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To appear in:

Rev Colomb Cienc Pecu (33,3)

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Influencia de diferentes suplementos proteicos en la recuperación y maduración in vitro de ovocitos bovinos

Influência de diferentes suplementações proteicas sobre a recuperação e maturação in vitro de oócitos bovinos

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To cite this article:

Fernandes-França PH, Oliveira-Santos MV, Oliveira-Lira GP, Azevedo-Borges A, Fernandes-Pereira A. Influence of different protein supplements on the recovery and *in vitro* maturation of bovine oocytes. Rev Colomb Cienc Pecu 20xx; 3x(#):xxx-xxx.
DOI: https://doi.org/10.17533/udea.rccp.v33n3a01

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Abstract

Background: The oocyte quality and maturation are influence by protein supplementation. **Objective:** To evaluate the influence of fetal bovine serum (FBS) and bovine serum albumin (BSA) concentrations on the recovery and *in vitro* maturation (IVM) of bovine oocytes. **Methods**: The study was divided into step 1 (oocyte recovery), and step 2 (IVM). In the first step, three experiments were performed according to the recovery medium: (R1) 10 *vs*. 20% FBS; (R2) 5 *vs*. 10% BSA; and (R3) better results of R1, R2, and the combination of FBS+BSA (5+5%). Within the second step, the maturation medium was supplemented according to three experiments: (M1) 5 *vs*. 10% FBS; (M2) 0.4 *vs*. 0.8% BSA; and (M3) better results of M1, M2, and the combination of FBS+BSA (5+0.8%). **Results:** In step 1 (R1 and R2), the media with 10% FBS and 10% BSA showed better oocyte quality results and were defined for experiment R3. In R3, the 10% FBS and the combination of FBS+BSA (5+5%) allowed recovery of better-quality oocytes. In step 2 (M1 and M2), media with both levels of FBS (5 and 10%) and 0.8% BSA were defined as better according to the maturation and viability rates of *cumulus* cells, so they were defined for experiment M3. In M3, no difference was noted among the supplements. **Conclusions:** For the oocyte recovery, 10% FBS and the combination of FBS+BSA (5+5%) can be used to obtain immature oocytes. For the *in vitro* maturation, FBS (both levels, 5 and 10%) and BSA (0.8%) can be used alone or in combination.

Keywords: *bovine serum albumin; brilliant cresyl blue; fetal bovine serum; in vitro embryo production; meiotic competence; oocyte maturation; oocyte quality; oocyte recovery.*

Resumen

Antecedentes: La calidad y maduración de los ovocitos son influenciados por la suplementación proteica. **Objetivo:** Evaluar la influencia de concentraciones de suero fetal bovino (FBS) y albúmina sérica bovina (BSA) en la recuperación y maduración *in vitro* (IVM) de ovocitos bovinos. **Métodos:** El estudio se dividió en el paso 1 (recuperación de ovocitos) y el paso 2 (IVM). En la primera etapa, tres experimentos se realizaron de acuerdo con el medio de recuperación: (R1) 10 *vs*. 20% FBS; (R2) 5 *vs*. 10% BSA; y (R3) mejores resultados de R1, R2 y la combinación de FBS+BSA (5+5%). En la segunda etapa, el medio de maduración fue suplementado para tres experimentos: (M1) 5 *vs*. 10% FBS; (M2) 0,4 *vs*. 0,8% BSA; y (M3) mejores resultados de M1, M2 y la combinación de FBS+BSA (5+0,8%). **Resultados:** En el paso 1 (R1 y R2), los medios con 10% FBS y 10% BSA mostraron mejores resultados de calidad oocitaria y fueron definidos para el experimento R3. En R3, 10% FBS y

la combinación de FBS+BSA (5+5%) permitieron la recuperación de ovocitos de mejor calidad. En la etapa 2 (M1 y M2), los medios con ambos niveles de FBS (5 y 10%) y 0,8% de BSA se definieron como mejores de acuerdo con las tasas de maduración y viabilidad de las células del *cumulus*, por lo que se definieron para el experimento M3. En M3, no se observó diferencia entre los suplementos. **Conclusiones:** Para la recuperación de ovocitos, se puede utilizar 10% de FBS y la combinación de FBS+BSA (5+5%) para obtener ovocitos inmaduros. Para la maduración *in vitro*, FBS (ambos niveles, 5 y 10%) y BSA (0,8%) se pueden usar solos o en combinación.

Palabras clave: *albúmina sérica bovina; azul de cresil brillante; calidad de los ovocitos; competencia meiótica; maduración de ovocitos; producción de embriones in vitro; recuperación de ovocitos; suero fetal bovino.*

Resumo

Antecedentes: A qualidade e a maturação de oócitos são influenciadas pela suplementação proteica. **Objetivo:** Avaliar a influência de concentrações de soro fetal bovino (FBS) e albumina sérica bovina (BSA) sobre a recuperação e maturação *in vitro* (IVM) de oócitos bovinos. **Métodos:** O estudo foi dividido em etapa 1 (recuperação de oócitos) e etapa 2 (IVM). Na primeira etapa, três experimentos foram realizados de acordo com o meio de recuperação: (R1) 10 *vs*. 20% FBS; (R2) 5 *vs*. 10% BSA; e (R3) melhores resultados de R1, R2 e a combinação de FBS+BSA (5+5%). Na segunda etapa, o meio de maturação foi suplementado de acordo com três experimentos: (M1) 5 *vs*. 10% FBS; (M2) 0,4 *vs*. 0,8% BSA; e (M3) melhores resultados de M1, M2 e a combinação de FBS+BSA (5+0,8%). **Resultados:** Na etapa 1 (R1 e R2), os meios com 10% FBS e 10% BSA mostraram melhores resultados de qualidade oocitária e foram definidos para o experimento R3. Em R3, 10% FBS e a combinação de FBS+BSA (5+5%) permitiram a recuperação de oócitos de melhor qualidade. Na segunda etapa (M1 e M2), meios com ambos os níveis de FBS (5 e 10%) e 0,8% BSA foram definidos como os melhores de acordo com as taxas de maturação e viabilidade de células do *cumulus*, então foram definidos para o experimento M3. No M3, não houve diferença entre os suplementos. **Conclusões:** Para a recuperação oocitária, 10% FBS e a combinação de FBS+BSA (5+5%) podem ser usados para obter oócitos imaturos. Para maturação *in vitro*, FBS (ambos os níveis, 5 e 10%) e BSA (0,8%) podem ser usados sozinhos ou em combinação.

Palavras-chave: *albumina sérica bovina; azul cresil brilhante; competência meiótica; maturação oocitária; produção in vitro de embriões; qualidade do oócito; recuperação do oocitária; soro fetal bovino.*

Introduction

In vitro embryo production (IVEP) has been prominent over the years, especially in the production of domestic animals, such as cattle (Boruszewska *et al*., 2016). Nevertheless, the IVEP has limitations that render it a less efficient technique, generating about 30–50% of viable blastocysts (Gonçalves *et al*., 2007; Pereira *et al*., 2010). Thus, improvements are required in the culture conditions of various stages of IVEP such as oocyte recovery, *in vitro* maturation (IVM) of the selected oocytes, and fertilization (IVF) and development (IVD) of the presumed zygotes to the blastocyst stage, which can be transferred or cryopreserved according to the requirement (Varago *et al*., 2008).

These initial steps of oocyte recovery and maturation are of great importance for better results, as the quality of *cumulus*-oocyte complexes (COCs) obtained determines the later steps of the technique (Santos *et al*., 2016; Santos *et al*., 2017). Therefore, to ensure nutrition and energy support in *in vitro* oocyte development, it is necessary to supplement the media with external protein sources. In this regard, protein sources such as fetal bovine serum (FBS) and bovine serum albumin (BSA) have been added (Ambrogi *et al*., 2017).

FBS is an undefined protein source consisting of a combination of proteins, growth factors, hormones, vitamins, and other nutrients present in the blood plasma (Van Der Valk *et al*., 2010; Leivas *et al*., 2011). In contrast, BSA is a purified protein that is present at high concentrations in blood plasma with high capacity and affinity for binding with other biomolecules (Muñoz and Mischler, 2015). Despite the extensive usage, the concentration of FBS and BSA in the recovery and maturation media varies among protocols, whether used alone or in combination (Sugimura *et al.*, 2018). Therefore, establishing the ideal concentration according to the influence on oocyte quality and maturation rate can contribute to increase the success of IVEP.

Thus, the present study aimed to evaluate the quality of bovine immature oocytes recovered and selected in medium containing different concentrations of FBS (10 *vs*. 20%) and BSA (5 *vs*. 10%), as well as to analyze the effect of these protein sources on the IVM of bovine oocytes (FBS: 5 *vs*. 10%; BSA: 0.4 *vs*. 0.8%).

Materials and methods

Ethical considerations

The research was conducted according to the institutional committee of animal usage (CEUA/UFERSA, no. 23091.001069/2015-79). All reagents used were from Sigma-Aldrich® (Sigma, St Louis, MO, USA), except when indicated.

Study design

The study was divided into two steps. In step 1, protein sources were added in the recovery medium and in step 2 in the IVM medium. In the first step, oocytes were obtained in the recovery medium composed of phosphate buffered saline (PBS) solution, and three experiments were performed according to the following supplements: (R1) 10 *vs*. 20% FBS (Gibco, Carlsbad, Kentucky, EUA); (R2) 5 *vs*. 10% BSA; and (R3) better results from experiments R1, R2, and the combination of FBS+BSA (5+5%). Thus, the better results were defined according to better recovery rate, viable oocytes by morphology, and viable oocytes by brilliant cresyl blue (BCB) of each experiment. Additionally, experiments with higher percentage of viable oocytes were considered more significant during the evaluation of the results. After recovery, oocytes were evaluated for morphological appearance and BCB assay.

In the second step, we aimed to evaluate protein sources in the IVM medium; three experiments were carried out to compare the following: (M1) 5 *vs*. 10% FBS; (M2) 0.4 *vs*. 0.8% BSA; and (M3) better results from experiments M1, M2 and the combination of FBS+BSA (5+0.8%). Thus, the better results were defined according to better rates of expansion and viability of *cumulus* cells (CCs), presence of the first polar body (1PB) and metaphase plate (MII) of each experiment. For this purpose, oocytes were recovered in TCM199 supplemented with 10% FBS, 40 µg/mL gentamicin sulfate, and 0.2 mM sodium pyruvate. They were subjected to IVM and evaluated for the expansion and viability of CCs, presence of the 1PB and MII.

Ovarian collection and oocyte recovery

Bovine ovaries (n = 735) were collected at a local slaughterhouse (Mossoró, Rio Grande do Norte, Brazil). Immediately after slaughter, the ovaries were transported in saline solution (NaCl, 0.9%) at a temperature of 35 °C for up to 30 min. In the laboratory, follicular aspiration was performed with the aid of 21G needles coupled to 5 mL syringes for 10–20 min. Thus, only visible follicles on the ovarian surface with a diameter of 2–8 mm were counted and aspirated. The follicular fluid obtained was kept at rest for 15 min for

sedimentation of the oocytes, which were then recovered. The time between the ovarian collection and oocyte recovery was approximately 60 min.

Morphological evaluation and bright cresyl blue assay

The recovered COCs were morphologically classified as either viable (one or more layers of CCs and homogeneous cytoplasm) or non-viable (less than one layer of CCs and heterogeneous or degenerated cytoplasm), according Santos *et al*. (2016).

For the BCB assay, oocytes selected by morphological criteria were washed in PBS and incubated in drops of BCB diluted in PBS (26 μM) for 60 min at 38.5 °C. Then, under stereomicroscope (Bel Photonics, Monza, BM, Italy), oocytes with blue staining in their cytoplasm were considered BCB^+ and more competent. Those with unchanged cytoplasm were considered BCB^- and less competent. This test is based on the ability of the enzyme glucose-6-phosphate dehydrogenase (G6PDH) to reduce BCB from blue to colorless in growing oocytes, whereas in oocytes grown at low activity the enzyme allows them to remain blue (Maia *et al*., 2017).

In vitro maturation (IVM) and evaluation of mature oocytes

In step 2, after oocyte retrieval, 20–30 morphologically classified as viable oocytes were transferred to droplets (covered with mineral oil) of the IVM medium consisting of TCM199 supplemented with 100 μM cysteamine, 0.2 mM sodiumm pyruvate, 40 μg/mL gentamicin sulfate, 10 μg/mL FSH/LH (Pluset®, Callier, Buenos Aires, ARG), and a protein source. The IVM was performed in petri dishes (Corning, Corning, NY, USA) at a temperature of 38.5 °C in a humid atmosphere containing 5% CO_2 for 24 h.

After IVM, oocytes with expanded CCs, identified under a stereomicroscope, were considered mature and those that did not show expansion were considered immature. For evaluation of the viability of CCs, oocytes were denuded by repeated pipetting and the resulting cell suspension was stained with trypan blue (0.2%). The cells were counted in the four quadrants at the edges of the Neubauer chamber, where the blue stained and non-stained cells were considered as dead and live, respectively.

After CC removal, the oocytes were morphologically evaluated for the presence of 1PB under stereomicroscope. Structures showing the 1PB were considered mature. Furthermore, the

nuclear stage of the oocytes was evaluated by fluorescence microscopy. For this, oocytes were fixed in paraformaldehyde (4%) and stained with Hoechst 33342 (10 µg/mL) for 15 min. Only oocytes with nuclear stage in metaphase II were classified as mature.

Statistical analysis

Recovery rates (oocytes recovered on the total of aspirated follicles), oocyte quality (viable oocytes on the total oocytes recovered), and maturation rates (oocytes showing expanded CCs, presence of 1PB, and MII on total oocytes evaluated) were compared among the groups and the results were analyzed by the Fisher exact test (GraphPad Software INSTAT 3.06). The viability rate of CCs (live cells/total cells counted × 100) was analyzed by the Chi-square test. Data were expressed as mean ± standard error and considered different when $p<0.05$. For each experiment, five replicates were considered.

Results

In step 1 (Table 1), which consisted of the evaluation of protein supplementation in immature bovine oocyte collection media, a total of 266 ovaries resulted in 1.978 oocytes, making a general mean of 7.4 oocytes retrieved per ovary. Thus, in experiment R1, 10% FBS was superior ($p<0.05$) compared to the 20% FBS group in the morphological evaluation and lower in the BCB test. Nevertheless, despite the superior result in the BCB test for the concentration of 20% FBS, it was observed that this result occurred due to the higher amount of morphologically non-viable oocytes. Already in the experiment R2, 10% BSA presented better result in the morphological evaluation when compared to the 5% BSA ($p<0.05$). Nevertheless, the two concentrations of BSA showed statistically similar results in the BCB assay. Thus, 10% FBS and 10% BSA were used in R3.

In the latter experiment, 10% FBS and 10% BSA were compared to the 5+5% FBS+BSA combination. The 10% BSA group had a higher recovery rate than the other groups. However, the combination of protein sources obtained a higher number of viable oocytes per morphology. In contrast, 10% FBS medium showed a higher percentage of viable oocytes using the BCB assay. Therefore, supplements that achieved positive results in oocyte quality, that is, FBS+BSA (5+5%) combination and 10% FBS, were considered better.

In step 2 (Table 2), which aimed to evaluate these supplements in the IVM media, a total of 469 ovaries resulted in 1.040 viable oocytes per morphology, making a mean of 2.2 viable

oocytes per ovary. As for experiment M1, there was no difference ($p>0.05$) between the 5% FBS and 10% FBS groups regarding the expansion and viability of CCs, presence of 1PB and MII. Therefore, 5 and 10% FBS were evaluated in the M3 experiment.

In experiment M2, 0.8% BSA was superior ($p<0.05$) only in the viability of CCs compared to the 0.4% BSA, and the 0.8% BSA was used in the subsequent experiments. In experiment M3, 5, 10% FBS, 0.8% BSA and the FBS+BSA (5+0.8%) combination were similar ($p>0.05$) for all evaluated parameters.

Table 1. Evaluation of different concentrations of protein supplements in bovine immature oocyte recovery medium (R): Recovery 1 (10% FBS *vs*. 20% FBS), R2 (5% BSA *vs*. 10% BSA), and R3 (the combination of FBS+BSA (5+5%) *vs*. 10% FBS *vs*. 10% BSA).

Parameters	Recovery 1 (R1)		Recovery 2 (R2)			Recovery 3 (R3)	
	10% FBS	20% FBS	5% BSA	10% BSA	FBS+BSA (5+5%)	10% FBS	10% BSA
Recovery rate, % (n)	56.2 ± 6.9[a] (168/299)	63.0 ±5.9[a] (216/343)	53.5 ± 5.0[a] (283/529)	45.3 ± 6.4[b] (203/448)	40.9 ± 4.8[b] (201/491)	41.9 ± 3.1[b] (239/570)	53.0 ± 5.5[a] (219/413)
Viable oocytes by morphology, % (n)	75.6 ± 4.9[a] (127/168)	58.8 ± 6.9[b] (127/216)	64.3 ± 8.4[b] (182/283)	73.9 ± 3.6[a] (150/203)	79.6 ± 2.0[a] (160/201)	68.6 ± 4.8[b] (164/239)	63.6 ± 4.5[b] (148/219)
Viable oocytes by BCB, % (n)	38.7 ± 8.9[a] (65/168)	49.5 ± 8.3[b] (107/216)	11.0 ± 3.8[a] (31/283)	17.2 ± 1.8[a] (35/203)	18.4 ± 3.7[b] (37/201)	29.3 ± 5.5[a] (70/239)	11.4 ± 3,9[b] (25/219)

Values are means (%) ± Standard Errors (n). Different superscript letters ([a, b]) in each experiment (R1, R2 and R3) within the same row indicate significant difference ($p<0.05$).

Table 2. Evaluation of different concentrations of protein supplements in the *in vitro* maturation medium (IVM) of bovine oocytes: Maturation 1 (5% FBS *vs*. 10% FBS), M2 (0.4% BSA *vs*. 0.8% BSA), and M3 (the combination of FBS+BSA (5+0.8%) *vs*. 5% FBS *vs*. 10% FBS *vs*. 0.8% BSA).

Parameters	Maturation 1 (M1)		Maturation 2 (M2)		Maturation 3 (M3)			
	5% FBS	10% FBS	0.4% BSA	0.8% BSA	FBS+BSA (5+0.8%)	5% FBS	10% FBS	0.8% BSA
				***Cumulus* cells**				
Expansion, % (n)	100.0 ± 0.0[a] (84/84)	97.8 ± 2.7[a] (92/94)	100.0 ± 0.0[a] (116/116)	97.8 ± 0.0[a] (113/113)	98.1 ± 1.5[a] (103/105)	99.0 ± 1.4[a] (104/105)	97.6 ± 1.4[a] (109/110)	93.3 ± 2.3[a] (98/105)
Viability, % (n)	73.0 ± 13.9[a] (842/1154)	71.0 ± 9.7[a] (719/1012)	56.0 ± 4.2[b] (1740/3109)	59.2 ± 14.3[a] (1458/2464)	56.1 ± 4.0[a] (1325/2361)	56.9 ± 3.3[a] (1151/2024)	57.6 ± 2.2[a] (1352/2348)	58.5 ± 3.6[a] (1149/1965)
				Nuclear stage				
1PB, % (n)	84.1 ± 6.0[a] (69/84)	78.3 ± 4.0[a] (72/92)	80.2 ± 9.5[a] (93/116)	87.6 ± 4.9[a] (99/113)	89.7 ± 5.4[a] (78/87)	88.7 ± 5.2[a] (86/97)	79.5 ± 6.4[a] (83/98)	87.5 ± 3.0[a] (91/104)
MII, % (n)	60.3 ± 3.9[a] (41/68)	54.0 ± 6.6[a] (47/87)	69.7 ± 13.1[a] (76/109)	60.2 ± 1.8[a] (56/93)	62.7 ± 11.3[a] (42/67)	60.8 ± 4.3[a] (48/79)	65.8 ± 9.9[a] (52/79)	66.7 ± 4.5[a] (48/72)

Values are means (%) ± Standard Error (n). Different superscript letters ([a, b]) in each experiment (M1, M2 and M3) within the same row indicate significant difference ($p<0.05$). 1PB: first polar body. MII: metaphase II.

Discussion

In this study, different concentrations of two protein sources (FBS and BSA) were evaluated as supplementation in bovine oocyte recovery and IVM media. In the recovery medium (R1), the 10% FBS concentration presented the best result for morphological quality in comparison with the 20% concentration. This fact demonstrates that even at a low concentration, FBS is able to provide good nutrition to the oocytes during the recovery while maintaining a high quality for IVM. In contrast, a better effect of BSA (R2) was observed at the highest concentration tested (10%), probably due to the simpler composition of this supplement. Nevertheless, in the third experiment (R3), when comparing the 10% FBS, 10% BSA, and the FBS+BSA (5+5%) combination, the most positive supplements for oocyte quality in the recovery were 10% FBS and FBS+BSA (5+5%) combination.

In general, the concentration of FBS in the oocyte recovery media is variable. Chasombat *et al*. (2015) used 10% FBS in bovine oocyte retrieval medium and obtained a good blastocyst rate (30.9%) at the end of IVF. On the other hand, Chen *et al*. (2016) used a concentration of 2% FBS during the recovery and reached 23.0% of blastocysts after 8 days of culture of the embryos generated. Furthermore, with respect to BSA, previous studies show variations in the quantities used. As an example, Ulloa *et al*. (2014) compared bovine oocyte harvesting methods using a concentration of 0.1% BSA and obtained 79.8% of maturation after follicular aspiration. In the study by Carrocera *et al*. (2016), the bovine oocyte recovery medium was supplemented with 0.4% BSA and, after IVF, up to 37.5% blastocyst was obtained. In other species, such as pigs and mice, the use of 0.4% (Galeati *et al*., 2010) and 10% (Dinopoulou *et al*., 2016) of BSA has also been reported. In addition, the conjugation of FBS+BSA (5+5%) in the medium of recovery may be an interesting supplement, since it allows a great nutritional contribution to the oocytes and showed a better rate of BCB^+ oocytes.

The use of the BCB assay for the selection of viable bovine oocytes proved to be an efficient method by allowing the semi-quantification of the G6PDH enzyme, indicative of the stage of oocyte development. According to Ashry *et al*. (2015), oocytes classified as BCB^+ in the recovery step are shown to be more competent for embryonic development. From this, it is suggested that this technique be performed in conjunction with the morphological evaluation, since morphologically non-viable oocytes for IVEP can also be stained using the dye.

In the IVM step, different concentrations of FBS and BSA were evaluated separately and associated, then, the best results were compared with a group containing both supplements. Regarding FBS, the two concentrations (5 and 10%) tested showed no difference in the parameters evaluated. In the experiment with BSA (M2), the concentration of 0.8% presented superiority compared to 0.4% regarding the viability of CCs. Previously, Ali and Sirard (2002) compared the presence of different protein sources during maturation. They verified the effect of the presence of free fatty acid BSA (0.8%), isolated BSA (0.8%), chicken egg albumin, polyvinylpyrrolidone (PVP) (0.8%), and 10% FBS in the media, and obtained a low maturation rate (44.0%) when using 0.8% BSA concentration, but achieved the best results using PVP supplementation, which is different from the results of the present study.

The cells that surround the oocyte are of great importance for oocyte maturation, as they present receptors for gonadotrophins (FSH and LH), and perform communication and exchange of nutrients and molecules important for oocyte development (Crocomo *et al*., 2011). Thus, the evaluation of CCs important for efficient IVM, because the viability parameter reveals the normal functioning of oocyte communication and media efficiency, and the expansion is related to cytoplasmic maturation (Araújo *et al*., 2014).

In the M3 experiment, concentrations of 5 and 10% of FBS, 0.8% BSA, and FBS+BSA (5+0.8%) combination were compared. According to Del Collado *et al*. (2014), low concentrations of FBS used in IVM can generate results similar to that obtained under high FBS concentrations. Thus, the lowest concentration of FBS (5%) was used in the medium containing the conjugation of the protein sources. After the evaluations of the CCs and nuclear stage, all groups presented similar results. This may be justified because the concentrations of FBS and BSA present enough nutrients to ensure oocyte development. Furthermore, Ambrogi *et al*. (2017) compared the transport of bovine oocytes in media supplemented with 10% FBS or 0.6% BSA followed by IVM with 10% FBS and found no statistical differences in the rates of MII, blastocysts, cryotolerance, levels of reactive oxygen species, and mitochondrial distribution (cytoplasmic maturation). This shows that the two supplements have similar efficiency.

The use of more than one protein source during the same stage of IVEP has already been reported previously by other authors. Leivas *et al*. (2011) used a medium supplemented with BSA and FBS for the culture of bovine embryos, where they obtained blastocyst rates of up to

43%. Del Collado *et al*. (2015) compared the presence of FBS+BSA (5+0.6%) conjugation during IVM where the rate of 89.6% of maturation was reached in the presence of 1PB, a result very similar to that obtained in the present study (89.7%). These authors suggest that only the presence of BSA in the IVM medium has lower effectiveness compared to the medium containing both protein sources. Therefore, it is believed that the supplementation of FBS together with BSA presents a greater protein supply for the development of COCs, by combining positive characteristics of the two supplements. However, when used alone at suitable concentrations, FBS and BSA show positive and similar results and can be used for bovine oocyte IVM.

In conclusion, supplementation with FBS+BSA (5+5%) in combination and 10% FBS is beneficial for the recovery medium, allowing a greater number of viable oocytes to be obtained by morphology and BCB assay. At the IVM stage, only addition of 0.4% BSA would not be of interest as a supplementation of the IVM medium of bovine oocytes. Thus, FBS (both levels, 5 and 10%) and BSA (0.8%) can be used alone or in combination with similar positive effect on oocyte maturation.

Funding

This research was supported by grants from the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq, Brazil, grant number 477710/2013-1) and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES, Finance Code 001). Alexsandra F Pereira is CNPq investigator (Process no.306963/2017-5).

Author contribution

PH Fernandes-França has designed the study, acquired and analyzed the data, and drafted the paper, MV Oliveira-Santos, GP Oliveira-Lira, and A Azevedo-Borges contributed with experiments, A Fernandes-Pereira designed and guided the experimental work, acquisition and analyzed data, drafted paper, revising it critically.

Acknowledgments

We thank the slaughterhouse and industrial refrigerator of Mossoro (Brazil) for supplying the biologic material.

Conflicts of interest

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

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