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ORIGINAL ARTICLE

Influence of corpus luteum and ovarian volume on the number and quality of bovine oocytes

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ABSTRACT

In order to evaluate whether ovarian volume, presence and diameter of the corpus luteum (CL) have effects on the number and quality of bovine recovered oocytes, 110 ovaries were obtained from the slaughterhouse. Cumulus oocytes complex were aspirated and evaluated under stereomicroscope. Oocytes were counted and classified according to their quality (Grades I, II, III and IV). Ovarian volume was weakly correlated to the number of good quality oocytes ($P < 0.05$). Ovaries with CL showed greater numbers of good quality oocytes than ovaries without CL ($P < 0.05$). Further, presence of CL and its diameter positively influenced the probability of recovering good quality oocytes ($P < 0.05$). In conclusion, ovarian volume is not a good parameter itself to predict important ovarian characteristics; moreover, analysis of CL, its presence and diameter, may be a good tool to improve efficiency on *in vitro* embryo production programs.

Key words: *bovine reproduction, embryo production, ovary, reproductive efficiency.*

INTRODUCTION

Despite the great advances achieved in the biotechnology of reproduction, the productive efficiency of transferable embryos, derived from *in vitro* fertilization (IVF), is still low (de Wit *et al.* 2000; Havlicek *et al.* 2010). There are strong indications that this fact is more related to the source of oocytes than to the conditions of IVF and culture (Bols *et al.* 1997) or due to the method of *in vitro* maturation of collected oocytes (Leibfried-Rutledge *et al.* 1987).

According to Reis *et al.* (2002), Simmental heifers have a higher number of collected oocytes when the corpus luteum (CL) is active. Furthermore, the presence of CL in Holstein cows was reported as required in order to improve *in vitro* embryo production (Reis *et al.* 2006) and Manjunatha *et al.* (2007) found higher cleavage and transferable embryo rates from ovaries with CL. Conversely, de Wit *et al.* (2000) showed no difference in the quality and number of oocytes collected from abattoir ovaries in the follicular, early luteal and late luteal phases.

The following studies indicate that plasma progesterone affects oocyte quality (Leibfried-Rutledge *et al.* 1987; Hendriksen *et al.* 2004; Pfeifer *et al.* 2009, 2011); oocytes collected in the late phase of diestrus are more competent than oocytes collected in early diestrus or follicular phase in which progesterone levels are lower (Machatkova *et al.* 1996, 2004; Pfeifer *et al.* 2009). Progesterone allows the follicle to be exposed for a longer period to luteinizing hormone (LH) pulses of low amplitude, getting a better quality oocyte (Greve *et al.* 1995; Pfeifer *et al.* 2009).

Ovarian volume shows correlation to some important ovarian aspects, such as number and quality of oocytes. Thus, the ovarian volume may be used in attempting to establish a parameter for clinical examination of the ovaries (rectal palpation) and to associate with other important ovarian characteristics (Fernandes *et al.* 2001).

The visual evaluation in relation to compaction, number and appearance of the cumulus cells and the uniformity of the cytoplasm, have been used as a way to select and qualitatively classify the oocytes (Pavlok *et al.* 1992; Moreno *et al.* 1993). Cumulus cells may control oocyte meiotic arrest and this control may be exerted via the cumulus processes that penetrate the zona pellucidae and interact with the oocyte through the gap junctions (de Wit *et al.* 2000).

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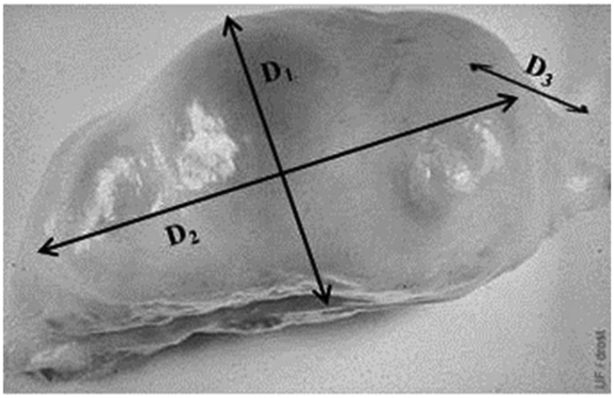

Figure 1 Schematization of measurement of the ovarian volume.

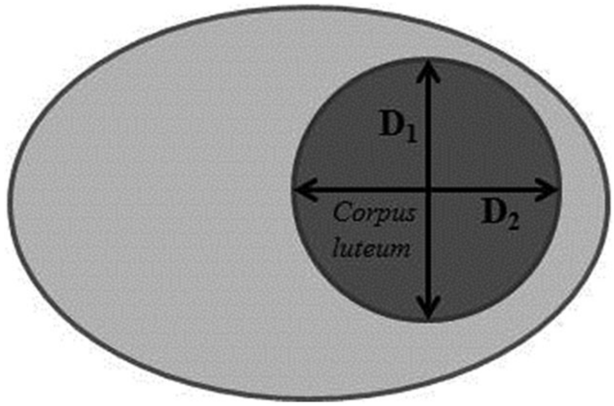

Figure 2 Schematization of measurement of the corpus luteum diameter.

Thus, this study aimed to evaluate whether ovarian volume, and CL presence and diameter would influence the number and the quality of the recovered bovine oocytes.

MATERIALS AND METHODS

Collection of ovaries

The experiment was conducted at the Animal Reproduction Laboratory, Department of Animal Science, Universidade Federal de Viçosa/MG, Brazil.

One hundred and ten ovaries from crossbred females (*Bos taurus taurus* × *Bos taurus indicus*) without defined age were collected from the slaughterhouse. The period between slaughter and collection of the ovaries did not exceed 20 min and they were transported to a laboratory in thermos flasks with saline at 38°C.

Assessment of ovarian volume and CL diameter

With a caliper, the diameter of each ovary was measured in three axes (Fig. 1). The volume of the ovary (1) was calculated from the formula for the sphere volume and expressed in cm^3.

$$\text{Volume of the ovary} = \frac{4}{3} \times \pi \times \left(\frac{D_1}{2}\right) \times \left(\frac{D_2}{2}\right) \times \left(\frac{D_3}{2}\right) \quad (1)$$

The diameters of the CL were also measured with a caliper, taking measurements on two axes (Fig. 2). The diameter was obtained by the arithmetic mean of two measurements (D_1 and D_2) and expressed in mm (2).

$$\text{Diameter} = \frac{(D_1 + D_2)}{2} \quad (2)$$

Follicular aspiration, screening and classification of the oocytes

The cumulus oocyte complexes (COCs) were aspirated from the follicles (2–8 mm diameter) using a 10 mL hypodermic syringe with an 18G needle. Immediately after aspiration, follicular fluid was placed in a Petri dish for screening oocytes under a stereomicroscope at 30× increase. The COCs were counted and classified according to Stringfellow and Givens (2010):

- Grade I: oocytes with compact cumulus and more than three layers of cells. Ooplasm with fine and homogeneous granules, filling the interior of the zona pellucidae, and brown color.
- Grade II: oocytes with less than three layers of cumulus cells. Ooplasm with heterogeneously distributed granules that may be more concentrated in the center and lighter on the periphery, or condensed in one place appearing as a dark spot. The ooplasm filled the entire space inside the zona pellucidae.
- Grade III: Oocytes have cumulus, but it was expanded. Ooplasm was contracted, with space between the cell membrane and zona pellucidae, irregularly filling the perivitelline space.
- Grade IV: oocytes without cumulus cells, cytoplasm with abnormal color and granulation or expanded and apoptotic cells.

Statistical analysis

For data analysis, the Statistical Analysis System (SAS 2002) was used. The ovarian volume and numbers of Grade I, II, III and IV oocytes with and without CL were compared by analysis of variance (PROC ANOVA). The association between the proportions of Grade I, II, III and IV oocytes and the presence of CL in the ovary was evaluated by chi-square test (PROC FREQ). The correlation among ovarian volume and CL diameter with the number of recovered oocytes was performed by Pearson's correlation (PROC CORR), and for better understanding of the relationship between variables, linear regression analysis (PROC REG) was done.

To evaluate the effect of the CL presence and diameter and ovarian volume on the probability of recovering good quality oocytes, a logistic regression was used (PROC LOGISTIC). The significance level was $\alpha = 0.05$.

RESULTS

As expected, ovaries with CL had great volume compared to those without CL. A total of 975 oocytes were recovered and analyzed, being the average per ovary equal to 8.86 ± 0.71, and the ovaries with CL showed a greater number of recovered oocytes and higher number of good quality oocytes (Table 1).

Table 1 Ovarian volume (cm^3) and number of Grade I, II, III and IV oocytes among ovaries with and without corpus luteum (CL) (mean ± standard error of mean)

Variables	Ovaries without CL (*n* = 59)	Ovaries with CL (*n* = 51)	*P*-value
Ovarian volume	5.73 ± 0.39^b	10.03 ± 0.58^a	<0.0001
Grade I	0.93 ± 0.18^b	1.67 ± 0.24^a	0.0158
Grade II	1.22 ± 0.21^b	1.88 ± 0.27^a	0.0486
Grade III	2.41 ± 0.39^a	3.22 ± 0.46^a	0.1827
Grade IV	2.86 ± 0.36^a	3.76 ± 0.70^a	0.2363
Total	7.42 ± 0.74^b	10.53 ± 1.24^a	0.0283
Grade I + II	2.15 ± 0.36^b	3.55 ± 0.43^a	0.0144

Different letters in the same row differ at $P < 0.05$.

Table 2 Proportion of Grade I and II oocytes (good quality) and Grade III and IV (poor quality) oocytes in ovaries with and without corpus luteum (CL)

Oocytes	Ovaries without CL	Ovaries with CL
Grade I + II	29.0% (127/438)	33.7% (181/537)
Grade III + IV	71.0% (311/438)	66.3% (356/537)

$P > 0.05$.

Table 3 Pearson's correlation of ovarian volume and corpus luteum (CL) diameter with the number and quality of recovered oocytes

	GI	GII	GIII	GIV	Total	GI+GII
Vol. ov.	0.35*	0.25*	0.36*	0.18^{ns}	0.39*	0.33*
Diam. CL	0.16^{ns}	0.18^{ns}	-0.04^{ns}	-0.16^{ns}	-0.03^{ns}	0.20^{ns}

Vol. ov. = volume of the ovary; Diam. CL = diameter of the corpus luteum; GI = Grade I; GII = Grade II; GIII = Grade III; GIV = Grade IV. *$P < 0.05$; ^{ns} not significant ($P > 0.05$).

There was no association ($P > 0.05$) between CL presence and oocyte quality. Furthermore, proportion of Grade I and II (good) and Grade III and IV (poor) oocytes was not affected by the presence of CL ($P > 0.05$; Table 2).

The CL diameter was not correlated with the number of recovered oocytes; however, ovarian volume showed positive mild correlation with the number of Grade I oocytes (Table 3).

Linear regression analysis was significant between ovarian volume and the number of Grade I oocytes and number of Grade I and II oocytes. The functions that best fit the model were a quadratic function for both (3, Figure 3; 4, Figure 4; respectively).

$$\text{N° GI} = 0.75178 + 0.00678 \times Vol.ov^2 \quad (3)$$

where:
GI = Grade I oocytes; Vol.ov = volume of the ovary (cm^3).

$$\text{N°GI} + \text{GII} = 1.90829 + 0.0116 \times Vol.ov^2 \quad (4)$$

where:
GI + GII = Grade I and II oocytes; Vol.ov = volume of the ovary (cm^3).

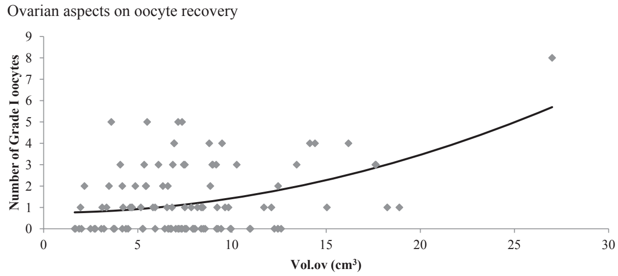

Figure 3 Number of Grade I oocytes according to ovarian volume ($R^2 = 0.16$; $P < 0.05$).

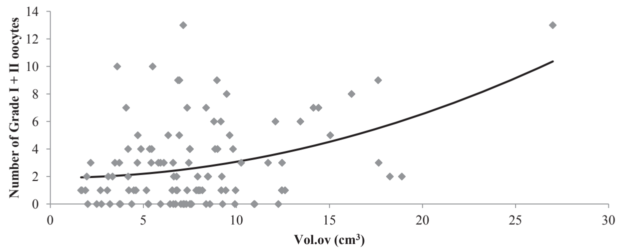

Figure 4 Number of Grade I and II oocytes according to ovarian volume ($R^2 = 0.14$; $P < 0.05$).

Table 4 Probability to obtain Grade I or II oocytes in ovaries with and without corpus luteum (CL)

Probability (%) to obtain at least:	Ovaries		*P*-value
	With CL	Without CL	
1 Grade I oocyte	59.3	36.4	0.0359
2 Grade I oocytes	30.8	19.1	0.1694
3 Grade I oocytes	13.9	8.5	0.2966
1 Grade I or II oocytes	82.1	65.7	0.0991
2 Grade I or II oocytes	58.3	34.2	0.0264
3 Grade I or II oocytes	49.2	24.1	0.0129
4 Grade I or II oocytes	32.0	14.6	0.0347
5 Grade I or II oocytes	14.0	7.6	0.1983

The low coefficients of determination ($R^2 = 0.16$ and $R^2 = 0.14$, respectively) make clear that ovarian volume is not by itself a reliable parameter for predicting the number of good quality recovered oocytes. Despite there being no association between presence of CL and proportion of good and poor oocytes by chi-square test, when data were analyzed by logistic regression analysis it is apparent that the presence of CL has a greater influence on the probability to recover good quality oocytes than ovarian volume.

While ovarian volume did not influence the likelihood of recovering good quality oocytes ($P > 0.05$), the presence of CL increased by 2.5 times the chance of getting at least one Grade I oocyte and 2.8 times the chance of getting at least four Grade I or II oocytes (Table 4).

The CL diameter also influenced the probability of obtaining good quality oocytes. The larger the CL diameter, the greater the probability of obtaining at

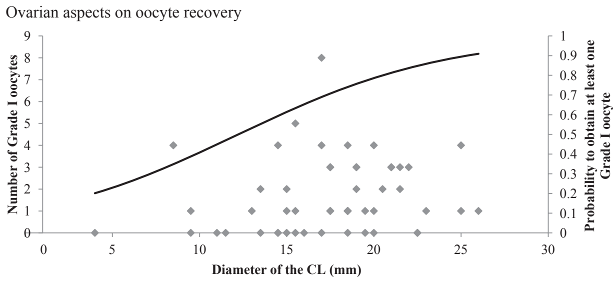

Figure 5 Dark line: probability to obtain at least one Grade I oocyte according to corpus luteum (CL) diameter. Gray points: number of recovered Grade I oocytes. $\text{Probability value} = \frac{e^{(-2.0456)+(0.1673\times\text{diameter})}}{1+e^{(-2.0456)+(0.1673\times\text{diameter})}}$.

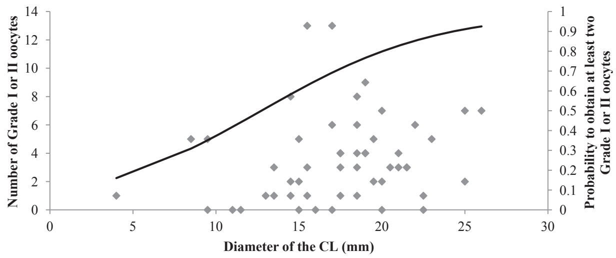

Figure 6 Dark line: probability to obtain at least two Grade I or II oocytes according to corpus luteum (CL) diameter. Gray points: number of recovered Grade I or II oocytes. $\text{Probability value} = \frac{e^{(-2.4161)+(0.1897\times\text{diameter})}}{1+e^{(-2.4161)+(0.1897\times\text{diameter})}}$.

least one Grade I oocyte and at least two Grade I or II oocytes ($P < 0.05$, Figs 5,6).

DISCUSSION

The use of ovarian volume to estimate gonadal function is limited, as the follicles are randomly arranged in the ovarian parenchyma and are not always detected by rectal palpation. Furthermore, correlation between ovarian volume and total oocytes in this study were low, indicating a marked influence of other factors. Therefore, other aspects become relevant, such as the category or type of ovarian follicle. Most of the follicles in this study were from a heterogeneous population with less than 8 mm. The developmental dynamics of these follicles is dependent on factors such as the presence of a dominant follicle in the ovary (Adams *et al.* 1993; Varisanga *et al.* 1998), mitotic index in granulosa cells (Lussier *et al.* 1987) and day of the estrous cycle (Vassena *et al.* 2003) among others. Moreover, the profile of ovarian activity can affect these correlations, such as variation in the size and growth rate of follicles between follicular waves (Viana *et al.* 2000). Regarding to the presence of a dominant follicle, it was reported that in cattle the developmental competence of oocytes from small antral follicles is not adversely affected by the presence of a dominant follicle (Smith *et al.* 1996) despite Varisanga *et al.* (1998) having found that the presence of a dominant follicle in either one or both ovaries of a pair has a negative effect on the IVF-produced bovine embryos. According to Vassena *et al.* (2003) no differences were detected in the proportion of either healthy or degenerate oocytes collected from the ovary containing the dominant follicle or from the contralateral ovary; however, fewer denuded oocytes were collected from the ovary containing the CL than from the contralateral ovary.

Another important factor refers to body condition of the cow; according to Dominguez (1995), the presence of follicles greater than 5 mm and the quality of the oocytes increase with body condition.

The influence of CL on bovine oocyte recovery rate and quality has been reported with variable results.

The CL presence may improve the quality of recovered oocytes due to progesterone production. Although the progesterone level was not evaluated in this study, several studies have demonstrated the relationship between CL and progesterone serum levels (Baruselli *et al.* 2001; Bo *et al.* 2002; Ferreira *et al.* 2006). Progesterone allows that the follicle be exposed for a longer period to low-amplitude LH pulses, getting a better quality oocyte (Greve *et al.* 1995; Pfeifer *et al.* 2009, 2011).

Reis *et al.* (2002) found in Simmental heifers higher numbers of recovered oocytes when the CL was active in the ovary; further, Reis *et al.* (2006) reported that the presence of CL in Holstein Friesian cows is fundamental to improve important characteristics in *in vitro* embryo production, such as number of collected oocytes from both ovaries, percentage of viable oocytes and cleaved zygotes and number of embryos at 7 days after *in vitro* fertilization.

Ovaries from the slaughterhouse showed higher rates of cleavage and transferable embryos when they had CL (Manjunatha *et al.* 2007). However, in crossbred bovine females, also with ovaries obtained from a slaughterhouse, the presence of CL did not cause significant changes in the number and quality of recovered oocytes (de Wit *et al.* 2000).

Extrapolation of the results found in this study for conditions of an IVF program should be done cautiously. Nevertheless, it is possible to hypothesize that the use of exogenous progesterone or maintenance of a functional CL in bovine females may provide efficient gains in embryo production, including commercial programs.

Conclusions

Since the results reported by other authors do not allow definitive conclusions about the effect of the presence of CL on the number and quality of recovered oocytes, as well on the efficiency of *in vitro* embryo production, it is possible to say that the question regarding the effects of the CL presence on follicular development, oocyte recovery and efficiency of *in vitro* embryo production, still deserves attention, requiring more research.

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