the blastocyst stage was recorded.

Experiment IV: Effect of G6PDH-activity on expression of apoptosis-related genes

The interactions between G6PDH-activity in goat oocyte and expression of apoptosis-related (Table 1) genes were examined. For this, immature oocytes were classified into BCB⁺, BCB⁻, and control groups and relationship between G6PDH-activity of immature oocytes with relative abundance of *Bax* and *Bcl-2* transcripts in mature oocytes, their surrounding cumulus cells, and in blastocysts were investigated.

Statistical analysis

The nucleus status of oocytes after IVM, cleavage and blastocyst rates after parthenogenetic activation was compared between groups of oocytes by χ^2 analysis. The intracellular GSH content of BCB⁺, BCB⁻, and control oocytes, and relative gene expression levels of different genes among the groups were analyzed by one-way ANOVA followed by Tukey's pairwise comparison. A *P* value of less than 0.05 was considered statistically significant.

Results

Experiment I: Interaction of G6PDH-activity with intracellular GSH content after IVM

The intracellular concentration of GSH was measured after IVM of BCB⁺ (*n*=40), BCB⁻ (*n*=48), and control (*n*=46) oocytes. The intracellular content of GSH in BCB⁺ oocytes were higher (*P*<0.05) than those of BCB⁻ and control oocytes. However the difference was not significant (*P*>0.05) between BCB⁻ and control oocytes (Fig. 2).

Experiment II: Effect of G6PDH-activity on meiotic competence

At 24 h after IVM, 87, 95 and 110 oocytes were used to determine the nuclear status of BCB⁺, BCB⁻ and control groups, respectively. The data for meiotic competence after IVM is presented in Table 2. Our results showed that more (*P*<0.05) oocytes of BCB⁺ and control groups reached MII

Fig. 2 Effect of glucose-6-phosphate dehydrogenase (G6PDH) activity in goat oocytes on intercellular GSH level after in vitro maturation (IVM). *BCB* brilliant cresyl blue. ^{a,b}Bars with different letters represent groups that are significantly different ($P<0.05$)

stage compared to BCB^- group. In contrast, the rate of oocytes with MI nuclear status was lower ($P<0.05$) in BCB^+ and control groups compared to the BCB^- group. Moreover, a lower ($P<0.05$) rate of oocytes from BCB^- and control groups showed GVBD nuclear status compared to the BCB^+ group. However, there was no difference ($P>0.05$) among the BCB^+ , BCB^- and control groups in terms of the rate of oocytes with GV nuclear status.

Experiment III: Effect of G6PDH-activity on in vitro development after parthenogenetic activation

After maturation in vitro, 125, 118 and 145 oocytes were activated for BCB^+ , BCB^- and control groups, respectively. Data for cleavage rate and development to blastocysts are presented in Table 3. Regarding the rate of cleavage, there was no difference ($P>0.05$) among BCB^+ , BCB^- and control groups. However, blastocyst rate of BCB^+ oocytes was higher ($P<0.05$) as compared to both the BCB^- and control groups. In addition, control group yielded more ($P<0.05$) blastocyst compared to the BCB^- group.

Table 2 Association between G6PDH-activity and nuclear status in goat oocytes after IVM (cumulative results of five replicates)

Group	No. oocytes	Nuclear status			
		GV % (n)	GVBD % (n)	MI % (n)	MII % (n)
BCB^+	87	4.6 (4) ^a	1.1 (1) ^a	24.1 (21) ^a	70.1 (61) ^a
BCB^-	95	10.5 (10) ^a	7.3 (7) ^b	31.5 (30) ^b	50.2 (48) ^b
Control	110	3.6 (4) ^a	9.1 (10) ^b	13.6 (15) ^b	73.6 (81) ^a

Different superscripts within the columns indicate a significant difference ($P<0.05$)

Experiment IV: Effect of G6PDH-activity on expression of apoptosis-related genes

Two apoptosis-related (*Bax* and *Bcl-2*) genes were analyzed by quantitative real-time PCR in mature oocytes, their surrounding cumulus cells, and in blastocysts after screening the oocytes using BCB staining test. At matured stage, the relative transcript abundance of *Bax* gene was not different ($P>0.05$) between control and both BCB^+ , and BCB^- oocytes. In addition, no difference ($P>0.05$) also in *Bax* transcript levels was observed among BCB^+ and BCB^- oocytes. The expression of *Bcl-2* gene was lower ($P<0.05$) for BCB^+ oocytes when compared with control oocytes. However, the difference was not significant ($P>0.05$) for the expression of *Bcl-2* gene when comparison was made between BCB^- and control oocytes (Fig. 3). Moreover, no difference ($P>0.05$) in relative transcript abundance of *Bcl-2* gene was observed between BCB^+ and BCB^- oocytes.

In cumulus cells, expression of *Bax* and *Bcl-2* genes was lower ($P<0.05$) for both BCB^+ and BCB^- cumulus cells when compared with control cumulus cells. In this regard, expression of both *Bax* and *Bcl-2* genes was also decreased ($P<0.05$)

Table 3 Association between G6PDH-activity and embryo development for matured goat oocytes subjected to parthenogenetic activation (cumulative results of three replicates)

Group	No. oocytes	Embryo development	
		Cleaved % (n)	Blastocyst % (n)
BCB^+	125	94.4 (118) ^a	47.4 (56) ^a
BCB^-	118	80.5 (95) ^b	11.5 (11) ^b
Control	145	88.9 (129) ^b	27.9 (36) ^c

Different superscripts within the columns indicate a significant difference ($P<0.05$)

Fig. 3 Relative mRNA expression of *Bax* and *Bcl-2* genes for mature oocytes selected on their glucose-6-phosphate dehydrogenase (G6PDH) activity. *BCB* brilliant cresyl blue. *Asterisk shows significant difference between the groups ($P<0.05$)

in BCB^+ cumulus cells when comparison was made between BCB^+ and BCB^- group (Fig. 4).

Our data showed that, the transcript level of *Bax* gene in blastocyst was not different ($P>0.05$) between BCB^+ , BCB^- , and control blastocysts. Our findings showed that expression of *Bcl-2* mRNA was increased ($P<0.05$) in blastocysts derived from BCB^+ oocytes in comparison with BCB^- and control blastocysts. However, as shown in Fig. 5, expression of *Bcl-2* mRNA in BCB^- blastocyst was decreased ($P<0.05$) compared with the control blastocysts. An increase ($P<0.05$) of *Bcl-2* mRNA was also observed when BCB^+ blastocysts compared with BCB^- blastocysts.

Discussion

Using BCB staining test present study expands upon the findings that oocytes with low G6PDH-activity (BCB^+) are

more competent than those of high enzyme activity (BCB^-). Previous reports have suggested the importance of G6PDH-activity during follicular growth and maturation in vitro by studying different species [8–12, 15, 16, 26, 28–31]. For instance, Rodríguez-González et al. (2002) using pre-pubertal goat as an experimental animal concluded that BCB test is a useful way to select more competent oocytes for embryo production in vitro [12].

Thereafter, numerous studies [8–12, 28–30, 32–35] including our own [14, 16] have paid much interest to this method for assessing oocyte quality and evaluating the outcome of in vitro fertilization. However, the predictive value of the BCB approach used in these studies is still controversial [36]. Therefore, it is still too early to apply the BCB tool in human ARTs and further experimental studies in animal model are needed to clarify whether the method is generally applicable.

Evidence presented in this study suggests that G6PDH-activity in goat oocyte is associated with meiotic competence.

Fig. 4 Relative mRNA expression of *Bax* and *Bcl-2* genes for cumulus cells of mature oocytes selected on their glucose-6-phosphate dehydrogenase (G6PDH) activity. *BCB* brilliant cresyl blue. *Asterisk shows significant difference between the groups ($P<0.05$)

Fig. 5 Relative mRNA expression of *Bax* and *Bcl-2* genes for blastocysts derived from oocytes that were selected on their glucose-6-phosphate dehydrogenase (G6PDH) activity. BCB brilliant cresyl blue. *Asterisk shows significant difference between the groups ($P<0.05$)

Our data showed that more oocytes from BCB⁺ group reached MII stage compared with BCB⁻ group, but not in comparison to those in the control group. This pattern of findings is in accordance with previous studies showing that activity of G6PDH is more strongly linked with nuclear maturation during culture in vitro [14, 16, 37, 38]. It has been speculated that BCB⁺ oocytes acquire better cytoplasmic maturation during the final phases of folliculogenesis and this is why the performance for this subgroup of oocyte is far better than BCB⁻ oocytes [8, 32, 39]. As concluded in a previous report [40], only oocytes that completed their cytoplasmic maturation are of sufficient quality, and thus able to complete normal developmental processes successfully.

Although completion of meiosis, or nuclear maturation is of great importance, there are a variety of other processes occurring within the cytoplasm of the oocyte that are required for complete developmental competence following fertilization [41]. Successful completion of these events is independent of nuclear maturation and is collectively referred to as cytoplasmic maturation [42]. Therefore, in this study, the higher nuclear maturation recorded for BCB⁺ oocytes compared to those of BCB⁻ oocytes compel the question whether differences associated with G6PDH-activity can be reflected in cytoplasmic maturation as well. It has been shown that glutathione is one of the key indices of cytoplasmic maturation. GSH is well known to play an important role in protecting cells against the destructive effects of reactive oxygen species, and to regulate protein and DNA synthesis by altering redox status [24]. Our data support the concept that the lower activity of G6PDH found in BCB⁺ oocytes is associated with increased content of intracellular GSH in comparison with BCB⁻ and control groups. To the best of our knowledge, using epifluorescence microscopy, our study is the first to show such an association between G6PDH-

activity and intracellular GSH content, although this relationship has been shown in a previous study by extraction of the cytoplasmic glutathione, in mice model [39].

To evaluate the effect of G6PDH-activity on developmental competence of goat oocytes, COCs were categorized based on their cytoplasmic coloration, followed by IVM, parthenogenetic activation, and subsequent culture of the produced embryos up to the blastocyst stage. As expected cleavage rate and blastocyst yield were higher in the BCB⁺ group compared with the BCB⁻ and control groups. These results are in accordance with several previous studies [8–12, 14, 16, 30, 39] demonstrated that oocytes with high G6PDH-activity (BCB⁺) have higher developmental competence than those with low enzyme activity (BCB⁻). The most plausible explanation for this finding is that COCs with a high G6PDH-activity had better cytoplasmic maturation and were thereby better able to support embryo development [8, 9, 14, 16, 39].

Apoptosis is a normal phenomenon during oogenesis and are evidently dependent upon the actions and interactions of a subset of multiple gene products [43]. Among these genes, members of *Bcl-2* family are small α -helical proteins that can be either pro-apoptotic or anti-apoptotic [44]. Expression of *Bax* (as pro-apoptotic) gene and *Bcl-2* (as anti-apoptotic) gene were investigated in this study to associate the activity of G6PDH with apoptosis in mature oocytes, their surrounding cumulus cells, and in blastocyst. At mature stage, whereas expression of *Bax* was found not to be different between groups, the *Bcl-2* gene showed lower expression in BCB⁺ oocytes compared with the control oocytes. This is in great accordance with previous study which reported the higher transcript level for *Bcl-2* in mature control oocytes in compare with BCB⁺ oocytes [34].

Our results for *Bcl-2* gene in cumulus cells quite aligned with the results of this gene in mature oocytes. In this regard,

not only the expression of *Bcl-2* gene was lower for BCB⁺ cumulus cells, but also *Bax* mRNA was decreased in BCB⁺ cumulus cells when compared with control and BCB⁻ cumulus cells. In contrast to this study, Janowski et al. (2012) did not observe any differences in the expression of apoptosis-related genes belonging to the *Bcl-2* or caspase gene family between the BCB groups [45]. While these authors believe that quantifying the expression of apoptotic genes in general has little significance for apoptosis detection, in accordance to our finding, Li et al. (2008) in their study concluded that expression abundance of the *Bax* and *Bcl-2* genes are good markers for oocyte apoptosis and subsequent embryo development [46]. At the blastocyst stage, results of this study also showed that *Bcl-2* mRNA was increased in BCB⁺ blastocysts compared with the control and BCB⁻ blastocysts, and expression of this gene was decreased in BCB⁻ blastocysts in comparison with control blastocysts. To the best of our knowledge, current report is the first to document this relationship in blastocyst. Furthermore, studies are currently underway in the authors' laboratory to evaluate the functional consequences of this change in *Bcl-2* and *Bax* mRNA following BCB test which might explain the existed discrepancy among the different studies.

In conclusion, we have used an in vitro embryo production system in the goat to examine the effects of G6PDH-activity on developmental potential of parthenogenetically activated oocytes. Screening goat oocytes with BCB staining test improved the ability of oocytes to reach to full maturation (MII) stage. After IVM, the more competent oocytes (BCB⁺) presented higher intracellular GSH level and capacity to develop to the blastocyst stage. Relative expression of *Bcl-2* and *Bax* in mature oocytes, their surrounding cumulus cells, and in blastocyst was influenced by G6PDH-activity in immature oocytes. The BCB staining test may offer a great benefit in the identification of oocytes with superior developmental capacity. However, this conclusion needs more experimental evidence to support, if this promising approach has to be used in human clinical protocols.

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