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Intraovarian influence of bovine corpus luteum on oocyte morphometry and developmental competence, embryo production and cryotolerance

Daniel E. Argudo [a], Milton A. Tenemaza [b], Shirley L. Merchán [b], José A. Balvoa [b], Maria S. Méndez [b], Manuel E. Soria [b], Luis R. Galarza [b], Luis E. Ayala [b], Hugo J. Hernández-Fonseca [c, 1], Mariana S. Perea [c], Fernando P. Perea [d, *]

[a] Unidad Académica de Ciencias Agropecuarias, Universidad Católica de Cuenca, Ecuador
[b] Laboratorio de Biotecnologías de la Reproducción Animal, Facultad de Ciencias Agropecuarias. Universidad de Cuenca, Ecuador
[c] Facultad de Ciencias Veterinarias, Universidad del Zulia, Venezuela
[d] Departamento de Ciencias Agrarias, Universidad de Los Andes, Trujillo, Venezuela

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ABSTRACT

Three experiments were conducted to determine influence of the bovine corpus luteum (CL) on morphometric and functional characteristics of oocytes, and subsequent embryonic development. Cumulus-oocyte complexes were aspirated from two types of cows: 1) with a CL in one ovary (CL+) and without a CL in the contralateral ovary (CL-), 2) and from cows without CL in either ovary (C). Intracellular activity of the enzyme glucose-6-phosphate dehydrogenase (G6PDH), oocyte diameter and thickness of the zona pellucida were determined (Experiment 1). Then, the rate of *in vitro* oocyte maturation for each ovarian category was evaluated and oocyte diameter and zona pellucida thickness were measured after maturation (Experiment 2). In Experiment 3, *in vitro* embryo production and cryotolerance were assessed. The oocyte diameter was greater ($P < 0.01$) and the zona pellucida was thinner in CL+ than in CL- ($P > 0.05$) or C ($P = 0.0131$) ovaries. Activity of G6PDH was lower in oocytes from CL+ than CL- ($P < 0.01$) and C ($P = 0.0148$) ovaries. Rate of oocyte maturation, oocyte diameter and thickness of the zona pellucida after maturation did not differ among groups. Rate of cleavage was greater in zygotes from CL+ than from CL- or C ($P < 0.01$); and CL+ ovaries produced more total embryos on day 7 ($P < 0.05$) and more blastocysts ($P < 0.01$) than CL- and C ovaries. Rate of expansion and hatching of day-7 vitrified-warmed blastocysts at 24 and 48 h of culture did not differ among groups. In conclusion, oocytes collected from CL+ ovaries were larger and metabolically more prepared to continue maturation than those from ovaries lacking a CL. Also, rates of cleavage and yield of blastocysts were greater for oocytes from CL+ ovaries than from CL- and C ovaries. These findings indicate that a CL influenced oocyte developmental competence and embryonic development, presumably through intraovarian interactions.

1. Introduction

The corpus luteum (CL) is a transient, highly vascularized ovarian tissue that develops in a few days from a corpus hemorrhagicum to a mature CL producing progressively greater amounts of progesterone (P4). Its functional activity is essential in all mammals for implantation and maintenance of early pregnancy [1].

Evidence in several ruminant species indicates that luteal function affects oocyte developmental competence and early embryonic development [2–6]. The presence of a CL and its physiologic stage at the time of oocyte retrieval seems to influence the number and quality of recovered COCs. For instance, number and

* Corresponding author. Francisco de Orellana. Residencias Yanuncay, Torre D, Dpto. 6B, Cuenca, Ecuador.
E-mail addresses: ferromi9@gmail.com, ferromi@ula.ve (F.P. Perea).
[1] Current address: As July 1st 2020. Anatomy, Physiology and Pharmacology Department, School of Veterinary Medicine, Saint George's University, Grenada, West Indies.

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quality of oocytes retrieved from abattoir-collected ovaries of pregnant cows were significantly greater from ovaries with a CL than from ovaries without a CL or from ovaries of open cows with or without a CL [7].

In another study, more total and high quality oocytes were recovered from ovaries bearing a CL than those lacking a luteal structure [8]. A greater proportion of blastocysts developed more rapidly from bovine oocytes collected from cows slaughtered on days 14–16 of the estrous cycle than at earlier or later stages [2]. In lactating dairy cows reduced progesterone concentration during the first follicular wave decreased embryo quality [9] and pregnancy per insemination [10]. Other studies, however, did not show an effect of CL on the number of recovered oocytes or on the subsequent embryo development after fertilization [11,12], or reported an adverse effect on the developmental competence of bovine oocytes and/or embryo production [13–15].

In buffaloes, a greater cleavage rate and proportion of transferable blastocysts were obtained from oocytes retrieved from ovaries bearing a corpus hemorrhagicum or a CL without dominant follicles in the same ovary [6].

In sheep, presence of a CL throughout progestogen treatment prior to administration of FSH as part of a superovulatory protocol increased the number of transferable embryos 1.8 fold and the viability of such embryos 1.3 fold compared to ewes without previous knowledge of luteal status [4]. Oocytes from ovaries with a CL showed greater rates of fertilization, development to blastocyst and hatching rates after vitrification than sheep without CL [4].

To date, a mechanism by which a CL influences oocyte developmental capacity or the subsequent embryonic development is unknown. Receptors for P4 (PR) present in bovine cumulus oocyte complexes suggest an important role of this hormone on oocyte competence to mature, to cleave and to reach advanced stages of embryonic development after *in vitro* fertilization [16]. Inhibition of P4 synthesis by cumulus cells or blocking the activity of PR decreased embryo production indicating a key role of P4 modulating acquisition of developmental competence of oocytes [16].

Greater blood flow to an ovary bearing a CL [17,18] could increase distribution of nutrients, hormones and growth factors into surrounding tissue, favoring follicular growth and oocyte development. Although there is no concrete evidence that CL influences the oocyte development by local mechanisms, luteal tissue produces numerous factors [19–22], that have been shown to influence growth and developmental capacity of oocyte [23–27]. One study showed that medium and large bovine follicles in ovaries bearing a CL had greater concentrations of P4 than those without a luteal structure [28], strengthening the idea that P4 could influence oocyte developmental competence by intraovarian interactions.

Given the conflicting results, the present study was designed to investigate developmental capacity and morphometric characteristics of oocytes recovered from bovine ovaries with or without a CL and subsequent embryonic development and cryotolerance after *in vitro* fertilization. Whether CL influence occurred by an intraovarian or systemic pathway was also assessed.

2. Material and methods

2.1. Research location

The experiments were conducted at the Laboratory of Reproductive Biotechnologies in the farm Irquis, University of Cuenca, Ecuador, located in Azuay province, 20 km from the city of Cuenca, at 2648 m above sea level (79° 4′ 32″ longitude west and 3° 4’ 48” latitude south).

2.2. Description of the experiments

Ovaries selected for these experiments were recovered from a local commercial abattoir from apparently healthy non-pregnant cows. Oocytes were collected from ovaries of two types of cows: 1) with a CL in one ovary (group 1, CL+) and without CL in the contralateral ovary (group 2; CL-), and 2) without CL in either of the two ovaries (control, group 3; C). The animals used in these experiments were mainly Holstein and dairy crossbred with a high proportion of Holstein breed, cows and heifers, with a body condition score between 2.0 and 4.0 on a 1 to 5 scale. Only ovaries with luteal structures that had the appearance and morphological characteristics of a functional CL (days 5 through 17 of the estrous cycle), as described by Ireland et al. [29], were included as group 1 in the experiments. All ovaries and corpora lutea were weighted with an electronic balance (Boeco, Germany).

In Experiments 1 and 2, cumulus-oocyte complexes (COC) from each type of ovary were selected and cultured (CO_2 Incubator, Memmert, Büchenbach, Germany) separately in 50-μL droplets covered with mineral oil. In experiment 3, COC and presumed zygotes from each type of ovary were cultured (Heracell™ 150i Trigas CO_2 Incubators, Thermo Fisher Scientific, Massachusetts, USA) in groups of no greater than 12 structures in 50-μL droplets covered with mineral oil.

In Experiment 1 (n = 551 oocytes in 5 replicates), intracellular activity of the enzyme glucose-6-phosphate dehydrogenase (G6PDH) oocyte diameter and thickness of the zona pellucida were determined. Cumulus-oocyte complexes of each ovarian group were incubated with 26 μM of brilliant cresyl blue (BCB) (Sigma-Aldrich, B-5388) diluted in synthetic oviductal fluid buffered with Hepes (hSOF) (NaCl 107.63 mM; KCl 7.16 mM; KH_2PO4 0,29 mM; $CaCl_2H_2O$ 1.7 mM; $NaHCO_3$ 5 mM; Sodium lactate 5.35 mM; Na-pyruvate 4.99 mM, Hepes Acid Free 10 mM; Hepes Sodium Salt 10 mM and BSA 3 mg mL^{-1} and embryo tested ultra-pure water) for 90 min at 38.5 °C in an atmosphere with 90% humidity and 5% CO_2. Then oocytes were classified into two groups according to whether the cytoplasm was stained blue (BCB+) or not (BCB-), and number of oocytes in each category was recorded. The BCB test was used to assess the activity of G6PDH because of the ability of this enzyme to metabolize the BCB stain. Accordingly, the BCB test allowed differentiation of oocytes that had completed the growth phase (blue cytoplasm; BCB+; low G6PDH activity) from those still growing (colorless cytoplasm; BCB-; high G6PDH activity) [30].

Thereafter, COC of each group were denuded of cumulus cells by gentle pipetting in hSOF supplemented with 1 mg mL^{-1} of hyaluronidase. Oocytes were photographed with a digital camera (Exelis AU-600-HD, Connecticut, USA) coupled to a phase contrast microscope at 100 X (Olympus CX31, Tokyo, Japan). Thickness of the zona pellucida and oocyte diameter (excluding zona pellucida) were measured using software (AmScope V.3.7) designed and calibrated to perform microscopic measurements.

In Experiment 2 (n = 851 oocytes in 6 replicates), rate of *in vitro* oocyte maturation for each ovarian category was evaluated and the oocytes measured as described above. After maturation and denudation, the COC were incubated at 38.5 °C for 10 min in darkness in hSOF containing 1 μg/mL of bisbenzimide (Hoechst 33342) and placed between a glass slide and a coverslip. Progression of meiosis was assessed under an inverted fluorescence microscope at 400 X (Olympus CKX41, Tokyo, Japan) and oocytes with a polar body extruded into the perivitelline space were considered matured.

In Experiment 3 (n = 2532 oocytes in 13 replicates), *in vitro* embryo production and cryotolerance were assessed. On day 2 after *in vitro* fertilization (IVF), cleavage rate was recorded, and 5 days later rate of embryo production was assessed. Embryos were

categorized according to developmental stage as morulae (M), early blastocysts (EB), regular blastocysts (RB), expanded blastocysts (ExB) and hatched blastocysts (HB). Immediately, a representative number of blastocysts was vitrified to assess cryotolerance; warmed blastocysts were cultured for 48 h, and the rate of embryos reaching a more advanced developmental stage (expanded or hatched blastocysts) was assessed at 24 and 48 h post-warming.

2.3. Chemicals

Chemicals were purchased from Sigma Chemical Co. (St Louis, MO, USA) unless otherwise indicated.

2.4. COC collection and in vitro maturation (IVM)

Ovaries were transported from the abattoir to the laboratory in sterile saline (0.9% NaCl) at around 30 °C within 3 h after slaughter, and immediately washed in warmed sterile saline. Cumulus-oocytes complexes were aspirated from antral follicles of 3–8 mm in diameter with an 18-gauge needle attached to a disposable 12-mL syringe. Only oocytes with 3 or more compact layers of cumulus cells, smoothly granular oocyte cytoplasm, and no irregularities of the oocyte nucleus, described as healthy oocytes by Vassena et al. [31], were used. After selecting and washing in hSOF, COC were cultured in an IVM medium composed of TCM-199 supplemented with 10% fetal bovine serum (FBS), 2.2 mg mL^{-1} sodium bicarbonate, 0.1 mg/mL L-glutamine, 0.01 UI/mL rh-FSH (Gonal-f®, Merck, Italy), 100 μM cysteamine, 1 μg/mL estradiol and 50 μg/mL gentamicin. Incubation was performed for 24 h at 38.5 °C in a humidified atmosphere of 5% CO_2.

2.5. In vitro fertilization and embryo culture (IVC)

In vitro matured COC were transferred to IVF medium that consisted of synthetic oviductal fluid (NaCl 107.63 mM; KCl 7.16 mM; KH_2PO_4 0.29 mM; $CaCl_2H_2O$ 1.7 mM; $NaHCO_3$ 25 mM; Sodium lactate 5.3 mM; Gentamicin 50 μg/mL; Na-pyruvate 4.99 mM; Fructose 0.5 mM; BSA-FAF 8 mg mL^{-1}; MEM amino acids 10 μL mL^{-1} and embryo tested ultra-pure water) supplemented with 10 μg/mL heparin. Frozen semen from a bull of proven fertility previously used in our laboratory was employed in all experiments. Motile spermatozoa were selected by centrifugation (10 min at 600×*g*) of thawed semen on a Percoll discontinuous density gradient (200 μL of 90, 60 and 30% Percoll in a 1.5-mL microtube). The pellet was reconstituted into 1 mL of IVF medium without heparin and centrifuged at 200×*g* for 10 min. Thereafter, the pellet was diluted with IVF medium and an aliquot added to fertilization droplets (final concentration of 1×10^6 spermatozoa/mL). Gamete incubation was performed for 18–20 h at 38.5 °C in a humidified atmosphere of 5% CO_2.

After IVF, presumptive zygotes were stripped from cumulus cells by gently pipetting, washed three times in hSOF, and transferred to IVC medium (synthetic oviductal fluid medium modified, SOFaaci, as described Holm et al. [32]) (NaCl 107.63 mM; KCl 7.16 mM; KH_2PO_4 1.19 mM; $MgSO_4$ $7H_2O$ 1.51 mM; $CaCl_2H_2O$ 1.78 mM; $NaHCO_3$ 25 mM; Sodium lactate 5.3 mM; Gentamicin 50 μg/mL; Na-pyruvate 7.27 mM; Glutamine 0.20 mM; Trisoduim citrate 0.34 mM; Myoinositol 2.77 mM; MEM amino acids 10 μL mL^{-1}; BME amino acids 30 μL mL^{-1} and BSA-FAF 3 mg mL^{-1} and embryo tested ultra-pure water), and incubated for 6 days at 38.5 °C in a humidified atmosphere of 5% CO_2, 5% O_2 and 90% N_2.

2.6. Vitrification and warming

Vitrification was performed using open pulled straws (OPS) as described Vajta et al. [33]. After IVC, embryos were placed into 100 μL droplet of vitrification solution 1 [VS1; 7.5% ethylene glycol (EG) 7.5% dimethyl sulfoxide (DMSO) in hSOF supplemented with 20% FBS] for 3 min. Next, embryos were transferred with a minimum volume of VS1 into 40 μL droplet of vitrification solution 2 (VS2; 16.5% EG, 16.5% DMSO and 0.5 M sucrose in hSOF with 20% of FBS) for <30 s. Embryos were loaded by placing the narrow end of the OPS into approximately 0.5 μL droplet of VS2, and immediately plunged into liquid nitrogen for 30–120 days.

Warming was performed by placing the narrow end of the OPS into four-well dish with 800 μL of 0.5 M sucrose and 20% FBS solution (WS1) for 5 min. Next, embryos were transferred into a 400 μL droplet of 0.25 M sucrose solution (WS2) for 5 min (WS1 and WS2 elaborated with hSOF), washed twice in hSOF, and then incubated in SOFaaci supplemented with 20% of FBS for 48 h at 38.5 °C in a humidified atmosphere of 5% CO2, 5% O2 and 90% N2.

2.7. Statistical analysis

Proportions of healthy oocytes (number of healthy oocytes/total number of recovered oocytes), BCB+ and matured oocytes, and proportions of cleavage and total embryos, embryos in different developmental stages, and rates of expansion and hatching of blastocysts were analyzed by logistic regression of SAS (V 9.3; SAS Institute, Inc., Cary, NC, USA).

Oocyte diameter, zona pellucida thickness and number of embryos per ovary were tested for normal distribution and homogeneity of variance and were log transformed when these criteria were not met. These data were analyzed by GLM procedure of SAS. Means were compared by LS means method of SAS. Significance was considered as $P < 0.05$, and P values between 0.051 and 0.1 were considered as tendency.

3. Results

3.1. Experiment 1. influence of corpus luteum on oocyte morphometry and activity of the enzyme glucose-6-phosphate dehydrogenase

As expected, ovarian weight was significantly greater in CL+ than in CL- and C (Table 1). Corpora lutea weighed an average of 4.1 ± 0.06 g. Oocyte diameter was larger in CL+ than in CL- and C ($P < 0.01$). Zona pelucida was thinner in CL+ than in CL- ($P > 0.05$) or C ($P = 0.0131$). Activity of the enzyme G6PDH was significantly lower in oocytes recovered from ovaries bearing a CL than in contralateral ($P < 0.01$) or control ovaries ($P = 0.0148$).

3.2. Experiment 2. influence of corpus luteum on rate of maturation and post maturation morphometry

Ovaries bearing a CL were significantly heavier than contralateral and control ovaries ($P < 0.01$). The average weight of the CL was 4.5 ± 0.08 g. Rate of oocyte maturation, oocyte diameter and thickness of the zona pellucida after maturation did not differ among groups (Table 2).

3.3. Experiment 3. influence of corpus luteum on rate of cleavage, embryo production and cryotolerance

As indicated previously, ovaries bearing a CL were significantly heavier than contralateral and control ovaries (11.4 ± 0.69 versus 6.4 ± 0.87 and 5.9 ± 0.85 g respectively; $P < 0.01$). Corpora lutea weighed an average of 4.2 ± 0.15 g. The proportion of healthy oocytes (563/845; 66.6%) recovered from CL+ ovaries was greater than in CL- (504/814; 61.9%; $P < 0.05$) or C (548/873; 62.7%;

Table 1
Oocyte morphometry and activity of the enzyme glucose-6-phosphate dehydrogenase in oocytes from ovaries bearing or not a corpus luteum (CL) at the time of the oocyte recovery.

Ovary	No. of oocytes	Ovary weight (g)	Oocyte morphometry (μm)		BCB+ (%)
			Diameter	Zona pellucida	
CL +	203	10.9 ± 0.19[a]	126.6 ± 0.62[a]	15.1 ± 0.21[b,c]	(163) 80.3[d]
CL -	190	5.9 ± 0.18[b]	124.2 ± 0.59[b]	15.6 ± 0.20[c,d]	(122) 64.2[e,f]
C	158	5.4 ± 0.22[b]	123.6 ± 0.72[b]	16.0 ± 0.25[d]	(110) 69.6[f]

Values with different letters in the same column differ [a, b] $P < 0.01$; [b, d] $P = 0.0131$; [d, e] $P < 0.01$; [d, f] $P = 0.0148$.
Cumulus-oocyte complexes were collected from ovaries of two types of cows: 1) with a CL in one ovary (CL+) and without CL in the contralateral ovary (CL-), and 2) without CL in either of the two ovaries (C). Experiment 1 was completed in 5 replications and 30 ovaries per group.

$P = 0.0519$) . Rate of cleavage was greater in CL+ than in CL- and C ($P < 0.01$) (Table 3). Oocytes recovered from ovaries bearing a CL produced significantly more total embryos on day 7 ($P < 0.05$) and more blastocysts ($P < 0.01$) than CL- and C ovaries (1.13 ± 0.10 versus 0.43 ± 0.14 and 0.55 ± 0.14 embryos per ovary; $P < 0.01$) (Table 3).

Regarding embryonic developmental stages on day 7 of culture, CL+ ovaries produced proportionally fewer morulae and early blastocysts ($P < 0.01$; $P < 0.05$) and more expanded and hatched blastocysts ($P < 0.01$) than CL- and C ovaries (Fig. 1). Rate of expansion and hatching of vitrified-warmed blastocysts at 24 (expanded: 62.2, 69.2 and 83.3% for group CL+, CL- and C, respectively; hatched: 15.5, 15.4 and 8.3% for group CL+, CL- and C, respectively) and 48 h (expanded: 57.7, 30.7 and 33.3% for group CL+, CL- and C, respectively; hatched: 17.7, 15.4 and 16.6% for groups CL+, CL- and C, respectively) of culture were statistically similar among groups.

4. Discussion

Considerable evidence supports the hypothesis that luteal tissue in an ovary enhances quality and developmental competence of oocytes aspired from that ovary [5,7,8], as well subsequent embryonic development [4,6,34,35]. However, several studies reported no effects [11,12] or opposing results [13–15]. Utilizing a different experimental approach, this study was conducted to determine whether the CL influences morphometric and functional characteristics of oocytes and subsequent embryonic development and, if so, whether this effect was local or systemic.

In Experiment 1, two findings supported that a CL stimulates oocyte growth. First, oocytes retrieved from ovaries bearing a CL were significantly larger than those recovered from ovaries without functional luteal tissue [36,37]. Second, a greater proportion of oocytes from CL+ ovaries were metabolically prepared to proceed with maturation than those from ovaries without luteal tissue, as indicated by activity of the enzyme G6PDH [30,38]. These two findings have important functional implications related to the developmental competence of oocytes. Moreover, in Experiment 3, a greater proportion of morphologically healthy oocytes [31] was obtained from ovaries bearing a CL than from those without luteal tissue, irrespective of whether they were contralateral or control ovaries, as previously demonstrated [7,8]. Taken together, these findings are consistent with subsequent results (Experiment 3), in which oocytes collected from CL+ ovaries underwent a greater rate of cleavage and embryo production.

Fair et al. [39] found that bovine oocytes achieved complete meiotic competence when they reached a diameter of 110 μm. Smaller oocytes had significantly less transcriptional activity indicating that they were still in a growing phase [39]. Also, oocytes with diameters of 110–120 and ≥ 120 μm reached MII in similar proportions (76 and 81%, respectively) [39]. Otoi et al. [36] indicated that meiotic competence was attained once oocytes reached 115 μm in diameter, while full developmental capacity was obtained at a size of at least 120 μm. Most oocytes in experiment 1 were ≥120 μm, and therefore, according to findings by Otoi et al. [36], they would have obtained full developmental capacity. It is likely that oocyte diameters of 2.4 and 3 μm greater in CL+ than in CL- and C respectively (Table 1), constituted a functional advantage that allowed CL+ oocytes to attain greater rates of cleavage and blastocyst production as demonstrated in experiment 3. Supporting these findings, nuclear maturation in pigs [40], buffalos [41] and camels [42], and blastocyst production in cows were correlated positively with oocyte diameter [36,43,44].

The zona pellucida plays several important roles during fertilization and early embryonic development. Although no studies were found linking the thickness of the zona pellucida with oocyte competence [45], it was stated that polarized light increased proportionally with increases in thickness of the inner and outermost layers of this structure. A denser ultrastructure and greater birefringence of the zona pellucida impair cumulus-oocyte interaction, and reduce sperm binding and penetration during fertilization, affecting negatively the rate of success [46]. Instead, lower birefringence of the zona pellucida in immature oocytes was associated with greater rates of cleavage and embryo production [47,48]. Furthermore, BCB+ immature oocytes of bovines had lower birefringence [47,48] than their counterpart BCB- oocytes, and achieved greater cleavage and embryo rates [48]. These findings are consistent with those observed in Experiment 1, in which CL+ oocytes

Table 2
Oocyte morphometry and rate of oocyte maturation from ovaries bearing or not a corpus luteum (CL) at the time of the oocyte recovery.

Ovary	No. of oocytes	Ovary weight (g)	Oocyte morphometry after maturation (μm)		Rate of nuclear maturation (%)
			Diameter	Zona pellucida	
CL +	271	11.1 ± 0.19[a]	126.0 ± 0.49[a]	15.9 ± 0.19[a]	(151) 55.7[a]
CL -	315	8.0 ± 0.27[b]	126.7 ± 0.73[a]	15.6 ± 0.28[a]	(158) 50.1[a]
C	265	7.8 ± 0.29[b]	127.0 ± 0.79[a]	16.0 ± 0.30[a]	(134) 50.6[a]

Values with different letters in the same column differ [a, b] $P < 0.01$.
Cumulus-oocyte complexes were collected from ovaries of two types of cows: 1) with a CL in one ovary (CL+) and without CL in the contralateral ovary (CL-), and 2) without CL in either of the two ovaries (C). Experiment 2 was completed in 6 replications and 48 ovaries per group.

Table 3
Rate of cleavage and embryo development from ovaries bearing or not a corpus luteum (CL) at the time of the oocyte recovery.

>Ovary	Oocytes (n)	Cleavage (n) %	Total embryos on day 7 * (n) %	Blastocyst ** (n) %
CL +	506	(323) 63.8[a]	(126) 39.0[b]	(86) 68.2[a]
CL -	417	(180) 43.2[b]	(49) 27.2[c]	(16) 32.6[b]
C	444	(208) 46.6[b]	(62) 29.8[c]	(24) 38.7[b]

Values with different letters in the same column differ: [a, b] $P < 0.01$; [b, c] $P < 0.05$.
*Correspond to percentages calculated out of the cleaved embryos.
**Correspond to percentages calculated out of total embryos on day 7.
Cumulus-oocyte complexes were collected from ovaries of two types of cows: 1) with a CL in one ovary (CL+) and without CL in the contralateral ovary (CL-), and 2) without CL in either of the two ovaries (C). Experiment 3 was completed in 13 replications and 104 ovaries per group.

had a thinner zona pellucida and a greater proportion of them showed low activity of the G6PDH, and with results of Experiment 3, in which these oocytes showed a greater developmental capacity in terms of cleavage and yield of blastocysts.

Activity of the G6PDH enzyme was lower in oocytes recovered from ovaries bearing a CL than those without a CL. This enzyme, involved in the pentose phosphate pathway, has high activity in growing oocytes, but low activity in oocytes that have completed the growth phase [49]. During the growing phase G6PDH is involved in producing NADPH for lipid synthesis [50]. Greater amounts of cytoplasmic lipids were associated with more mitochondrial activity [51] and mtDNA content [52] in BCB+ oocytes. Also, following *in vitro* maturation, BCB+ bovine oocytes had greater peripheral distribution of cortical granules than BCB- oocytes [53], indicating usefulness of the BCB method for selecting fully grown oocytes for *in vitro* embryo production [30,52,54]. According to Alm et al. [30] the proportion of bovine oocytes attaining MII after *in vitro* maturation was greater in BCB+ than in BCB- oocytes. Furthermore, BCB+ bovine [54] and porcine [55] oocytes were significantly larger than those that remained colorless. Numerous studies have reported a greater developmental competence of BCB+ oocytes compared with those BCB- in terms of cleavage rate and blastocyst yield [30,38]. In agreement with previous reports, BCB+ oocytes from CL+ ovaries were larger before *in vitro* maturation and reached a greater cleavage rate and blastocyst development than oocytes obtained from ovaries without a CL.

Before IVM (experiment 1), oocytes from CL+ ovaries had a greater diameter, less thickness of the zona pellucida and were metabolically more advanced (as indicted by activity of G6PDH enzyme). Nevertheless, after IVM (experiment 2), oocyte diameter and zona thickness did not differ among groups and were not associated with an improved nuclear maturation rate in the CL+ oocytes. These differences are not easy to explain, especially because no publications were found assessing these two morphometric characteristics before and after IVM. However, it has been noted that the zona pellucida undergoes changes in its ultrastructure and birefringence [47,48]. Accordingly, during IVM morphological and physical properties of the zona pellucida change from a fairly compact structure with high birefringence to a more porous one exhibiting lower birefringence [47]. Likely, changes in the ultrastructural characteristics of the zona pellucida during maturation could explain the variation of these two oocyte measurements when results of experiments 1 and 2 are analyzed together. However, additional studies are necessary to elucidate changes occurring in these characteristics in bovine oocytes before and after IVM.

Even though our data failed to show a statistical difference in nuclear maturation rate between oocytes from ovaries with or without a CL (experiment 2), there was at least a 5% difference in nuclear maturation in favor of the CL+ oocytes. As in our study, rate of IVM did not differ between buffalo oocytes recovered from CL+ and CL-ovaries (38.4 versus 40.0% respectively) [56]. Given that no specific studies were found about the influence of bovine CL on nuclear maturation, we compared our data with studies in which IVM was performed in slaughterhouse bovine oocytes categorized by the BCB method. Accordingly, rate of nuclear maturation in the present study was considerably lower than that indicated by Lamas-Toranzo [52], but greater than that reported by Silva et al. [53]. Furthermore, although nuclear maturation rate was statistically similar between BCB- and BCB+ oocytes, the developmental ability of BCB+ oocytes after IVF was clearly superior, with greater rates of cleavage and blastocyst production [52]. Ultimately, the better demonstration of the increased developmental competence of CL+ oocytes shown in Experiment 1 was corroborated in Experiment 3, in which CL+ oocytes had greater rate of cleavage and yielded more blastocysts than CL- oocytes. Under the experimental conditions of the present study, it was not possible to know the specific cause of the lower rates of nuclear maturation observed in experiment 2. As a different batch of ovaries was obtained in a local slaughterhouse, the quality of the oocytes may have been affected by nutritional, physiological/endocrine or health status, or the age of cows at slaughter [57–63].

Several studies have associated the presence of a CL or the luteal phase of the estrous cycle with obtaining oocytes of better quality and with greater rates of blastocyst production [2,5,6,8,34,35]. For instance, Boediono et al. [34], showed that bovine ovaries bearing a CL at the time of collecting oocytes produced significantly more blastocysts than those from the follicular phase or that did not contain a CL. Pirestani et al. [35] categorized bovine ovaries into three groups: A) ovaries with a large follicle, B) ovaries with at least one CL and C) those with neither a large follicle nor a CL. Ovaries of groups A and B produced significantly more blastocysts than ovaries without either structure (52.3, 54.8 and 32.9% for groups A, B and C, respectively). Also, oocytes collected from cyclic cows (with a CL in the ovary) underwent greater rate of cleavage and yield more 8-cell and 16-cell embryos than the counterpart acyclic cows (without CL in either ovary) [64]. In the current study, greater cleavage rate was associated with presence of a functional CL, as

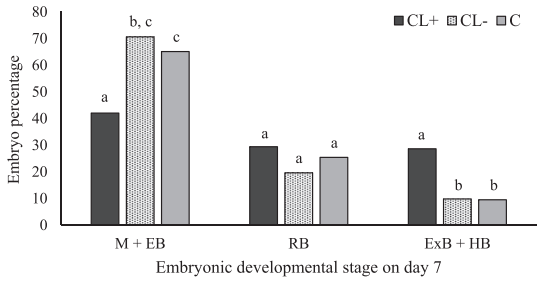

Fig. 1. Developmental stage of embryos obtained from ovaries bearing or not a corpus luteum at the time of the oocyte recovery ([a, b] $P < 0.01$; [a, c] $P < 0.05$). M: morulae; EB: early blastocysts; RB: regular blastocysts; ExB: expanded blastocysts; HB: hatched blastocysts.

described by Ireland et al. [29], which resulted in the production of proportionally more total embryos and blastocysts. In addition, as shown in Fig. 1, luteal tissue seems to accelerate embryonic development, as on day 7 of culture there were fewer morulae and early blastocysts and more regular, expanded and hatched blastocysts from CL+ ovaries than from ovaries without CL.

The precise mechanism(s) by which a CL affects the ability of oocytes to mature, to cleave and to reach advanced stages of embryonic development after *in vitro* fertilization is still unknown. Even though it is unclear whether a CL influences oocyte developmental competence by either intraovarian or systemic interactions or both, the outcomes of our study indicate that the influence of CL occurs through intraovarian mechanisms, because the morphometric and functional characteristics of the oocytes prior to maturation and the rates of cleavage and embryo production were quite similar among contralateral and control ovaries.

Several mechanisms associated with regulation of development of oocytes and embryos are mediated by P4 [reviewed by Ref. [65]]. It is known that molecular interactions between the oocyte and surrounding granulosal cells are indispensable to regulate oocyte growth and maturation [66]. In cattle, P4 receptor (PR) is expressed in the cumulus oocyte complex and expression of this protein is subject to significant changes after *in vitro* maturation and in response to supplementation with LH, FSH and P4 [16]. Inhibition of P4 synthesis by cumulus cells *in vitro* with trilostane, an inhibitor of 3 beta-hydroxysteroid dehydrogenase, decreased bovine embryo development, and demonstrated that P4 intracellular signaling is mediated by interactions with nuclear and membrane PR. These finding have important implications for acquisition of developmental competence by oocyte and further development of blastocysts [16]. Apparently, a mechanism by which P4 could regulate the acquisition of developmental competence of oocytes may be based on its capacity to inhibit apoptosis [67], as blockage of PR in COC induced increased caspase 9 and caspase 3 / 7 activities [68]. Additional amounts of progesterone may be available within the CL+ ovary through the countercurrent mechanism (retrograde transfer of P4 and other hormones) demonstrated in several species of domestic animals [69–71]. In fact, since the 1960s, it has been known that concentrations of P4 measured in the ovarian vein ipsilateral to the ovary containing a CL were greater compared to those in the contralateral ovarian vein (draining an ovary without a CL) (Erb et al., 1962). Consistent with those concepts, a significantly greater concentration of P4 in follicular fluid of medium and large follicles was reported in bovine CL+ ovaries than those lacking a luteal structure [28].

On the other hand, as the bovine CL is highly vascularized [17,18] increased blood flow to a CL+ ovary could improve distribution of nutrients, hormones and growth factors into the surrounding tissue, which in turn may favor oocyte development and follicular growth. Moreover, luteal tissue is composed of different types of cells: luteal cells, smooth muscle cells, pericytes, fibrocytes and immune cells, indicating that the CL is a heterogeneous tissue [72]. This mix of cells produces several substances such as insulin-like growth factor [19], vascular endothelial growth factor [20], angiotensin II [21], and prostaglandins [22], which are involved in regulation of oocyte growth and developmental capacity [23–27]. Interestingly, P4, angiotensin II and prostaglandins, 3 substances produced by CL, mediate the resumption of meiotic progression in bovine oocytes [73].

From a systemic point of view, low circulating concentrations of progesterone prior to AI have been associated repeatedly with poor fertility and have been identified as a very important factor causing low conception rates in cattle [74]. Reduced P4 concentration during growth of the first follicular wave affected *in vitro* embryo quality [9], and reduced pregnancy after AI in lactating dairy cows [10]. Thus, circulating progesterone plays an essential role in creating favorable conditions to assure survival of early embryos in the uterus [74]. In the current study, we used an experimental approach to assess whether the influence of CL (if any) was local or systemic. For this purpose, we used two types of ovaries without CL to make comparisons: 1) contralateral ovary to that containing a CL, which according to CL morphology and appearance [29] was exposed to the elevated circulating concentrations of progesterone typical of diestrum, and 2) ovary not bearing a CL obtained from cows without CL in either ovary, which was not exposed at all to luteal progesterone. In light of the current findings, influence of a CL occurred through local mechanisms, because either type of ovary without CL showed similar outcomes in terms of oocyte competence and subsequent embryo production at day 7 of culture. As stated previously, two mechanisms may explain the results of the two types of ovaries without CL (CL- and C): 1) they lacked the positive effect of an increased blood flow demonstrated in ovaries with CL [17,18], in terms of availability of greater amounts of growth factors, hormones and other important substances for follicular and oocyte development, and/or 2) they did not have the positive influence of numerous substances produced by CL [19–22] that have been shown to stimulate oocyte development capacity [22,27], and as a consequence, to improve quality and quantity of blastocysts.

In addition to what was previously discussed, considering P4 as the central component of the positive actions of CL, it is currently not clear how CL influence developmental capacity of oocytes in a local/paracrine manner (further research is needed to elucidate this topic). The simplest and more likely answer to this issue would be that there is a greater likelihood of harvesting better quality oocytes when there are greater concentrations of P4, either systemically or locally [9,10,64,74]. It is plausible that this physiological context improves follicular environment [75], changes follicular fluid composition [76], improves blood flow and/or inhibits follicular atresia [77,78]. Even so, local concentrations of progesterone, produced by the cumulus cells, may stop cumulus cell apoptosis through different intrafollicular pathways [78], increasing ability of oocytes to grow and eventually become transferable embryos. Thus, considering the evidences indicated above, lower oocyte competence in the CL- and C groups, may be explained by low or absent amounts of intraovarian progesterone as hypothesized by Cerri et al. [76], and suggested by current findings.

In sheep, CL not only increased cleavage rate and blastocyst production, by oocytes from that ovary, but also improved the proportion of hatched blastocysts after vitrification [4]. Even though a positive effect of CL on oocyte developmental competence and subsequent embryo production was observed in this study, as occurred in sheep, no influence of the CL was observed after vitrification/warming, as the rates of blastocyst expansion and hatching did not differ among groups. Two reasons could explain this inconsistency: 1) the positive effect of CL on oocyte competence and embryonic development was not intense enough to be evident in cryotolerance; 2) and more likely, the number of embryos in groups CL- and C were not sufficient to allow a solid statistical analysis in this aspect of the present research.

General, these data support the hypothesis that a CL influences the developmental capacity of bovine oocytes. During the reproductive cycle, various biological mechanisms interact and cooperate in order to achieve the purposes of reproductive function: fertilization, gestation and birth of a healthy offspring assuring survival of the species. Thus, it is logical that luteal tissue interacts with follicles through local and systemic mechanisms that stimulate oocyte developmental competence and subsequent embryonic development, as demonstrated in this and other studies.

In conclusion, oocytes collected from ovaries bearing a CL were larger and a greater proportion of them had completed the growth

phase than those lacking a CL. In addition, CL+ ovaries yielded healthier oocytes, had greater rates of cleavage, and produced more total embryos and more advanced blastocysts on day 7 than ovaries without luteal tissue, irrespectively of whether these were contralateral (CL-) or control ovaries. These findings indicate that CL influenced oocyte developmental competence and embryo development likely through intraovarian interactions.

CRediT authorship contribution statement

Daniel E. Argudo: Writing - original draft, Methodology. **Milton A. Tenemaza:** Investigation, Data curation. **Shirley L. Merchán:** Investigation, Data curation. **José A. Balvoa:** Investigation, Data curation. **Maria S. Méndez:** Conceptualization, Validation. **Manuel E. Soria:** Conceptualization, Resources, Validation. **Luis R. Galarza:** Resources, Software. **Luis E. Ayala:** Formal analysis, Supervision. **Hugo J. Hernández-Fonseca:** Project administration, Supervision. **Mariana S. Perea:** Visualization, Investigation. **Fernando P. Perea:** Writing - review & editing, Formal analysis.

Declaration of competing interest

The authors have no conflicts of interest to declare.

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