Original Articles

rccp Revista Colombiana de Ciencias Pecuarias

Effect of seasonality on the quality of bovine oocytes selected by the brilliant cresyl blue method¤

Efecto de la estacionalidad en la calidad de los ovocitos seleccionados por el método de azul cresil brillante

Efeito da sazonalidade na qualidade dos ovócitos bovinos selecionados pelo método azul cresil brilhante

Roger C Maia[1], MV, MSc; Edgar M Mogollón Waltero[2], MV, PhD; Diego F Dubeibe Marin[2], MV, MSc; Bruno Pena Carvalho[3], MSc, DrSc; Ángelo J Burla Dias[1*], MV, PhD.

[1]*Laboratório de Reprodução e Melhoramento Genético Animal, Universidade Estadual do Norte Fluminense Darcy Ribeiro, Campos dos Goytacazes, Brasil.*

[2]*Laboratorio de Biotecnología de la Reproducción Animal, Universidad Cooperativa de Colombia, Bucaramanga, Colombia.*

[3]*Setor de Implementação da Programação de Transferência de Tecnologia, Empresa Brasileira de Pesquisa Agropecuária (Embrapa), Rio Branco, Brasil.*

(Received: November 21, 2016; accepted: June 6, 2017)

doi: 10.17533/udea.rccp.v30n4a01

Abstract

Background: The brilliant cresyl blue (BCB) staining is a non-invasive test to select the best-suited oocytes for embryonic development. This makes it a useful tool to select best-quality oocytes at the times of the year when there is forage restriction. **Objective:** To evaluate the effect of seasonality on the nuclear maturation and quality of oocytes selected by the BCB test. **Methods:** The *cumulus-oophorus* complexes (COCs) were obtained in summer and winter of 2010 and 2011. Selected COCs were maintained for 90 min at 38.5 °C in a CO_2 incubator, in TCM 199 medium containing 10% fetal bovine serum and antibiotics, and supplemented with 26 µM brilliant cresyl blue. Afterwards, they were divided according to the ooplasm staining (BCB^+ —blue; BCB^- —unstained). Subsequently, COCs were matured for 22 h. Nuclear maturation was evaluated at 22 h of culture. **Results:** The proportion of BCB^- oocytes was higher in the winter of 2010, but there was no increase in this group in the winter of 2011. The percentage of oocytes that reached metaphase II was higher in control and BCB^+ groups in relation to oocytes from BCB^- group. **Conclusion:** The season of the year influences the percentage of oocytes best suited for embryonic production in situations in which

¤ To cite this article: Maia RC, Mogolllón EM, Dubeibe DF, Pena B, Dias, Burla AJ. Effect of seasonality on the quality of bovine oocytes selected by the brilliant cresyl blue method. Rev Colomb Cienc Pecu 2017; 30:259-266.

* Corresponding author: Ángelo José Burla Dias. Universidade Estadual do Norte Fluminense Darcy Ribeiro, Avenida Alberto Lamego, 2000 – Parque Califórnia, CCTA, LRMGA, 28035-200, Campos dos Goytacazes, Rio de Janeiro, RJ. E-mail: aburla@uenf.br.

oocyte donors receive pasture-based feeding, since the method was effective in determining the effect of seasonality on the competence of bovine oocytes to reach nuclear maturation.

Keywords: *colorimetric test*, *cumulus-oophorus complexes*, *in vitro maturation*, *pasture*, *seasons.*

Resumen

Antecedentes: La tinción con azul cresil brillante (BCB) es un método no invasivo para seleccionar ovocitos aptos para el desarrollo embrionario. Por tanto, es una herramienta útil para selecionar los ovocitos de mejor calidad en temporadas de restricción de forraje. **Objetivo:** Evaluar el efecto de la estacionalidad sobre la maduración nuclear y calidad de los ovocitos seleccionados por el test BCB. **Métodos:** Los complejos *cumulus-oophorus* (CCOs) fueron obtenidos durante el verano y el invierno de 2010 y 2011. Los CCOs seleccionados se mantuvieron durante 90 min a 38,5 ºC en una incubadora de CO_2 en un medio TCM 199 con 10% de suero fetal bovino y antibióticos, suplementado con 26 µM de azul cresil brillante. Luego se separaron según el color del citoplasma (BCB^+ —azul y BCB^- —incoloro). Posteriormente, los CCOs se maduraron durante 22 h. La evaluación de la maduración nuclear se realizó a las 22 h de cultivo. **Resultados:** La proporción de ovocitos BCB^- fue mayor en el invierno de 2010, pero no hubo un aumento de ese grupo en el invierno de 2011. El porcentaje de ovocitos que alcanzaron la etapa de metafase II fue mayor en el grupo control y BCB^+ con respecto al grupo BCB^-. **Conclusión:** La estación del año influye en el porcentaje de ovocitos más aptos para la producción de embriones en situaciones donde las donadoras de ovocitos reciben alimentación a base de pastos, ya que este método fue eficaz para determinar el efecto de la estacionalidad en la competencia de ovocitos bovinos en alcanzar la maduración nuclear.

Palabras clave: *complejos de cumulus-oophorus*, *estaciones*, *maduración in vitro*, *pastura*, *test colorimétrico.*

Resumo

Antecedentes: O método do azul cresil brilhante (BCB) não é invasivo e seleciona ovócitos mais aptos ao desenvolvimento embrionário. Portanto é ferramenta útil para selecionar ovócitos de melhor qualidade em épocas do ano que ocorre restrição de pastagem. **Objetivo:** Avaliar o efeito da sazonalidade sobre a maturação nuclear e a qualidade dos ovócitos selecionados pelo teste BCB. **Métodos:** Os complexos *cumulus oophorus* (CCOs) foram obtidos no verão e inverno de 2010 e 2011. Os CCOs selecionados foram mantidos por 90 min, a 38,5 °C, em incubadora de CO_2, em meio TCM 199 contendo 10% de soro fetal bovino e antibióticos, e suplementado com 26 µM de azul cresil brilhante. Em seguida, estes foram divididos de acordo com a coloração do citoplasma (BCB^+ —azuis e BCB^- —incolores). Então os CCOs foram maturados durante 22 h. A avaliação da maturação nuclear foi realizada às 22 h de cultivo. **Resultados:** A proporção dos ovócitos BCB^- foi maior no inverno de 2010, mas não houve aumento desse grupo no inverno de 2011. O percentual de ovócitos que atingiu o estágio de metáfase II foi maior no controle e no grupo BCB^+ em relação ao grupo BCB^-. **Conclusão:** A estação do ano influencia o percentual de ovócitos mais aptos a produção de embriões, em situações onde as doadoras de ovócitos recebem alimentação baseada em pastagens, já que o método se mostrou efetivo para determinação do efeito da sazonalidade sobre a competência de ovócitos bovinos em atingirem a maturação nuclear.

Palavras chave: *complexos de cumulus-oophorus*, *estações do ano*, *maturação in vitro*, *pastagem*, *teste colorimétrico.*

Introduction

Bovine *in vitro* embryo production (IVP) has contributed to develop the Brazilian cattle herd (Rodrigues, 2014). However, the percentage of oocytes that reach the blastocyst stage is low (35-45%). Evidence suggests the oocyte quality is the main factor for blastocyst yield, thus the best oocytes should be selected (Lonergan and Fair, 2016).

The criteria for *cumulus-oophorus* complexes (COCs) selection have been based on morphology, not on the complete evaluation of oocyte competence (Van Den Hurk and Zhao, 2004). Therefore, several studies have been looking for methods to demonstrate oocyte competence in a non-invasive manner. Currently, only invasive methods that damage the oocyte can predict its quality, precluding its use for IVP.

The search for non-invasive biomarkers was reviewed in an earlier paper (Goovaerts *et al*., 2010). The brilliant cresyl blue (BCB) staining is one of these methods. It determines glucose-6-phosphate dehydrogenase enzyme (G6PDH) activity, which is increased in growing oocytes and decreased in full-grown ones (Rodriguez-Gonzales *et al*., 2002). Thereby, oocytes with lower enzymatic activity do not degrade BCB, turning blue (BCB^+), and thus have greater developmental competence. Furthermore, researchers have identified genes that could be used as biomarkers of oocyte quality (O'Shea *et al*., 2012). Markers of oocyte quality were proposed in oocytes selected by BCB, with BCB^+ oocytes showing different gene expression profile (Ashry *et al*., 2015). Several authors (Alm *et al.,* 2005; Wu, 2007; Mirshamsi *et al.,* 2013) have evaluated selection of oocytes via BCB. In these studies, they concluded that oocytes could be stained by BCB without changing their competence and favorable results are obtained in selection of competent oocytes, when associated with morphological selection.

Some Brazilian regions have marked seasonality, characterized by temperature and humidity variations that impose different environmental and nutritional standards to cattle throughout the year (Nicoloso *et al*., 2006). Studies relating seasonality to oocyte quality have focused on morphological analysis, or used harmful substances, which impede oocyte fertilization (Castaneda *et al*., 2013). Thus, the present study adopted BCB staining to relate bovine oocyte maturation capacity with seasonal variations. Although studies have demonstrated the effect of seasons on feed restriction and cattle reproduction, to the best of our knowledge this is the first attempt of using this method to select oocytes that are more competent for IVP in different seasons, since BCB^+ oocytes have a higher maturation rate.

Materials and methods

Location

The experiment was conducted at the Laboratório de Reprodução e Melhoramento Genético Animal Northern Rio de Janeiro (UENF), located in Campos dos Goytacazes, RJ, Brazil.

Climatic conditions

The animals originated from the Northern region of Rio de Janeiro, where the climate is tropical wet-dry (Aw) according the Köppen classification, with a rainy summer and a dry winter. The annual average temperature is 24 ºC. The driest quarter occurs in the June/July/August period and the rainy season in December/January/February, being the annual average rainfall 1,055 mm (Mendonça *et al*., 2012). In summer (December to March) the days are longer than the nights and the solar radiation favors the rise in temperature, precipitation and relative humidity. Winter (June to September) has the lowest rainfall index and the lowest temperatures. In general, rain is five times lower in wintertime and air humidity is low (Emidio, 2016).

Collection and morphological selection of COCs

The COCs recovered from ovaries obtained at slaughterhouses in the city of Campos dos Goytacazes (21°45'15" S latitude, 41°19'28" W longitude) were transported to the laboratory in thermal bottles, where the ovaries were washed in saline solution (0,9% NaCl) with antibiotics (100 IU/mL penicillin and 100 µg/mL streptomycin). Ovarian follicles of 3 to 8 mm in diameter were aspirated and the follicular fluid was placed in a 50-mL conical tube. After sedimentation, the pellet was transferred to a Petri dish (Corning, 100 × 20 mm) with 3-5 mL of handling medium [199 with Earle's salts plus 25 mM Hepes and antibiotics (100 IU/mL penicillin and 100 µg/mL streptomycin) supplemented with 10% fetal calf serum (FCS)] to search, retrieve, and classify the oocytes. Only degree I and II oocytes (Loos *et al*., 1989) —with three or more compact layers of *cumulus* cells and uniform cytoplasm— were used.

The experiment was conducted in the summer and winter of 2010 and 2011. In 2010, the study was carried out in January, February, March, June, July, and August, while in 2011 it took place in February, March, June, and August.

Selection of COCs by BCB test

After selection, COCs were incubated in incubation solution (199 medium supplemented with bicarbonate with antibiotics and 5% FBS) containing 26 µM of BCB (860867, Sigma-Aldrich Brasil Ltda, São Paulo, Brazil).

The COCs were maintained for 90 min in 5% CO_2, at 38.5 °C (Manjunatha *et al*., 2007). Oocytes incubated with BCB were separated according to the cytoplasm staining: Oocytes with blue staining in the cytoplasm (BCB^+) and oocytes with colorless cytoplasm (BCB^-). The COCs in the control group were kept under the same conditions except for the absence of BCB. Next, COCs were washed twice in PBS and placed in the incubator for *in vitro* maturation.

Oocyte culture

The COCs were transferred to 100 µL drops of maturation medium [199 medium with Earle's Salts (M-5017), plus 20 mM sodium bicarbonate solution (S6297), 5.0 mg/mL LH (Lutropin-V, Bioniche, Beleville, Canada), 0.5 µg/mL FSH (Folltropin-V, Bioniche, Belevile, Canada), 0.2 mM pyruvate (P4562), 100 IU/mL penicillin (P3032), and 100 µg/mL streptomycin (S1277). Twenty oocytes per drop remained under mineral oil, for 22 h, at 38.5 °C, in 5% CO_2.

Evaluation of oocyte maturation

To determine the effect of seasonality on nuclear competence of oocytes, the meiosis stage was evaluated at 22 h of maturation. After IVM, COCs were denuded mechanically by pipetting in PBS solution (0.01 g/mL PVA), subsequently placed on slide and coverslips and fixed for 72 h in acetic acid and ethanol (1:3, v:v). Afterwards, the material was stained with 2% acetic orcein. Oocytes were evaluated by light microscopy. The stages of nuclear maturation were defined according to Hewitt and England (1997), based on the morphology of chromatin, such as germinal vesicle (GV, prophase I stage), germinal vesicle breakdown (GVBD), metaphase I (MI), and metaphase II (MII).

Experimental design

Experiment one. The COCs were incubated in the BCB solution and then divided into two groups according to the staining of cytoplasm. The COCs from complexes from control group underwent the same treatment as the others, except for the absence of the BCB in the incubation medium. Those with blue cytoplasm were classified as BCB^+, and those with colorless cytoplasm were classified as BCB^-. Thus, the proportion of these two groups was determined in different seasons of 2010 and 2011.

Experiment two. The exposure of COCs to BCB and the treatment of control group were performed as described in Experiment I. After selection of COCs by the cytoplasm staining (BCB^+, BCB^-, and control) they were set for *in vitro* maturation for 22 h and subsequently evaluated for nuclear maturation stage.

Statistical analysis

The rates of oocytes matured in different treatments and the proportions of BCB^+ and BCB^- oocytes in summer and winter were compared by the Chi-square test at 95% confidence level. All data were analyzed using BioEstat 5.0 software (2007). Four replicates were used in each season of 2010 and 2011 to evaluate the proportion of BCB^+ and BCB^- oocytes, and five replicates to evaluate the nuclear maturation rate.

Results

Proportion of BCB^+ and BCB^- oocytes

Results showed a higher percentage ($p<0.05$) of BCB^+ oocytes obtained in the summer of 2010 compared with those obtained in the winter of the same year. Additionally, the percentage of BCB^- oocytes was lower ($p<0.05$) in the summer compared with the winter (Table 1).

Table 1. Percentage of BCB^+ and BCB^- oocytes obtained in summer and winter of 2010.

	BCB^+ *n* (%)	**BCB^- *n* (%)**
Summer	142/250 (56.8)^a	108/250 (43.2)^a
Winter	109/240 (45.4)^b	131/240 (54.6)^b

Values followed by different superscripts in the same column differ significantly by the chi-square test ($p<0.05$).

In the year 2011, there were no differences ($p>0.05$) between the percentages of BCB^+ or BCB^- oocytes obtained in summer and winter (Table 2).

Table 2. Percentages of BCB^+ and BCB^- oocytes obtained in summer and winter of 2011.

	BCB^+ *n* (%)	BCB^- *n* (%)
Summer	122/207 (58.9)	85/207 (41.1)
Winter	112/219 (51.1)	107/219 (48.9)

Data were analyzed by the chi-square test at the 5% significance level.

Evaluation of nuclear maturation in different seasons

We also evaluated the effect of seasonality on the ability of oocytes selected by the BCB method to reach MII after 22 h of *in vitro* maturation. Data related to nuclear maturation of different experimental groups in summer and winter of 2010 and 2011 were not significantly different (Tables 3 and 4).

Table 3. Percentages of BCB^+ bovine oocytes matured *in vitro* that reached metaphase II during the summer and winter of 2010 and 2011.

	2010 *n* (%)	2011 *n* (%)
Summer	96/123 (78.0)	85/107 (79.4)
Winter	56/76 (73.7)	75/94 (79.8)

Data were analyzed by the chi-square test at the 5% significance level.

Table 4. Percentages of BCB^- bovine oocytes matured *in vitro* that reached metaphase II during the summer and winter of 2010 and 2011.

	2010 *n* (%)	2011 *n* (%)
Summer	38/80 (47.5)	35/75 (46.6)
Winter	54/113 (47.8)	45/96 (46.8)

Data were analyzed by the chi-square test at the 5% significance level.

Evaluation of nuclear maturation in BCB^+ and BCB^- oocytes

The percentage of metaphase II in BCB^- oocytes was lower ($p<0.05$) than those of control and BCB^+ groups (Tables 5 and 6).

Table 5. Meiotic configuration of bovine oocytes matured *in vitro* obtained during the summer and winter of 2010.

	Summer - MII *n* (%)	Winter - MII *n* (%)
Control	71/102 (69.6)[a]	69/104 (66.3)[a]
BCB^+	96/123 (78)[a]	56/76 (73.8)[a]
BCB^-	38/80 (47.5)[b]	54/113 (47.8)[b]

[1]MII: Metaphase II. Values followed by different superscripts in the same column differ significantly by the chi-square test ($p<0.05$).

Table 6. Meiotic configuration of bovine oocytes matured *in vitro* obtained during the summer and winter of 2011.

	Summer - MII *n* (%)	Winter - MII *n* (%)
Control	79/98 (80.6)[a]	69/98 (70.4)[a]
BCB^+	85/107 (79.4)[a]	75/94 (79.8)[a]
BCB^-	35/75 (46.6)[b]	45/96 (46.8)[b]

[1]MII: Metaphase II. Values followed by different superscripts in the same column differ significantly by the chi-square test ($p<0.05$).

Discussion

Success in *in vitro* bovine embryo production is directly related to the quality of the gametes used, which is largely influenced by nutrition (Sales *et al*., 2015). In general, oocyte donors receive a controlled diet without significant changes in quantity and composition throughout the year, which makes it a negligible factor in the variation of the process. However, *in vitro* embryo production from ovaries obtained from slaughterhouses is dependent upon the forage supply, which may be influenced by the effects of seasonality on forage production. (Sartori and Guardieiro, 2010).

Due to its extension, Brazil has pronounced climatic variability across regions. The regions nearest to the equator have lower temperature variations throughout the year, but seasonality is mainly due to the volume and distribution of rainfall during the year (Sansigolo *et al*., 2010). Therefore, due to the high spatial variability in the rainfall regime these data should be validated in other regions and countries (Alvares *et al*., 2013).

To evaluate the effect of seasonality on the quality of bovine COCs, we used BCB, a vital stain, substrate from glucose-6-phosphate dehydrogenase (G6PD). The BCB has been used as a noninvasive method for the selection of oocytes that reach a stage of development consistent with the ability of these gametes to be fertilized and reach embryonic and fetal development. Glucose-6-phosphate dehydrogenase is active in growing oocytes, but has low activity in those oocytes that have completed this phase. Since the latter have a low G6PD activity, they do not degrade the BCB and turn blue (BCB^+; Alm *et al*., 2005). Oocytes considered BCB^-, by contrast, have a higher G6PD activity and thus degrade the stain,

remaining colorless. These oocytes have a lower ability to complete maturation and to be fertilized *in vitro* since they are still growing and have a smaller pool of mRNA, proteins, lipids, and sugars (Mota *et al.*, 2009).

Oocyte maturation is a crucial event comprising the progression of meiotic cycle. The major parameter to know oocyte has achieved nuclear maturation is a meiotic configuration of MII (Fulka *et al.,* 1998). In this stage oocytes can be fertilized and support embryonic development (Smitz *et al*., 2004).

In this study, values found during the summer of the two years assessed are similar to those described in 2005 by Alm *et al*. (57.9% BCB^+) and in 2009 by Mota *et al*. (60.37% BCB^+), who used the BCB method to select more competent oocytes for IVM. The total annual rainfall in the municipality of Campos dos Goytacazes in 2010 and 2011 was 597 and 739 mm (Evapotranspiration Station at CCTA/ UENF —unpublished data), respectively. These volumes were lower than the historical annual average rainfall volume (1,055 mm).

Despite the low volume of rainfall in the two years under study, in 2011, the rainfall was slightly higher than in the previous year. This situation may have provided a greater supply of forage to animals in the dry season, which may have increased the percentage of BCB^+ oocytes obtained in the winter.

Ovaries were obtained from cows from the north region of Rio de Janeiro State, in which the use of pastures for cattle feeding predominates. These pastures are subject to seasonal variations, with maximum yield occurring in the rainy season and a significant decline in production during the dry season. (Euclides *et al*., 2007; Teixeira *et al*., 2011).

Thus, cattle are not feed uniformly during the year, and this affects reproductive characteristics, since follicular growth, maintenance of pregnancy, and estrous cyclicity are low-priority activities on a nutrient-distribution scale and only work normally when functions like growth and nutrient reserves are maintained (Zeron *et al*., 2001).

In this work, BCB^- oocytes showed a lower nuclear maturation rate compared with control and BCB^+ groups (Tables 5 and 6). As the progression of oocyte meiosis to metaphase II is a requirement for fertilization and subsequent embryonic development, these data support the hypothesis that BCB^- oocytes are less able to develop, reinforcing the fact that the BCB method is an important tool to select oocytes for *in vitro* embryo production. We found no difference in maturation rate between BCB^+ and control oocytes, proving the lack of deleterious effect from BCB exposure and reinforcing the fact that BCB is a vital dye (Rodríguez-González *et al*., 2002).

In this regard, Ashry *et al.* (2015) demonstrated that abundance of oocyte gene transcripts related to maturation and proper development (such as JY-1 and BMP15) was higher in BCB^+ oocytes compared with BCB^-. On the other hand, BCB^- COCs showed increased expression of cathepsins B, S, and Z of *cumulus* cells. Thus, the authors correlated the abundance of transcripts of these genes with BCB^+ and BCB^- oocytes to prove that the BCB method can be used to acquire more competent oocytes (Ashry *et al*., 2015). Moreover, the use of this colorimetric method in oocytes and zygotes increases the possibility of predicting the early embryonic development potential, since oocytes with blue cytoplasm and unstained zygotes significantly increase embryo production (Mirshamsi *et al*., 2013).

These data confirm the efficacy of the BCB test in selecting oocytes able to undergo maturation. Oocytes obtained from prepubertal goats (Rodriguez-Gonzalez *et al.*, 2002) and from swine (Wongsrikeao *et al.*, 2006) have shown increased maturation and fertilization rates of BCB^+ oocytes in relation to BCB^- and control. However, Alm *et al*. (2005) found similar nuclear maturation rates between BCB^+ oocytes and the control group in cattle. Nevertheless, BCB^+ oocytes resulted in a significantly higher ($p<0.05$) blastocyst rate (34.1%) than BCB^- (3.9%) and the control (18.3%) groups.

Data from Tables 3 and 4 indicate that the nuclear maturation rates of BCB^+ and BCB^- oocytes were not affected by seasonality, but the effect of seasonality on the proportion of these oocytes obtained in the summer and winter was remarkable. Adamiak *et al*. (2005), demonstrated that feeding donors twice their nutritional requirements for maintenance increases the number of follicles from 4 to 8 mm and the number

of embryos produced. By contrast, malnutrition due to seasonal issues has negative implications on the resumption of meiosis, number of follicles, and oocyte quality (Santos *et al*., 2008).

In this work, mainly cattle of *Bos primigenius indicus* breeds such as Nellore were used. These animals are more resistant to tropical conditions (Wolfenson *et al.*, 2000) like high temperature and humidity than breeds evolved in Europe (e.g. Angus and Holstein; Hansen, 2004; Paula-Lopes *et al.*, 2003). The ovaries were collected at slaughterhouses in Campos dos Goytacazes (RJ, Brazil) where the tropical conditions are intense. Thus, the effect of seasonality influenced by nutrition becomes more evident than the thermal stress during summer.

In view of the above considerations, it can be concluded that the season can influence the percentage of oocytes best suited for *in vitro* embryo production in situations in which oocyte donors receive feed exclusively on pasture. Additionally, the brilliant cresyl blue method is effective to determine the effect of seasonality on the competence of bovine oocytes to achieve nuclear maturation.

Acknowledgments

The authors thank the Carlos Chagas Filho Foundation for Research Support at Rio de Janeiro State (FAPERJ) for financial support.

Conflict of interest

The authors declare they have no conflicts of interest with regard to the work presented in this report.

References

Adamiak SJ, Mackie K, Watt RG, Webb R, Sinclair KD. Impact of nutrition on oocyte quality: Cumulative effects of body composition and diet leading to hyperinsulinemia in cattle. Biol Reprod 2005; 73:918-926.

Alm H, Torner H, Lohrke V, Ghone IM, Kanitz W. Bovine blastocyst development rate *in vitro* is influenced by selection of by brilliant cresyl blue staining before IVM as indicator for glucose-6-phosphate dehydrogenase activity. Theriogenology 2005; 63: 2194-2205.

Alvares CA, Stape JL, Sentelhas PC, Gonçalves JLM, Sparovek G. Köppen's climate classification map for Brazil. Meteorologische Zeitschrift 2013; 22(6):711-728.

Ashry M, Lee K, Mondal M, Datta TK, Folger JK, Rajput SK, Zhang K, Hemeida NA, Smith G. Expression of TGFβ superfamily components and other markers of oocyte quality in oocytes selected by brilliant cresyl blue staining: Relevance to early embryonic development. Mol Repro Dev 2015; 82(3):251-264.

Castaneda CA, Kaye P, Pantaleon M, Phillips NC, Norman S, Fry R, D'occhio M. Lipid content, active mitochondria and brilliant cresyl blue staining in bovine oocytes. Theriogenology 2013; 79:417-422.

De Loos F, Van Vliet C, Van Maurick P, Kruip TA. Morphology of immature bovine oocytes. Gamete Res 1989; 24:197-204.

Euclides VPB, Macedo MCM, Zimmer AH, Medeiros RN, Oliveira MP. Características do pasto de capim-tanzânia adubado com nitrogênio no final do verão. Pesq Agropec Bras 2007; 42(8):1189-1198.

Emidio ZPO. Estações do Ano. Ipmet 2016; [Access date: May 10th, 2017]. URL: https://www.ipmet.unesp.br/4estacoes/#

Ferreira AM. Nutrição e atividade ovariana em bovinos: Uma revisão. Pesq Agropec Bras 1993; 28:1077-1093.

Fulka JJr, First NL, Moor RM. Nuclear and cytoplasmic determinants involved in the regulation of mammalian oocyte maturation. Mol Hum Reprod 1998; 4:41-49.

Goovaerts IGF, Leroy JLMR, Jorsen EPA, Bols PEJ. Noninvasive bovine oocyte quality assessment: Possibilities of a single oocyte culture. Theriogenology 2010; 74:1509-1520.

Hansen PJ. Physiological and cellular adaptations of zebu cattle to thermal stress. Anim Reprod Sci 2004; 82:349-360.

Lonergan P, Fair T. Maturation of oocyte *in vitro*. Annu Rev Anim Biosci 2016. 4:255-268.

Manjunatha BM, Gupta PSP, Devaraj M, Ravindra JP, Nandi S. Selection of developmentally competent buffalo oocytes by brilliant cresyl blue staining before IVM. Theriogenology 2007; 68:1299-1304.

Mirshamsi SM, Karamishabankareh H, AhmadI-Hamedani M, Soltani L, Hajarian H, Abdolmohammadi AR. 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 2013; 136:245-251.

Mota GB, Batista RITP, Serapião RV, Boité MC. Developmental competence and expression of MATER and ZAR1 gene in immature bovine oocytes selected by brilliant cresyl blue. Zygote 2009; 26:1-8.

Nicoloso RS, Lanzanova ME, Lovato T. Manejo das pastagens de inverno e potencial produtivo de sistemas de integração lavoura-pecuária no estado do Rio Grande do Sul. Cienc Rural 2006; 36(6):1799-1805.

Paula-Lopes FF, Chase CC JR, Al-Katanani YM, Krininger CE, Rivera RM, Tekin S, Majewski AC, Ocon OM, Olson TA, Hansen PJ. Genetic divergence in cellular resistance to heat shock in cattle: Differences between breeds developed in temperate versus hot climates in responses of preimplantation embryos, reproductive tissues and lymphocytes to increase culture temperatures. Reprod 2003; 125:285-294.

Mendonça JC, Freitas RM, Shimabukuro YE, Marques VS. Avaliação de eventos de inundação na Região Norte Fluminense, Rio de Janeiro, utilizando imagens de sensores remotos. Ambi-Água 2012; 7:255-267.

O'Shea LC, Mehta J, Lonergan P, Hensey C, Fair T. Developmental competence in oocytes and *cumulus* cells: Candidate genes and networks. Syst Biol Reprod Med 2012, 58:88-101.

Rodríguez-Gonzalez E, Lopez-Bejar M, Velilla E, Paramio MT. Selection of prepuberal goat oocytes using the brilliant cresyl blue test. Theriogenology 2002; 57:1397-1409.

Rodrigues CFM. Historical context of cattle embryo transfer technique in Brazil. Anim Reprod 2014; 11(3):137-140.

Sales JN, Iguma LT, Batista RI, Quintão CC, Gama MA, Freitas C, Pereira C, Camargo LS, Viana JH, Souza JC, Baruselli PS. Effects of a high-energy diet on oocyte quality and *in vitro* embryo production in *Bos indicus* and *Bos Taurus* cows. J Dairy Sci 2015; 98:3086-3099.

Sansigolo CA, Kayano MT. Trends of seasonal maximum and minimum temperatures and precipitations in Southern Brazil for de 1913-2006 period. Theor Appl Climatol 2010; 101:209-216.

Santos J, Cerri R, Sartori R. Nutritional management of the donor cow. Theriogenology 2008; 69:88-97.

Sartori R, Guardieiro MM. Fatores nutricionais associados à reprodução da fêmea bovina. R Bras Zootec 2010; 39:422-432.

Smitz JEJ, Nogueira D, Vanhoutte Matos DG, Cortvrindt RN. Oocyte: *In vitro* maturation. In: Suh CS, Sonntag B, Erickson GF. The ovarian life cycle: A contemporany view. Rev Endocr Metab Disord 2004; 3:5-12.

Teixeira FA, Bonomo P, Pires AJV, Silva FF, Fries DD, Hora DD. Produção anual e qualidade de pastagem de *Brachiaria decumbens* diferida e estratégias de adubação nitrogenada. Acta Sci Anim Sci 2011; 33:241-248.

Van Den Hurk R, Zhao J. Formation of mammalian oocytes and their growth, differentiation and maturation within ovarian follicles. Theriogenology 2004; 63:1717-1751.

Wolfenson D, Roth Z, Meidan R. Impaired reproduction in heat stressed cattle: Basic and applied aspects. Anim Reprod Sci 2000; 60:535-547.

Wongsrikeao P, Otoi T, Yamasaki H, Agung B, Taniguchi M, Naoi H, Shimizu R, Nagai T. Effects of single and double exposure to brilliant cresyl blue on the selection of porcine oocytes for *in vitro* production of embryos. Theriogenology 2006; 66:366-372.

Wu Y-G, Liu Y, Zhou P, Lan G-C, Han D, Miao D-Q, Tan J-H. Selection of oocytes for *in vitro* maturation by brilliant cresyl blue staining: A study using the mouse model. Cell Res 2007; 17:722-731.

Zeron Y, Ocheretny A, Kedar O, Borochov A, Sklan D, Arav A. Seasonal changes in bovine fertility: Relation to oocyte developmental competence, membrane properties and follicle fatty acid composition. Reprod 2001; 121:447-454.