LIPID CONTENT, MITOCHONDRIAL ACTIVITY AND EARLY EMBRYO DEVELOPMENT IN OOCYTE COLLECTED FROM CROSSBRED COWS (*Bos taurus indicus*)

Yoeli Mendez[1]
Nohely Parra[2]
Francisco Baez[2]
Robert Valeris[3]
Patricia Villamediana[2]

ABSTRACT

The objective of this research was to evaluate the effect of phenotypic predominance on lipid content, mitochondrial activity and early developmental competence as indicators of oocyte quality. Cumulus-oocyte complexes (COCs) were recovered through follicular aspiration, and underwent *in vitro* maturation (IVM), *in vitro* fertilization (IVF), and *in vitro* culture (IVC) of presumptive zygotes. Lipid content and mitochondrial activity in immature and IVM oocytes were determined. A maturation rate of 80.6% and 69.3% was found for oocytes predominantly *B. indicus* and predominantly *B. taurus*, respectively. Total fertilization rate was 27.6%; 26.1% for predominantly *B. indicus* oocytes and 29% for predominantly *B. taurus* oocytes. A total of 55.5% and 57.5% of cleaved embryos after 48 and 72 h post-insemination (hpi) in predominantly *B. indicus* group were observed, respectively. As for the predominantly *B. taurus* group, 48.6% and 60.4% of cleaved embryos were found after 48 and 72 hpi, respectively. In both groups, immature oocytes showed a greater amount of small lipidic droplets ($p < 0.0001$); IVM decreased the number of small lipid droplets ($p < 0.0001$) and increased the number of medium and large lipid droplets ($p < 0.0001$). Predominantly *B. indicus* oocytes had a greater number of small and medium-sized lipid droplets, while there were no significant differences in large lipid droplets. IVM oocytes had higher mitochondrial activity than immature oocytes group ($p < 0.05$) without any effect of phenotypic predominance on this parameter. Assessment of lipid content was not a predictive factor of oocyte quality in crossbred cows.

Key words: Oocyte quality, lipid content, phenotypic predominance, *Bos indicus*, *Bos taurus.*

CONTEÚDO LIPÍDICO, ATIVIDADE MITOCONDRIAL E DESENVOLVIMIENTO EMBRIONÁRIO PRECOCE DE OÓCITOS COLETADOS DE VACAS MESTIÇAS (*Bos taurus indicus*)

RESUMO

O objetivo deste trabalho foi avaliar o efeito da predominância fenotípica no conteúdo lipídico como um indicador da qualidade do oócito. Os COC foram recuperados por aspiração folicular e submetidos a maturação in vitro (IVM), fertilização in vitro (FIV) e cultura in vitro (CIV). Determinou-se o conteúdo lipídico e atividade mitocondrial em oócitos imaturos e IVM. A taxa de maturação total era de 75%, com valores de 80,6% e 69,3% para oócitos predominantemente *B. Indicus* e predominantemente *B. taurus*, respectivamente. A taxa de fertilização total foi de 27,6%, para oócitos predominantemente *B. indicus* e

[1] Técnico de Área Inversiones Porcinas Venezuela. Correspondência.
[2] Laboratorio de Citogenética, Departamento de Biología, Facultad Experimental de Ciencias. Universidad del Zulia.
[3] Universidad del Zulia, Facultad de Ciencias Veterinarias.

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predominantemente *B. taurus*, este foi de 26,1% e 29%, respectivamente. Um total de 52,1% de embriões divididos e 58,9% foi observada após 48 e 72 horas após a inseminação (hpi), respectivamente. Além disso, para o grupo predominantemente *B. indicus* 55,5% e 57,5% embrião clivada após 48 e 72 hpi foi observado. Enquanto para o grupo predominantemente *B. taurus* 52,1% e 58,9% dos embriões divididos após 48 e 72 hpi respectivamente. Oócitos imaturos mostraram gotas lipídicas mais pequenas (p<0,0001), em contraste com oócitos IVM com maior número de gotas lipídicas médias e grandes (p <0,0001). Oócitos predominantemente *B. indicus* mostraram mais gotas lipídicas pequenas (p = 0,0005) e médias (p = 0,005), enquanto que para as gotas lipídicas grandes não foram observadas diferenças significativas. Oócitos IVM tiveram maior atividade mitocondrial que o grupo de oócitos imaturos (p < 0,05), não houve efeito de predominância fenotípica sobre este parâmetro. A avaliação do conteúdo lipídico não foi um preditor de qualidade dos oócitos nas vacas mestiças.

Palabras-Chave: Qualidade oocitária, conteúdo lipídico, predominância fenotípica, *Bos indicus, Bos taurus.*

CONTENIDO LIPÍDICO, ACTIVIDAD MITOCONDRIAL Y DESARROLLO EMBRIONARIO TEMPRANO DE OVOCITOS PROVENIENTES DE VACAS MESTIZAS (*Bos taurus indicus*)

RESUMEN

El objetivo de la presente investigación fue el de valorar el efecto de la predominancia fenotípica sobre el contenido lipídico como indicador de la calidad ovocitaria. Los COC's fueron recuperados mediante aspiración folicular y sometidos a maduración in vitro (MIV), fecundación in vitro (FIV) y cultivo in vitro (CIV). Se determinó el contenido lipídico y actividad mitocondrial en ovocitos inmaduros y MIV. La tasa de maduración total se ubicó en 75%, con valores de 80,6% y 69,3% para ovocitos predominantemente *B. indicus* y predominantemente *B. taurus*, respectivamente. La tasa de fecundación total fue de 27,6%, para ovocitos predominantemente *B. indicus* y predominantemente *B. taurus*, esta fue de 26,1% y 29%, respectivamente. Se observó un total de embriones divididos de 52,1% y 58,9% tras 48 y 72 horas post inseminación (hpi), respectivamente. Por otro lado, para el grupo predominantemente *B. indicus* se apreció un 55,5% y 57,5% de embriones divididos tras 48 y 72 hpi. Mientras que para el grupo predominantemente *B. taurus* se apreció un 52,1% y 58,9% de embriones divididos tras 48 y 72 hpi. Ovocitos inmaduros presentaron mayor número de gotas lipídicas pequeñas (p ‹0,0001), en contraste con los ovocitos MIV que presentaron mayor número de gotas lipídicas medianas y grandes (p < 0,0001). Ovocitos predominantemente *B. indicus* presentaron mayor número de gotas lipídicas pequeñas (p = 0,0005) y medianas (p = 0.005), mientras que para las gotas lipídicas grandes no se apreciaron diferencias significativas. Ovocitos MIV presentaron mayor actividad mitocondrial que el grupo de ovocitos inmaduros (p < 0,05), sin observarse efecto de la predominancia fenotípica sobre este parámetro. La valoración del contenido lipídico no resultó un factor predictivo de la calidad ovocitaria en hembras mestizas.

Palabras clave: Calidad ovocitaria, contenido lipídico, predominancia fenotípica, *Bos indicus, Bos taurus.*

INTRODUCTION

Intracellular lipids are a substrate that can be used as a source of energy during invitro embryo production (IVP) (1,2). Thus, alterations in the quantity and the type of lipid species may result in a decrease of competence in oocyte development (3), being a paramount condition for the success in IVP, as are sperm quality and culture system (4,5). Leroy et al. (6) reported that IVM oocytes matured under fatty acid concentrations similar to those of follicular fluid from dairy cows undergoing negative energy balance, showed a decrease in maturation, fertilization, cleavage, and blastocyst rates. Furthermore, the increase in lipid content may induce mitochondrial dysfunction, which results in abnormal chromosome segregation, alterations in mitochondrial morphology, distribution, and membrane potential, along with an increase in lipid droplets (7,8,9).

Oocyte quality is a major factor in the success of reproductive biotechnology implementation. Several studies resulted in the selection of morphologic and biochemical parameters that contribute as a whole to determine oocyte development competence. Recently, attention has been focused to the study of lipid content as an indicator of oocyte quality, due to the possibility of taking advantage of an endogenous energy source during IVP and the susceptibility to cryotolerance related to an increase in lipid droplets (10). An increment in concentration and variation of the composition of free fatty acids in the oocyte may impair its developmental competence (3,6).

In western Venezuela, dual-purpose cattle husbandry has been developed. It has resulted in crossbreeding programs mainly alternating *B. taurus* and *B. indicus*, with the aim of producing an animal capable of adapting to the particular environmental conditions of the tropics (11). Moreover, the effect of phenotypic predominance on oocyte quality is paramount for cattle husbandry in the tropics. Oocytes from predominantly *B. indicus* cows are more competent in the early stages of development *in vitro* than oocytes from cows with phenotypic predominance *B. taurus* in tropical settings (12).

Several studies have aimed to increase the success of IVP systems, from improvements in recollection methods of oocytes to the design of culture media similar to the physiological conditions in cows. However, few studies have focused on the effect of lipid content and mitochondrial activity over oocyte development competence, and especially in their relation with phenotypic component in tropical crossbred cows.

MATERIALS AND METHODS

Selection of females in the slaughterhouse

Before slaughter, all cow and heifer was classified according to grade crossing and phenotypic ratio according Isea-Villasmil e Aranguren-Méndez (13). Only animals with phenotypic predominances above 5/8 *Bos taurus* and *Bos indicus* were included in one of two groups, phenotypic predominance *Bos taurus* and *Bos indicus*, respectively.

Oocyte collection and IVM

Ovaries were collected from local slaughterhouses, in an area that has characteristics of tropical wet forest with annual rainfall between 550-1500 mm. After collection, ovaries were transported within 2hto the laboratory in 0.9% NaCl (w/v) at 35-37 ºC. For this study, bovine oocytes obtained from predominantly *B. indicus* (n=367) and predominantly *B. taurus* (n=369) crossbred cows, were used. COCs were recovered by aspiration of all visible follicles

(2-8 mm) and resuspended in TCM-199 (11150-059, Gibco Life Technologies, Grand Island, NY, USA) supplemented with $NaHCO_3$(2.2 mg/L), gentamicin sulfate (50 µg/mL), BSA (0.4 g/L), HEPES (25 Mm)and heparin (11.1 µg/mL). Oocytes enclosed in a compact cumulus with an evenly granulated cytoplasm were selected. Groups of 25 COCs were IVM in 100 µL drops of TCM-199 supplemented with FSH(0.5 µg/ml, Folltropin-V®, Bioniche), 17β estradiol (1 µg/mL) gentamicin sulfate (25 µg/mL) and 10% fetal calf serum (FCS) in plates covered by mineral oil. COCs were cultured for 23 h at 38.5 ºC in an atmosphere with 5% CO_2 in air saturated humidity.

Sperm preparation and IVF

Frozen semen from a single bull (*Bos taurus*) were thawed at 37 ºC for 30sec and washed in a 15 Falcon tube using Percoll® gradients of 45% and 90% prepared with Tyrode´s Albumin Lactate Pyruvate (TALP) solution (2 mL of 45% Percoll® at the top and 2 mL of 90% Percoll® in the bottom of the tube) by centrifugation at 325 g for 15 min; then the pellet was diluted with 0.5 mL TALP solution and centrifuged a second time at 325 g for 10 min. After maturation, batches of COCs were fertilized in 100 µL Talp Fert medium containing 0.6% fraction V fatty acid free BSA, 10 µg/mL heparin, 20 mM penicillamine, 10 mM hypotaurine, and 1 mM epinephrine. Spermatozoa were added to a concentration of 1 x 10^6 spermatozoa/mL. COCs and spermatozoa were incubated for 18 h at 38.5 ºC under an atmosphere with 5% CO_2 in air saturated humidity.

IVC

Presumptive zygotes were denuded of cumulus cells by manual pipetting. Droplets of 50 µL of modified synthetic oviduct fluid with amino acids, citrate and myo-inositol (mSOFaaci) supplemented with fatty acid free BSA (6 mg/mL), sodium pyruvate (11mg/mL) and gentamicin sulfate (50 µg/mL) were layered under mineral oil and embryos cultured in group of 25 at 38.5ºC in 5% CO_2 in air saturated humidity. The embryos were cultured for 3 days.

Oocytes denudation

Cumulus cells were mechanically removed from intact COCs by repeated pipetting with PBS supplemented with BSA (3mg/mL). Dispersion of cumulus was verified by a stereoscopic microscope.

Assessment of lipid content

Oocytes were previously fixed in 10% formaldehyde in PBS, pH 7.4, for 2 h at room temperature. After fixation, they were washed in distilled water containing 0.05% polivinyl-alcohol (PVA) and then transferred to drops of 50% ethanol. After 2 min, oocytes were stained in drops of 1% Sudan Black B (w/v; S668, Fisher) in 70% ethanol for 1–2 min, then they were washed three times with 50% ethanol, 5 min each, followed by a 5 min wash in 0.05% PVA in distilled water. Prepared oocytes were mounted in 10 µL glycerol on cover slips and examined under a light microscope at 600X magnification. To estimate the relative amount of lipid droplets in the cytoplasm in each oocyte, a grid with five squares of 1,600 μm^2 (40 x 40 µm) each was designed using ImageJ 14.1 software. Lipid droplets were classified as small, medium, and large (<2 µm, 2–6 µm, and >6 µm, respectively). The number of droplets per category in the 1,600 μm^2 square was counted, and the average

number of droplets from five squares for each embryo was calculated. Data regarding lipid accumulation are presented as number of lipid droplets per 1,000 μm^2 (14).

Evaluation of mitochondrial activity in oocytes

Oocytes were stained with 0.02% Janus Green B in maturation medium and maintained at 38.5° C in an atmosphere with 5% CO_2 in humidity saturated air for 30 min. After several washes in PBS and PVA (0.1 mg/mL), oocytes were observed under light microscope (600X) (15). To estimate mitochondrial activity, a photograph of each oocyte was taken and processed with Adobe® Photoshop® CS6 software. Color photographs of oocytes were converted to gray scale images and the average gray intensity (arbitrary units) was obtained.

Nuclear maturation

After denudation, oocytes were fixed with methanol + acetic acid (3:1) for 48 h at 4ºC, stained with 1% solution of lacmoid in 45% glacial acetic acid, evaluated under an optical microscope (Olympus CX31, Japan®) (400X), and classified according to the meiotic stage reached: mature (metaphase II + polar body, telophase I) and immature (anaphase I, metaphase I, chromosomal condensation and in GV). Oocytes that could not be included in previous groups were considered as degenerate.

Assessment of fertilization and cleavage rate

To evaluate the fertilization rate, oocytes were removed from culture at 17 hpi and fixed and stained as described in the previous section. Oocytes were examined under an optical microscope (Olympus CX31, Japan®) and classified as either (a) non fertilized – the presence of female and the absence of male chromatin; (b) normal fertilized: the presence of female and male chromatin in the cytoplasm, a decondensed sperm head, pronuclei or cleavage; (c) abnormal fertilized: asynchronous oocytes (marked alteration in the formation of pronuclei, as undecondensed sperm head or telophase II) and > 2 pronuclei (oocytes in which more than 2 pronuclei were observed in the cytoplasm); or (d) degenerate oocytes. Cleavage rate was evaluated at 48 and 72 hpi, taking into account the total of embryos of 2 or more cells obtained in relation to the total of oocytes that were fertilized. All embryos were observed under stereoscopic microscope (Olympus, SZX12, Japan®).

Experimental design

Oocytes obtained from each of the phenotypic predominance were divided into two groups, the first group (GI) corresponding to immature oocytes were fixed and stained for evaluation of lipid content and mitochondrial activity. The second group (GII) were oocytes for IVM, from which a pooled sample of 10 oocytes in each group were used for the evaluation of meiotic progression, lipid content and mitochondrial activity. The rest of IVM oocytes (GII) were used in IVF, and a pooled sample of 10 oocytes of each group were fixed for the evaluation of fertilization rate after 17 hpi. Finally, cleavage rate after 48 and 72 hpi was assessed.

Statistical analysis

Nuclear state, fertilization and cleavage rate were expressed as frequencies and analyzed using the Chi-square test. Lipid content and mitochondrial activity were expressed as mean ± SD and analyzed using General Linear Model (GLM PROC) and statistical LSMEANS of the SAS® Version 8.2 software (1999; SAS® Institute Inc., Cary, NC, USA). A value of $p< 0.05$ was considered to be statistically significant.

RESULTS

A total of 736 oocytes were included in this study; 367 oocytes represented the experimental group with *B. indicus* phenotypic predominance, and 369 oocytes represented the experimental group with *B. taurus* phenotypic predominance. Eighty-eight oocytes were analyzed for lipid content (Table 1). Immature oocytes showed a greater amount of small lipiddroplets; this condition changed as oocytes reached maturation, having a cytoplasm richer in medium and large lipid droplets ($p <0.0001$). No significant differences in large lipid droplets were found due to phenotypic predominance.

Table 1. Lipid content in immature and IVM oocytes from cows with phenotypic predominance of *B. indicus* and *B. taurus*.

Stage	Predominance	Total of evaluated oocytes	Small droplets <2 µm	Medium-sized droplets 2 a 6 µm	Large droplets > 6 µm
Immature oocytes	***B. indicus***	**29**	74.7 ± 37.1^{a}	12.4 ± 12.2^{ab}	0.1 ± 0.3^{a}
	B. taurus	**26**	41.7 ± 27.6^{b}	7.5 ± 6.8^{a}	0.2 ± 0.5^{a}
	Total	**55**	59.1 ± 36.6^{x}	10.1 ± 10.2^{y}	0.2 ± 0.4^{y}
IVM oocytes	***B. indicus***	**15**	21.6 ± 12.5^{bc}	26.4 ± 11.2^{c}	2.1 ± 1.1^{b}
	B. taurus	**18**	8.6 ± 9.4^{cd}	17.8 ± 8.7^{bc}	2.4 ± 1.4^{b}
	Total	**33**	14.5 ± 12.6^{y}	21.7 ± 10.7^{x}	2.3 ± 1.3^{x}

Mean ± SD. Lipid droplets /10^3 µm^2. ^{a,b,c,d} Values within the same column with different superscripts differ ($p < 0.0005$). $^{x, y}$ Values within the same column with different superscripts differ ($p < 0.0001$).

IVM oocytes showed greater mitochondrial activity (128.5 AU) than immature oocytes (133.9 AU). On the other hand, there was no effect of phenotypic predominance on this parameter (Table 2).

Table 2. Mitochondrial activity in bovine immature and IVM oocytes from cows with phenotypic predominance of *B. indicus* and *B. taurus.*

Stage	Predominance	Total of evaluated oocytes	Gray scale intensity
Immature oocytes	***B. indicus***	**24**	131.4 ± 11.2
	B. taurus	**26**	136.3 ± 9
	Total	**50**	133.9 ± 10.3^{b}
IVM oocytes	***B. indicus***	**15**	128.9 ± 15
	B. taurus	**17**	128.1 ± 12.5
	Total	**32**	128.5 ± 13.5^{a}

Mean ± SD. Arbitrary units. ^{a,b} Values within the same column with different superscripts differ ($p < 0.05$).

Maturation rate for predominantly *B. indicus* oocytes was 80.6%, whereas for the predominantly *B. taurus* group it was 69.3%. A 3.3% and 6.5% incidence of degenerate oocytes for predominantly *B. indicus* and predominantly *B. taurus* groups was observed, respectively. There were no significant differences in the above-mentioned parameters (Table 3).

Table 4 displays data corresponding the assessment of bovine oocyte fertilization, wherein a total of 145 presumptive zygotes were taken into account. The predominantly *B. indicus* group had 26.1% normal fertilized oocytes. As for the group predominantly *B. taurus*, 29% normal fertilized oocytes and 2.6% of degenerate oocytes, without any significant differences in any case.

Table 3. Nuclear maturation of bovine oocytes from cows with phenotypic predominance of *B. indicus*and *B. taurus.*

Predominance	**Total of evaluated oocytes**	**N° of mature oocytes**			**N° of immature oocytes**				**N° Deg.**
		Total n (%)	**TeloI n (%)**	**MII+CP n (%)**	**Total n (%)**	**GV n (%)**	**MI n (%)**	**AnaI n (%)**	**n (%)**
B. indicus	**62**	**50 (80.6)**	1 (1.6)	49 (79)	**10 (16.1)**	1 (1.6)	8 (12.9)	1 (1.6)	**2 (3.3)**
B. taurus	**62**	**43 (69.3)**	2 (3.2)	41 (66.1)	**15 (24.2)**	0	15 (24.2)	0	**4 (6.5)**
Total	**124**	**93 (75)**	3 (2.4)	90 (72.5)	**25 (20.2)**	1 (0.8)	23 (18.5)	1 (0.8)	**6 (4.8)**

TeloI: Telophase I. MII+CP: Metaphase II + polar body. GV: Germinal vesicle. MI: Metaphase I. AnaI: Anaphase I. N° Deg: degenerate oocytes.

Table 4. Assessment of fertilization in bovine oocytes from cows with phenotypic predominance of *B. indicus* and *B. taurus*

Predominance	**Total of evaluated presumptive zygotes**	**N° of adnormal fertilized oocytes**					**N° Deg.**
		Non Fert. Oocytes n (%)	**Norm. Fert. Oocytes n (%)**	**Activated n (%)**	**Async. n (%)**	**>2PN n (%)**	**n (%)**
B. indicus	**69**	**45 (65.2)**	**18 (26.1)**	**3 (4.3)**	**0**	**0**	**3 (4.4)**
B. taurus	**76**	**47 (61.8)**	**22 (29)**	**2 (2.6)**	**2 (2.6)**	**1 (1.3)**	**2 (2.6)**
Total	**145**	**92 (63.4)**	**40 (27.5)**	**5 (3.4)**	**2 (1.3)**	**1 (0.6)**	**5 (3.4)**

Non Fert. Oocytes: non fertilized oocytes. Norm. Fert. Oocytes: normal fertilized oocytes. Async: asynchronous oocytes. >2PN: oocytes with more than 2 pronuclei. Deg: degenerate oocytes.

The evaluation of embryo development was performed. After 48 and 72 hpi, 52.1% and 58.9% of total cleaved embryos, respectively, were observed (Table 5). The predominantly *B. indicus* group showed 55.5% and 57.5% cleaved embryos after 48 and 72 hpi, respectively; whereas, the predominantly *B. taurus* group had 48.6% and 60.4% cleaved embryos after 48 and 72 hpi, respectively. No statistically significant differences were observed.

Table 5. Evaluation of embryo development of bovine oocytes from cows with phenotypic predominance of *B. indicus* and *B. taurus*.

Predominance	**Total zygotes in IVC**	**Total cleaved embryos 48 hpi n (%)**	**2 Cells n**	**>2 Cells n**	**Total cleaved embryos 72 hpi n (%)**	**2 Cells n**	**>2 Cells n**
B. indicus	**153**	**85 (55.5)**	35	50	**88 (57.5)**	20	68
B. taurus	**144**	**70 (48.6)**	27	43	**87 (60.4)**	20	67
Total	**297**	**155 (52.1)**	62	93	**175 (58.9)**	40	135

DISCUSSION

Research focusing on determination of lipid content in bovine oocytes is scarce, especially in *B. indicus* oocytes. This study presents the first findings for quantification and dynamics of lipid droplets in oocytes from crossbred cows. In this research, it was observed that lipid content was affected by both phenotypic predominance and maturation stage of the oocyte. A previous study by Ballard et al. (16), reported the presence of oocytes with greater amount of lipid content in *B. indicus* cows, having in turn higher levels of cholesterol and triglycerides in blood compared with *B. taurus* cows, which suggests the use of these indicators as predictive parameters of oocyte lipid content. By contrast, Ordóñez-León et al. (17) observed a greater amount of lipid droplets in *B. taurus* oocytes, followed by *B. indicus* x *B. taurus*, and finally *B. indicus* oocytes. As to maturation stage, immature oocytes showed greater amounts of lipid droplets; an abundance of small lipid droplets could be observed. This condition varied toward an increase in the presence of medium-sized and large droplets as MII was reached. Those results coincide with the findings of Hyttel et al. (18) who reported that the number, size, and distribution of lipid droplets change during oocyte maturation: immature oocytes have small lipid droplets, whereas mature oocytes have larger droplets.

The recognition of lipid droplets as functional organelles in the cell that participate in the regulation of lipid storage and metabolism is quite recent (19). Moreover, models trying to reveal the mechanisms of biogenesis and growth of these structures are continually modified and updated. There are at least three models that seek to explain the process of growth of lipid droplets: a) incorporation of neutral lipids and phospholipids to lipid droplets through domains having close contact with endoplasmic reticulum; b) lipid synthesis by the enzymatic machinery present in lipid droplets; c) fusion of preexistent lipid droplets (20).

The findings of this research seem to support the model of fusion of lipid droplets (19,20). In this case, small lipid droplets are abundantly found in immature oocytes ($59.1/10^3$ μm^2), whereas medium-sized ($10.1/10^3$ μm^2) and large ($0.2/10^3$ μm^2) droplets are found in lesser amounts. After IVM, a decrease in the total number of lipid droplets occurred, but the amount of medium-sized ($21.7/10^3$ μm^2) and large ($2.3/10^3$ μm^2) lipid droplets increased (Table 1), in such a way that it is possible that the decrease in the total amount of lipid droplets is related to the fusion of small droplets that would integrate into larger droplets.

The presence of more lipid content in immature oocytes is associated with a greater number of mitochondria, higher division and blastocyst rate and greater numbers of total cells (21). Lipids in the oocyte may play a paramount role in its metabolism, due to the fact that triglyceride concentrations have been observed to decline in female gamete afterIVM, and the

inhibition of fatty acid oxidation during IVM results in alteration of embryo development (1,2).

In this research, mitochondrial activity was not affected by phenotypic predominance. No report about this parameter in oocytes from crossbred cows could be found. However, Esper and Barboza (In: 17), observed that IVP *B. indicus* embryos had a greater number of mitochondria than *B. taurus* embryos. Moreover, *B. indicus* oocytes had greater lipid content and tended to show higher mitochondrial activity after cows were administered fat enriched diets (22).

On the other hand, oocyte maturation stage is a factor that influences that parameter, IVM oocytes having higher mitochondrial activity than immature oocytes. These results coincide with previous studies wherein higher concentrations of ATP are observed in oocytes at MII (23,24,25). According to this, evaluation of oocyte morphology revealed that this trait is related to oocyte mitochondrial activity (23,24). Immature oocytes with brownish cytoplasm would have the ideal amount of lipid droplets and ATP to reach oocyte maturation; oocytes with pale cytoplasm would have few lipid droplets and lower concentrations of ATP, and finally, oocytes with dark cytoplasm would have a high amount of lipid droplets as well as high concentrations of ATP. Oocytes with high concentrations of ATP in the cytoplasm have a lower rate of first polar body extrusion after they are subjected to IVM, whereas those with low levels of ATP could reach blastocyst stage with a low number of cells (24).

Oocytes from predominantly *B. indicus* and predominantly *B. taurus* cows showed maturation rates of 80.6% and 69.3%, respectively. By contrast, Báez et al. (12) reported values of 66.17% and 50.94% for oocytes from predominantly *B. indicus* and predominantly *B. taurus* cows, respectively. Fertilization rate was not affected by oocyte lipid content. That was also the conclusion of Cerri et al. (26), who administered diets enriched with fatty acids from different sources to cows without observing significant variations in fertilization rates. Leroy et al. (6) observed that IVM in the presence of stearic or palmitic acids had no influence on oocyte lipid content, but resulting in decreasing fertilization rates, maybe as part of a collateral effect mediated by alterations of oocyte maturation.

Mitochondrial activity, determined by oxygen consumption and reactive oxygen species (ROS) production, is augmented during fertilization process in bovine oocytes and it is related to pronuclei formation (27). On the other hand, swine oocytes with positive staining for bright cresyl blue had a greater number of mitochondrial DNA copies and higher fertilization rates than those negative to that stain (28). However, Ge et al. (29) reported that oxidative phosphorylation inhibition, even though it diminishes mitochondrial membrane potential and ATP synthesis, has no effect on murine oocytes fertilization rates; in this case it is probable that the inhibitory effect was not enough to cause a critical decrease in ATP concentrations.

Camargo et al. (30) found differences between racial groups, so that oocytes from *B. indicus* cows showed higher cleavage rates than those from *B. taurus* cows, with values of 66.7% and 53.1%, respectively. However, Paula-Lopes et al. (31) and Hernández-Cerón et al. (32) failed to detect differences in cleavage rates between oocytes from *B. taurus*, tropicalized *B. taurus*, and *B. indicus* at 38.5°C.

A slight increase in oxygen consumption is seen during the beginning of the first embryo cleavage, along with an increase in ROS concentrations, which is an indicator of the mitochondrial activity needed to meet the energy demand inherent to embryo cleavage (27). It is well known that the inhibition of oxidative phosphorylation and the decrease in the amount of mitochondrial DNA copies induce the decline of blastocyst rates in bovines in a dose-dependent way possibly due to the decrease in ATP concentrations by alterations in mitochondria distribution, and by mitotic spindle disarrangement (29). Culture media supplementation with L-carnitine augments ATP content in the oocyte and induces lipid

droplets redistribution (33), as well as increases blastocyst rates (34) and enhances embryo development competence after vitrification (33).

The oocyte meiotic stage affects mitochondrial activity; IVM oocytes had higher mitochondrial activity that immature oocytes. Phenotypic predominance had no effect on this parameter. Studies undertaken to date have surmised the possibility of establishing lipid content determination as an indicator of oocyte quality. However, this research has proved that lipid content in slaughterhouse-derived oocytes determined through lipid droplets quantification vary according to phenotypic predominance, but they are not related to oocyte competence in crossbred cows; therefore, lipid content does not qualify as a parameter predictive of oocyte development potential for IVP in the tropics. However, the effect of phenotypic predominance on oocyte lipid content and the existence of studies that indicate a relationship between genotype and cryopreservation susceptibility, it would be worthwhile to consider the effect that lipid content could have on post-thawing survival in oocytes and embryos obtained from crossbred (*B. taurus indicus*) cows.

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