—Original—

In vitro embryo production of *Bos taurus* oocytes treated with brilliant cresyl blue (BCB) from small and large follicles

Jose L. Rodríguez[1, 2], Miguel Betancourt[3], Fahiel Casillas[4], Socorro Retana-Márquez[4], Eduardo Casas[3], and Filiberto Fernández[2]*

[1] *Biological and Health Sciences PhD Program, Metropolitan Autonomous University, Mexico City 04960, Mexico*
[2] *Department of Agricultural and Animal Production, Division of Biological and Health Sciences, Metropolitan Autonomous University-Xochimilco, Mexico City 04960, Mexico*
[3] *Department of Health Sciences, Division of Biological and Health Sciences, Metropolitan Autonomous University-Iztapalapa, Mexico City 09340, Mexico*
[4] *Department of Biology of Reproduction, Division of Biological and Health Sciences, Metropolitan Autonomous University-Iztapalapa, Mexico City 09340, Mexico*

Abstract: Oocyte quality is important for *in vitro* embryo production success. Morphological features such as follicular size, oocyte diameter, cytoplasm appearance, and zona pellucida integrity determine oocyte's developmental competence. Evaluations at the meiotic stage, and of metabolic state and viability have also been performed. Therefore, the aim of this study was to elucidate if *Bos taurus* oocytes from follicles of different sizes, identified by the brilliant cresyl blue staining (BCB), show improved *in vitro* embryo development. Oocytes from small (2.0–4.9 mm) and large (5.0–10.0 mm) follicles were classified according to their cytoplasm appearance, blue (BCB+) and colorless (BCB−), and by measuring their diameters. The analyzed parameters were viability before maturation and after fertilization, assessment of the meiotic stage, embryo development and blastocyst formation, and the results for BCB+ and BCB− oocytes from small and large follicles were compared. The results varied according to the oocyte classification, oocyte from small follicles showed an increase in blastocyst production. We conclude that BCB staining allows the select on of oocytes from small follicles that are competent of reaching the blastocyst stage in *Bos taurus*.

Key words: Brilliant cresyl blue, Oocyte viability, Oocyte diameter, Meiosis stage, *In vitro* embryo production

Received: August 4, 2018
Accepted: October 16, 2018
*To whom correspondence should be addressed.
e-mail: freyes@correo.xoc.uam.mx

Introduction

The evaluation of oocyte quality is critical for *in vitro* embryo production success. It is important to identify oocytes with developmental competence. This is commonly performed by assessment of morphological features [1], such as the oocyte diameter and follicular size [2, 3]; evaluations at the cellular level of the meiotic stage [4], metabolic activity [5] and viability have also been performed [6].

Morphologic assessment of oocyte quality is based on the thickness of the *cumulus oophorus* and cytoplasm homogeneity [7]. However, oocyte selection based on morphological characteristics provides an imprecise criterion of choice [8]. Oocytes with normal morphological appearance can be found at an early stage of degeneration [9].

The follicular size is another parameter used to identify oocytes with developmental capacity. It has been reported that follicular growth is a key process, because the oocytes of large follicles (LF) have greater developmental capacity than those obtained from small follicles (SF) [1, 3, 10]. However, it has also been reported that bovine follicles of the same follicular size exhibit different physiological stages [11]. Other studies, performed with bovine, goat and sheep oocytes, have reported an association between oocyte diameter and blastocyst development [12]. Bovine blastocyst production increases in full-grown oocytes with diameters greater than 120 μm compared those with diameters of 115 μm [13]. When oo-

cytes complete growth, they achieve a metabolic state considered more competent for *in vitro* maturation (IVM) and *in vitro* fertilization (IVF), therefore, identification of competent oocytes is a critical point for *in vitro* embryo development (IVED). The brilliant cresyl blue (BCB) technique for competent oocyte identification has been described [7]. BCB is a non-invasive intracellular dye used to identify oocytes that have completed their growth. It is an electron acceptor and it has been reported as an indicator of the glucose-6-phosphate dehydrogenase (G6PDH) activity [1, 5]. In oocytes that have completed their growth, the activity of G6PDH and the production of reduced nicotinamide adenine dinucleotide phosphate (NADPH) decreases, consequently, BCB cannot be reduced and the oocyte cytoplasm staining remains blue (BCB+). In oocytes that continue to grow, G6PDH activity and NADPH production is intense, and they reduce BCB, rendering the oocyte cytoplasm colorless (BCB−) [3].

It has been reported that BCB+ oocytes in both, bovine and buffalo, are larger in diameter [12, 14], and that they are capable of producing a greater number of embryos and blastocysts [8, 14]. Blastocyst production from BCB+ oocytes has been reported to be significantly better than that from control [8, 14–16]. However, it has also been reported that no differences were found between the production of blastocysts from BCB+ bovine oocytes and controls [17–19]. These conflicting results, in terms of the BCB staining efficacy in identifying competent oocytes, could be due to differences between the bovine species (*Bos indicus* vs. *Bos taurus*) investigated. One study reported blastocysts production of 28% from BCB+ oocytes obtained from the crossbred *B. taurus* x *B. indicus*; however, this value was not significantly different from that of the control (25%) [17]. Consequently, the bovine species could constitute a key factor in the identification of high-quality oocytes.

Worldwide *B. taurus* is an economically important species due to its higher production of milk and meat compared to *B. indicus* [20]. Milk production in Mexico was 11 billion liters in 2017 [21], and approximately 50.6% of is obtained from *B. taurus* [22]. For this reason, to preserve these species characteristics, crosses of *B. taurus* and *B. indicus* have been made. Oocytes from *B. indicus* have better quality than those from *B. taurus* mainly due to major adaptation characteristics. Besides, *B. indicus* has thermotolerant genes which protect their reproduction features of increased fertilization and blastocyst formation [23]. Another factor to be considered is that geographical distribution of *B. Indicus* is in Mexico's south-east, and that of *B. taurus* is in the north and center. It has been reported that *B. taurus* has greater sensitivity to high temperatures, i.e. heat stress, and this affects follicular growth, follicle number, steroidogenesis and gap junctions between the oocyte and cumulus cells [23]. These factors may lead to infertility; therefore, this species has become highly important in terms of the study of IVF efficiency. It has been reported that higher blastocyst rates were obtained in *B. indicus* than in *B. taurus* (36% vs. 12%, respectively) [24]. Most studies reporting high blastocyst rates were performed on *B. indicus*, but in several studies, the animal species was not specified. Therefore, the aim of this study was to elucidate if *B. taurus* oocytes from follicles of different sizes, identified by BCB staining, show increased IVED rates.

Materials and Methods

This study was approved under the regulations of the Committee for the Care and Use of Animals, Metropolitan Autonomous University-Xochimilco. The ovaries were obtained from *B. taurus* cows at the Temamatla slaughterhouse, State of Mexico, under the approval of the animal health federal law with the number: 025575-R. Frozen semen was obtained from one commercial Holstein bull (Semex, México).

Unless otherwise stated, all chemicals were purchased from Sigma-Aldrich (St. Louis, MO, USA). The incubation conditions for all processes were 38.5°C with an atmosphere of 5% CO_2, 95% air, and saturation humidity.

Oocyte collection

Ovaries were obtained from *B. taurus* (Holstein) cows and transported to the laboratory in a solution consisting of 0.157 M NaCl at 37°C supplemented with antibiotics: 7.5 IU/ml ampicillin, 7.5 *μ*g/ml streptomycin, and 0.015 *μ*g/ml amphotericin. The follicles were classified using a Vernier caliper in to small (SF; 2.0–4.9 mm) and large (LF; 5.0–10.0 mm). The follicular fluid was aspirated with Tyrode's modified medium, supplemented with 10 mM sodium lactate, 0.50 mM HEPES buffer, and 0.01% polyvinyl alcohol (TL-HEPES-PVA), with a pH of 7.3–7.4 [6].

Brilliant cresyl blue stain

Oocyte-cumulus cells complexes (COCs) from the two follicles sizes were exposed to BCB (26 *μ*M) diluted in modified Dulbecco's phosphate-buffered saline (mD-PBS), supplemented with 0.4% bovine serum albumin (BSA) for 90 min. COCs were classified according to the color of the cytoplasm: blue (BCB+) and colorless (BCB−) [1, 3].

Oocyte diameter assessment

To determine the diameter of the oocytes treated with the BCB, it was necessary to remove the cumulus cells. Each oocyte was photographed by a camera (Nikon COOLPIX S4) and all measures were obtained using ImageJ, image processing program [25], considering the center and two perpendicular axes, including the zona pellucida.

In vitro maturation of BCB+, BCB− and control oocytes

Both BCB treated and control oocytes were washed three times in TCM-199 supplemented with 26.2 mM sodium bicarbonate, 3.05 mM D-glucose, 0.91 mM sodium pyruvate, 0.1% PVA, 0.57 mM cysteine and 10 ng/ml epidermal growth factor [6]. The oocytes were transferred in batches of ten to 45 μL of the maturation medium same as above supplemented with 10% fetal bovine serum (FBS) and 0.075 IU/ml of human menopausal gonadotropin, then cultured for 24 h.

In vitro fertilization sperm preparation

Thawed semen was diluted 1:3 with modified Tris-buffered medium (mTBM) containing 113.1 mM NaCl, 3 mM KCl, 7.5 mM $CaCl_2$, 20 mM Tris, 11 mM glucose, 10 mM sodium pyruvate, 60 μg/ml penicillin, and 100 μg/ml streptomycin sulphate [26]. The semen suspension was centrifuged at 500×g for 20 min [27]. The pellet was resuspended in 1 ml of mTBM supplemented with 8 mg/ml BSA and incubated for 1 h (swim up). The supernatant was recovered (0.5 ml) and diluted in mTBM to obtain a concentration of 1×10^6 sperm/ml [5].

In vitro fertilization of BCB+, BCB− and control oocytes

The cumulus cells were partially removed from the oocytes after IVM, and the oocytes were transferred in batches of ten to 45 μL of the fertilization medium: Tyrode's medium supplemented with 6 mg/ml BSA, lactate, and pyruvate (TALP). The oocytes were incubated with sperm for 21 h [18].

In vitro embryo development of BCB+, BCB− and control oocytes

The remaining cumulus cells were mechanically removed and the zygotes were co-cultured with granulosa cells in sequential media SOF1-SOF2 (In vitro, Mexico) for 168 h [28]. SOF1 (50 μL) was used for 48 h and SOF2 (50 μL) for the remaining 120 h. The culture media were supplemented with 10% FBS [28]. Cleavage was evaluated after 72 h of incubation and blastocyst production at 168 h.

Assessment of the viability and meiotic stage

A double stain was performed. Thiazolyl blue solution (0.5 mg/ml) exposure for 2 h was used to determine the oocyte viability. Oocytes with violet cytoplasm was considered viable, and colorless ones were considered non-viable [6]. A second staining, Hoechst (40 μg/ml) diluted in PBS for 45 min, was performed to assess the meiotic stage: germinal vesicle (GV), metaphase I (MI), metaphase II (MII) [7] and fertilized (F), which was determined by the presence of male and female pronuclei. MII plus fertilized oocytes were considered mature. Observations were made at 400 x under an epifluorescence microscope (Nikon Eclipse E600).

Statistical analysis

To compare the viability, meiotic stage, IVED, and blastocyst rates among the oocytes groups from both follicles sizes, data were treated as non-parametric and analyzed using a multiple comparison Duncan test and Number Cruncher Statistical Software (NCSS[11]). Percentage data are presented as mean ± SD values. Differences were considered significant when $P < 0.05$.

Experimental design

Four experiments were performed to determine the viability, meiotic stage, oocyte diameter, IVM, IVF and IVED of BCB−treated oocytes from small (SF; 2.0–4.9 mm) and large (LF; 5.0–10.0 mm) follicles and untreated controls.

Experiment 1. Determination of the diameter of oocytes treated with BCB.

The diameters of 463 BCB−treated oocytes were measured (4 replicates) of which 322 were obtained from SF and 141 from LF.

Experiment 2. Assessment of the viability of oocytes before IVM and after IVF.

For oocyte viability before IVM and after IVF, a total of 406 BCB−treated oocytes were evaluated with thiazolyl blue solution (MTT) (4 replicates) before IVM: 263 and 143 from SF and LF, respectively. For the control, a total of 166 oocytes were evaluated (4 replicates) of which 96 and 70 oocytes were obtained from SF and LF, respectively. After IVF, 389 BCB−treated oocytes were evaluated (6 replicates) of which 275 and 114 were from SF and LF, respectively. For the control, 138 oocytes were evaluated (6 replicates): 103 and 35 oocytes from SF and LF, respectively.

Table 1. Results of BCB staining of oocytes from the two sizes of follicles

Follicle Size	Total n	Group	n (% ± SD)
Small	1,053	BCB+	221 (21 ± 17)[a]
		BCB−	832 (79 ± 19)[b]
Large	467	BCB+	220 (47 ± 18)[c]
		BCB−	247 (53 ± 21)[c]

n: number of oocytes. The percentages were calculated from number of oocyte analyzed. Different superscripts indicate a significant difference ($P < 0.05$).

Table 2. Diameters of BCB+ and BCB− oocytes from the two follicles sizes

Follicle Size	Total n	Group	n ($\overline{X}$ ± SD)
Small	322	BCB+	61 (154 ± 7)
		BCB−	261 (151 ± 7)
Large	141	BCB+	61 (153 ± 5)
		BCB−	80 (150 ± 6)

n: number of oocytes. $\overline{X}$ ± SD: oocyte diameter (μm) average ± SD. The diameters showed no significant differences among the groups ($P > 0.05$).

Table 3. Viability before IVM and after IVF of oocytes from the two follicles sizes

Follicle Size	Group	Before IVM		After IVF	
		n	Viability n (% ± SD)	n	Viability n (% ± SD)
Small	Control	96	92 (95 ± 9)	103	97 (94 ± 11)
	BCB+	50	44 (88 ± 11)	53	45 (85 ± 15)
	BCB−	213	204 (96 ± 4)	222	199 (89 ± 11)
Large	Control	70	68 (97 ± 4)	35	31 (88 ± 13)
	BCB+	71	70 (98 ± 4)	61	54 (88 ± 13)
	BCB−	72	70 (97 ± 3)	53	45 (85 ± 21)

n: number of oocytes. The percentages were calculated from the number of analyzed oocytes. There were no significant differences ($P > 0.05$).

Experiment 3. Assessment of the meiotic stage before IVM and after IVF.

A total of 388 BCB−treated oocytes were evaluated with Hoechst stain (4 replicates) before IVM: 248 and 140 oocytes from SF and LF, respectively. For the control, 160 oocytes were evaluated with Hoechst (4 replicates): 92 and 68 oocytes from SF and LF, respectively. After IVF, 343 BCB−treated oocytes were evaluated with Hoechst (6 replicates): 244 and 99 oocytes from SF and LF, respectively. For the control, 128 oocytes (6 replicates) were evaluated: 97 and 31 oocytes from SF and LF, respectively.

Experiment 4. Evaluation of the embryo development and blastocyst formation rates.

A total of 262 BCB−treated oocytes were cultured (4 replicates) to evaluate embryo development: 193 and 69 oocytes from SF and LF, respectively. For the control, 161 oocytes were cultured (4 replicates): 87 and 74 oocytes from SF and LF, respectively. The percentage of blastocysts was calculated from the total cleavage in each group.

Results

Oocytes treated with BCB

In the present study, 1,520 oocytes were obtained, 69% from SF (n=1,053) and 31% from LF (n=467). Those treated with BCB showed a higher percentage of BCB− in both follicle sizes ($P < 0.05$). A higher percentage of BCB+ oocytes was obtained from LF that from SF ($P < 0.05$) (Table 1).

Diameters of oocytes treated with BCB

The diameters of BCB+ and BCB− oocytes from SF and LF were similar ($P > 0.05$) (Table 2).

Viability of oocytes before IVM and after IVF

The viability of BCB+, BCB− and control oocytes groups from both follicle sizes did not show differences before IVM ($P > 0.05$). Also, after IVF no differences among the groups were found ($P > 0.05$). The result of comparisons of the viability before IVM and after IVF were also similar ($P > 0.05$) (Table 3).

Meiotic stage before IVM and after IVF

The percentage of oocytes in the GV and MI stages before IVM were similar among the BCB+, BCB− and control oocytes from both sizes of follicles ($P > 0.05$).

Table 4. Meiotic stage before IVM and after IVF of viable oocytes from the two follicles sizes

Follicle Size	Group	Before IVM n (% ± SD)			After IVF n (% ± SD)				
		n	GV	MI	n	MI	MII	F	Matured*
Small	Control	92	53 (58 ± 12)	39 (42 ± 12)	97	21 (21 ± 19)^{a}	25 (25 ± 16)^{a}	51 (53 ± 32)^{a}	76 (78 ± 19)^{a}
	BCB+	44	28 (64 ± 22)	16 (36 ± 22)	45	4 (9 ± 6)^{b}	6 (13 ± 0)^{b}	35 (78 ± 18)^{b}	41 (91 ± 6)^{b}
	BCB−	204	134 (66 ± 10)	70 (34 ± 10)	199	28 (14 ± 14)^{b}	57 (28 ± 17)^{a}	114 (57 ± 22)^{a}	171 (85 ± 14)^{b}
Large	Control	68	44 (65 ± 15)	24 (35 ± 15)	31	3 (10)^{b}	7 (22 ± 8)^{a}	21 (68 ± 8)^{c}	28 (90 ± 15)^{b}
	BCB+	70	42 (60 ± 10)	28 (40 ± 5)	54	7 (13 ± 10)^{b}	13 (24 ± 23)^{a}	34 (63 ± 27)^{c}	47 (87 ± 13)^{b}
	BCB−	70	42 (60 ± 20)	28 (40 ± 20)	45	5 (11 ± 8)^{b}	8 (18 ± 8)^{a}	32 (71 ± 16)^{b}	40 (89 ± 9)^{b}

n: number of oocytes. Meiotic stage: germinal vesicle stage (GV), metaphase I (MI), metaphase II (MII), and fertilized (F), which was identified by the presence of male and female pronuclei. *The MII plus fertilized oocytes were considered matured. The percentage was calculated from the number of analyzed oocytes. Different superscripts indicate a significant difference ($P < 0.05$).

Table 5. Embryo and blastocyst development rates of oocytes from the two follicles sizes

Follicle Size	Group	n	Cleavage n (% ± SD)	Blastocyst/Cleavage n (% ± SD)
Small	Control	87	50 (57 ± 6)^{a}	8 (16 ± 1)^{a}
	BCB+	57	35 (61 ± 20)^{a}	7 (26 ± 21)^{b}
	BCB−	136	73 (54 ± 15)^{b}	9 (12 ± 4)^{a}
Large	Control	74	47 (64 ± 19)^{a}	7 (24 ± 15)^{b}
	BCB+	27	17 (63 ± 21)^{a}	3 (21 ± 11)^{b}
	BCB−	42	32 (76 ± 13)^{a}	5 (25 ± 12)^{b}

n: number of oocytes. Different superscripts indicate a significant difference ($P < 0.05$).

After IVF, control oocytes from SF displayed higher MI rates compared to BCB+ and BCB− oocytes ($P < 0.05$). All the oocyte groups from LF had similar MI rates ($P > 0.05$). Percentages of matured oocytes (MII + F) from SF were significantly higher in the BCB+ and BCB− groups compared to the control; however, no significant differences were found among the oocyte groups from LF ($P > 0.05$). Concerning fertilization, in oocytes from SF, fertilization rates were higher in the BCB+ group compared to the control and BCB− groups ($P < 0.05$). However, in oocytes from LF, fertilization rates were higher in the BCB− group compared to the BCB+ and control groups ($P < 0.05$) (Table 4).

Embryo and blastocyst development

The embryo cleavage rates of the BCB+ and control oocytes from SF showed no difference ($P > 0.05$), but they were higher compared to the BCB− group ($P < 0.05$). On the other hand, the embryo cleavage rates of BCB+, BCB− and control group oocytes from LF were similar ($P > 0.05$) (Table 5).

The blastocyst development rates of the BCB+ oocytes from SF were higher than those of the control and BCB− group oocytes ($P < 0.05$). The blastocyst development rates of LF oocytes were similar among the BCB+, BCB− and control groups ($P > 0.05$) (Table 5).

Discussion

Reproductive technologies are important for the development of the livestock industry, especially that of *B. taurus* cows [5]. However, research results regarding efficacy BCB staining have been contradictory. Some studies did not specify the animal species studied [1, 8, 29, 30] and some were carried out on *B. indicus* [5, 24, 31] exclusively; therefore, it is difficult to draw definitive conclusions.

The genotype is an important aspect to be considered, because there are differences in the follicular development between *B. indicus and B. taurus*. During follicular recruitment, *B. indicus* cows recruit a greater number of follicles per follicular wave than *B. taurus* [32]; therefore, the number of oocytes recovered under hormonal treatment is higher in *B. indicus* than in *B. taurus* [24]. On the other hand, it is known that the quality of oocytes from *B. taurus* decreases in the summer [33]. Therefore, these characteristics should be considered when evaluating the developmental capacity of oocytes [5].

In the present study, a greater number of *B. taurus* oocytes were obtained from SF, but only 21% of them were BCB+. This suggests that oocytes from SF continue in the growth phase. In LF, fewer oocytes were recovered and no significant differences in their characteris-

tics were found between the BCB+ and BCB− groups, although higher BCB+ oocyte rates were obtained from LF compared to SF. It is clear that oocytes from LF would possess better developmental capacity because follicular growth is essential for oocyte maturation [34]. In agreement with our results, Karami-Shabankareh *et al.* [1] reported that bovine oocytes showed no correlation between the BCB+ percentage and follicular size.

In *B. taurus* and buffalo, it has been demonstrated that BCB+ oocytes are larger in diameter than BCB− oocytes [12, 35]. However, the present study found that the diameter was similar in BCB+ and BCB− oocytes obtained from both follicle sizes. In agreement with this, Castaneda et al. [25] reported the same. Also, some studies have demonstrated that bovine oocytes from 3 mm follicles have completed their growth, therefore they do not increase in diameter in relation to the follicular development [29, 34]. Accordingly, the diameter of the *B. taurus* oocytes, as a parameter of quality, cannot be used as a reliable method to predict the developmental capacity.

The meiotic stage before IVM is related to developmental capacity [36]. In the present study, GV oocyte rates were similar in BCB+, BCB− and control oocytes from both sizes of follicles. Another study of buffalo also reported that GV percentages of BCB+, BCB− and control oocytes from three follicles sizes were similar [7].

The ovulation of matured oocytes with the ability to carry out normal embryo development is the end point of *in vivo* maturation [37]. The results of the present study show that the maturation rates of BCB+ and BCB− oocytes from both SF and LF were similar. These results demonstrate that BCB staining does not increase the MII proportion of oocytes. Some studies have reported great similarities in the MII proportions of BCB+ and control oocytes [16, 38, 39, 40]. In controls, the maturation rate was slightly reduced, only in oocytes form SF, as reported by Manjunatha et al. [35] in buffalo.

Results concerning the efficacy of BCB staining of bovine oocytes are controversial. In the present study, the fertilization rate increased in BCB+ oocytes from SF (78%) and BCB− oocytes from LF (71%). It has been reported that BCB− bovine oocytes have higher fertilization rates than BCB+ [41]. Also, BCB+ buffalo oocytes have been reported to have higher fertilization rates than BCB− oocytes [35]. Another study of *B. taurus* oocytes reported a fertilization rate of 48%, lower than that seen in the present study [23].

The embryo cleavage rate of oocytes from SF were higher in the BCB+ and control groups than in the BCB− group; therefore, blue BCB staining identified the oocytes in SF with the greatest capacity for development. Some studies have reported similar results for *B. taurus* [42] and *B. indicus* [5]. With regard to oocytes from LF, BCB staining did not identify those with greater development capacity, as was previously reported by Karami-Shabankareh *et al.* [1].

The blastocyst production was higher in BCB+ (26%) than in BCB− (12%) oocytes from SF. Besides, the blastocyst production rate of BCB+ oocytes was similar to that of BCB+ oocytes (21%) from LF. These results agree with those reported by Muasa et al. [5] for *B. indicus*. Blastocyst production was similar in BCB+ and BCB− oocytes from LF. A likely explanation for this is that in LF, oocytes are selected naturally *in vivo* by endocrine and paracrine factors for follicular recruitment [43–45].

In summary, BCB staining of oocytes from SF increased blastocyst production by allowing the selection of competent oocytes that will reach the blastocyst stage. In LF, since oocytes have been selected naturally (by means of ovarian folliculogenesis), no significant differences were observed in blastocyst production rates among the groups. However, fewer oocytes were recovered from LF, resulting in fewer oocytes available for IVF procedures.

The results of this study suggest that selecting oocytes from SF, that are positively stained by BCB, increases the blastocyst rates in *B. taurus*.

Conflict of Interest

The authors declare no conflict of interest.

Acknowledgments

The authors thank the Temamatla slaughterhouse, State of Mexico, for the donation of the ovaries. This publication represents the fulfillment of the requirements for the degree of Doctor in Biological Sciences and Health for José Luis Rodríguez-Suastegui. Thanks to the CONACYT for the scholarship granted to José Luis Rodríguez-Suástegui (224661).

References

1) Karami-Shabankareh, H., Azimi, G. and Torki, M. (2014): Developmental competence of bovine oocytes selected based on follicle size and using the brilliant cresyl blue (BCB) test. Iran. J. Reprod. Med., 12, 771–778. [Medline]
2) Heleil, H., Kuzmina, T., Novikova, N., Torner, H. and Alm, H. (2010): Effect of prolactin on developmental competence of bovine oocytes selected by brilliant cresyl blue staining. J. Reprod. Infertil., 1, 1–7.
3) Ghoneimy, K., El-Naby, A., Elraay, M., Youssef, Y., Sosa,

G. and Abou El-Roos, M. (2017): Impact of using brilliant cresyl blue stain on oocyte and embryo selection. Egypt. J. Vet. Sci., 48, 43–51. [CrossRef]
4) Torner, H., Ghanem, N., Ambros, C., Hölker, M., Tomek, W., Phatsara, C., Alm, H., Sirard, M.A., Kanitz, W., Schellander, K. and Tesfaye, D. (2008): Molecular and subcellular characterisation of oocytes screened for their developmental competence based on glucose-6-phosphate dehydrogenase activity. Reproduction, 135, 197–212. [Medline] [CrossRef]
5) Muasa, S., Mutembei, M., Camargo, S. and Viana, M. (2015): Effect of follicle size of Boran Cows on Glucose 6 Phosphate Dehydrogenase activity and developmental competence. Int. J. Vet. Sci., 4, 161–165.
6) Fernández, F., Hernández, J., Romero, J. and Rodríguez, J. (2013): Viability after vitrification of sow and sheep embryos produced in vitro. Rev. Salud Anim., 35, 52–58.
7) Heleil, B. and Fayed, M. (2010): Developmental competence of buffalo oocytes from follicles of different diameters selected by brilliant cresyl blue staining. Glob. Vet., 4, 176–184.
8) Mirshamsi, S.M., Karamishabankareh, H., Ahmadi-Hamedani, M., Soltani, L., Hajarian, H. and Abdolmohammadi, A.R. (2013): Combination of oocyte and zygote selection by brilliant cresyl blue (BCB) test enhanced prediction of developmental potential to the blastocyst in cattle. Anim. Reprod. Sci., 136, 245–251. [Medline] [CrossRef]
9) Em, S., Kataria, M., S, B. and Yadav, P. (2014): Expression profile of developmentally important genes between hand-made cloned buffalo embryos produced from reprogramming of donor cell with oocytes extract and selection of recipient cytoplast through brilliant cresyl blue staining and in vitro fertilized embryos. J. Assist. Reprod. Genet., 31, 1541–1552. [Medline] [CrossRef]
10) Lonergan, P., Monaghan, P., Rizos, D., Boland, M.P. and Gordon, I. (1994): Effect of follicle size on bovine oocyte quality and developmental competence following maturation, fertilization, and culture in vitro. Mol. Reprod. Dev., 37, 48–53. [Medline] [CrossRef]
11) Sugulle, A., Dochi, O. and Koyama, H. (2008): Developmental competence of bovine oocytes selected by brilliant cresyl blue staining: effect of the presence of corpus luteum on embryo development. J. Mamm. Ova Res., 25, 50–55. [CrossRef]
12) Pujol, M., López-Béjar, M. and Paramio, M.T. (2004): Developmental competence of heifer oocytes selected using the brilliant cresyl blue (BCB) test. Theriogenology, 61, 735–744. [Medline] [CrossRef]
13) Otoi, T., Yamamoto, K., Koyama, N., Tachikawa, S. and Suzuki, T. (1997): Bovine oocyte diameter in relation to developmental competence. Theriogenology, 48, 769–774. [Medline] [CrossRef]
14) Bhardwaj, R., Ansari, M.M., Pandey, S., Parmar, M.S., Chandra, V., Kumar, G.S. and Sharma, G.T. (2016): GREM1, EGFR, and HAS2; the oocyte competence markers for improved buffalo embryo production in vitro. Theriogenology, 86, 2004–2011. [Medline] [CrossRef]
15) Silva, D.S., Rodriguez, P., Galuppo, A., Arruda, N.S. and Rodrigues, J.L. (2013): Selection of bovine oocytes by brilliant cresyl blue staining: effect on meiosis progression, organelle distribution and embryo development. Zygote, 21, 250–255. [Medline] [CrossRef]
16) Alm, H., Torner, H., Löhrke, B., Viergutz, T., Ghoneim, I.M. and Kanitz, W. (2005): Bovine blastocyst development rate in vitro is influenced by selection of oocytes by brillant cresyl blue staining before IVM as indicator for glucose-6-phosphate dehydrogenase activity. Theriogenology, 63, 2194–2205. [Medline] [CrossRef]
17) Mota, G.B., Batista, R.I., Serapião, R.V., Boité, M.C., Viana, J.H., Torres, C.A. and de Almeida Camargo, L.S. (2010): Developmental competence and expression of the MATER and ZAR1 genes in immature bovine oocytes selected by brilliant cresyl blue. Zygote, 18, 209–216. [Medline] [CrossRef]
18) Opiela, J., Lipiński, D., Słomski, R. and Katska-Ksiazkiewicz, L. (2010): Transcript expression of mitochondria related genes is correlated with bovine oocyte selection by BCB test. Anim. Reprod. Sci., 118, 188–193. [Medline] [CrossRef]
19) Fakruzzaman, M., Bang, J.I., Lee, K.L., Kim, S.S., Ha, A.N., Ghanem, N., Han, C.H., Cho, K.W., White, K.L. and Kong, I.K. (2013): Mitochondrial content and gene expression profiles in oocyte-derived embryos of cattle selected on the basis of brilliant cresyl blue staining. Anim. Reprod. Sci., 142, 19–27. [Medline] [CrossRef]
20) Arce, C., Aranda, E., Osorio, M., González, R., Díaz, P. and Hinojosa, J. (2017): Productive and reproductive parameters in dual-purpuse Zebu x Holstein cattle in Tabasco, Mexico. Rev. Mex. De Cienc. Pecu., 8, 83–91.
21) SAGARPA (2018): Milk Production from México in the 2017. (Online). April 26, 2018. http://www.numerosdelcampo.sagarpa.gob.mx/publicnew/productosPecuarios/cargarPagina/5.
22) Villamar, A.L. and Olivera, C.E. (2005): Situación actual y perspectiva de la producción de leche de Bovino en México. (Online) April 29, 2018. https://www.inforural.com.mx/leche-sistemas-de-produccion/.
23) Báez, F., Chávez, A., Hernández, H. and Villamediana, P. (2010): Evaluation of in vitro development capacity of bovine oocytes obtained from predominantly *Bos taurus* and *Bos indicus* cows. Rev. Cient., 20, 259–267.
24) Sales, J.N., Iguma, L.T., Batista, R.I., Quintão, C.C., Gama, M.A., Freitas, C., Pereira, M.M., Camargo, L.S., Viana, J.H., Souza, J.C. and Baruselli, P.S. (2015): Effects of a high-energy diet on oocyte quality and in vitro embryo production in *Bos indicus* and *Bos taurus* cows. J. Dairy Sci., 98, 3086–3099. [Medline] [CrossRef]
25) Castaneda, C.A., Kaye, P., Pantaleon, M., Phillips, N., Norman, S., Fry, R. and D'Occhio, M.J. (2013): Lipid content, active mitochondria and brilliant cresyl blue staining in bovine oocytes. Theriogenology, 79, 417–422. [Medline] [CrossRef]
26) Park, K. and Niwa, K. (2009): Bovine oocytes can be penetrated in modified tris-buffered medium. Asian-Australas. J. Anim. Sci., 22, 500–506. [CrossRef]
27) Sohel, M.M., Hoelker, M., Noferesti, S.S., Salilew-Wondim, D., Tholen, E., Looft, C., Rings, F., Uddin, M.J., Spencer,

T.E., Schellander, K. and Tesfaye, D. (2013): Exosomal and non-exosomal transport of extra-cellular microRNAs in follicular fluid: implications for bovine oocyte developmental competence. PLoS One, 8, e78505. [Medline] [CrossRef]
28) Gandhi, A.P., Lane, M., Gardner, D.K. and Krisher, R.L. (2000): A single medium supports development of bovine embryos throughout maturation, fertilization and culture. Hum. Reprod., 15, 395–401. [Medline] [CrossRef]
29) Janowski, D., Salilew-Wondim, D., Torner, H., Tesfaye, D., Ghanem, N., Tomek, W., El-Sayed, A., Schellyer, K. and Holker, M. (2012): Incidence of apoptosis and transcript abundance in bovine follicular cells is associated with the quality of the enclosed oocyte. Theriogenology, 78, 656–669. [Medline] [CrossRef]
30) Su, J., Wang, Y., Li, R., Peng, H., Hua, S., Li, Q., Quan, F., Guo, Z. and Zhang, Y. (2012): Oocytes selected using BCB staining enhance nuclear reprogramming and the in vivo development of SCNT embryos in cattle. PLoS One, 7, e36181. [Medline] [CrossRef]
31) Velez, I., Chica, A., Urrego, R., Torres, V., Jimenez, C. and Zambrano, J. (2017): Producción in vitro de embriones a partir de complejos cúmulos ovocitos tipo II en bovinos *Bos indicus*. Rev. CES Med. Vet. Zoot., 12, 79–87.
32) Motta, P., Ramos, N., González, C. and Castro, E. (2011): Follicular dynamics in the reproductive life of female livestock. Vet. Zootec., 5, 89–99.
33) Camargo, L., Viana, J., Sá, W., Ferreira, A., Ramos, A. and Vale, V. (2006): Factors influencing in vitro embryo production. Anim. Reprod., 3, 19–28.
34) Iwata, H., Hashimoto, S., Ohota, M., Kimura, K., Shibano, K. and Miyake, M. (2004): Effects of follicle size and electrolytes and glucose in maturation medium on nuclear maturation and developmental competence of bovine oocytes. Reproduction, 127, 159–164. [Medline] [CrossRef]
35) Manjunatha, B.M., Gupta, P.S., Devaraj, M., Ravindra, J.P. and Nandi, S. (2007): Selection of developmentally competent buffalo oocytes by brilliant cresyl blue staining before IVM. Theriogenology, 68, 1299–1304. [Medline] [CrossRef]
36) Labrecque, R. and Sirard, M.A. (2014): The study of mammalian oocyte competence by transcriptome analysis: progress and challenges. Mol. Hum. Reprod., 20, 103–116. [Medline] [CrossRef]
37) Fulka, J. Jr., First, N.L. and Moor, R.M. (1998): Nuclear and cytoplasmic determinants involved in the regulation of mammalian oocyte maturation. Mol. Hum. Reprod., 4, 41–49. [Medline] [CrossRef]
38) Lekola, K., Ng'ambi, J., Nkadimeng, M., Mphaphathi, M. and Nedambale, T. (2016): Effects of various concentrations of gonadotropins and 17β estradiol on the in vitro maturation of cattle oocytes selected using brilliant cresyl blue staining. S. Afr. J. Anim. Sci., 46, 321–326.
39) Maia, R., Mogollón, E., Dubeibe, D., Pena, B. and Burla, A. (2017): Effect of seasonality on the quality of bovine oocytes selected by the brilliant cresyl blue method. Rev. Colomb. Cienc. Pecu., 30, 259–266. [CrossRef]
40) Otero, R., Da Costa, E. and Pereira, E. (2017): In vitro nuclear maturation of bovine oocytes selected by the brilliant cresyl blue method. Rev. Colomb. Cienc. Anim., 9, 345–354.
41) Pawlak, P., Warzych, E., Chabowska, A. and Lechniak, D. (2014): Differences in cytoplasmic maturation between the BCB+ and control porcine oocytes do not justify application of the BCB test for a standard IVM protocol. J. Reprod. Dev., 60, 28–36. [Medline] [CrossRef]
42) Rodriguez-Villamil, P., Ongaratto, F., Moreira, G., Taranco, M. and Bó, G. (2016): Vitrification of immature and matured bovine oocytes: effect of brilliant cresyl blue selection and hyaluronan addition. Anim. Reprod., 13, 42–49. [CrossRef]
43) Silva, J.R., van den Hurk, R. and Figueiredo, J.R. (2016): Ovarian follicle development in vitro and oocyte competence: advances and challenges for farm animals. Domest. Anim. Endocrinol., 55, 123–135. [Medline] [CrossRef]
44) Sirard, M.A. (2011): Follicle environment and quality of in vitro matured oocytes. J. Assist. Reprod. Genet., 28, 483–488. [Medline] [CrossRef]
45) Maldonado, J.G., Agudelo, B. and Vasquez, A. (1997): Dinámica folicular en novillas y vacas *Bos indicus* y *Bos taurus*. Rev. Colomb. Cienc. Pecu., 10, 67–75.