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Influence of Dominant Follicle and Corpus luteum on Recovery of Good Quality Oocytes for *In vitro* Embryo Production in Cattle

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Authors' contributions

This work was carried out in collaboration between all authors. Author JMPF paper conception and edition. Authors EC, FAO, AMZ, CTO and ÍACS paper edition and correction. Author CAAT supervisor and final edition. All authors read and approved the final manuscript.

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ABSTRACT

The quality of oocytes is more critical for the success of bovine *in vitro* embryo production (IVP) than the conditions of *in vitro* culture of these structures. Despite knowledge about bovine follicular development, especially gonadotropin-dependent phase, be considerably wide, knowledge about effects of the estrous cycle phase, follicular or luteal, on recovery

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of oocytes may strongly contribute to improve IVP efficiency. Previous efforts to find the proper *cumulus*-oocyte complex (COC) *in vitro* maturation conditions did not improve this technique at level of the *in vivo* embryo production. In physiological conditions the oocyte to be fertilized is donated by healthy follicles during a specific stage of the estrous cycle. However, COCs collected for IVP are obtained from follicles disregarding the follicular phase; consequently, they may have been subjected to different levels of estradiol, progesterone, FSH and LH. Plasma progesterone affects oocyte quality probably because it allows the follicle is exposed for a long period to low amplitude LH pulses, resulting in good quality oocyte. Besides, the presence of a functional corpus luteum (CL) in the ovary may interfere with the amount and quality of oocytes, CL is related to a high vascularization of the ovary which can propitiate an optimal hormonal and nutritional environment for developing follicles. Moreover, the developmental ability of oocytes from small follicles can be influenced by a presence of dominant follicle. Therefore, the ovarian condition of regularly cyclic females needs to be a concern in collection of oocytes for commercial IVP.

Keywords: Estrous cycle; in vitro embryo production; progesterone; reproductive efficiency.

1. INTRODUCTION

In vitro embryo production (IVP) is still inefficient when compared to the *in vivo* production, according to Leibfried-Rutledge et al. [1] the *in vitro* oocyte maturation is the most important step for IVP. Some authors support the idea that the quality of oocytes can be more important for the IVP success than the *in vitro* culture conditions of these structures [2].

Previous efforts to find the proper maturation conditions did not improve this technique at level of the *in vivo* embryo production [3]. Furthermore, studies have been developed to distinguish the competent *cumulus*-oocyte complexes (COCs) from incompetent COCs [4]. These studies led to the conclusions: 1) The partial or total loss of *cumulus* reduces rates of development [5]; and 2) That “better looking” COCs (oocytes with multilayer and compact *cumulus*) do not necessarily have the greatest development capacity [6]. However, the characteristics of a relevant developing COC are not yet fully known [4].

In vivo, the oocyte to be fertilized is donated by healthy follicles during a specific stage of the estrous cycle. Nevertheless, COCs collected for IVP are obtained from follicles disregarding the follicular phase and estrous cycle. As a result, they may have been subjected to different levels of estradiol, progesterone, FSH and LH. These factors may affect the ability of *in vitro* development of the COC [4].

2. CORPUS LUTEUM AND PROGESTERONE

Corpus luteum (CL) is an endocrine gland that is formed in the ovary after ovulation and contributes to regulate estrous cycle and maintenance of pregnancy. The main function of the CL is to secrete progesterone (P4) during the estrous cycle and pregnancy [7].

The presence of a functional CL in the ovary may interfere with the quantity and quality of bovine oocytes [8]; however, with varying results.

Dairy cows with CL showed better performance than cows without CL in relation to the number of collected oocytes from both ovaries and better quality oocytes [9]. Furthermore, the presence of CL may be associated with increased production of embryos [10,11].

In Nelore cows without CL, but treated with P4 implants for 5 days and administration of $PGF_{2\alpha}$ on start of the estrous synchronization treatment, a reduction in the total number of aspirated oocytes compared to females containing CL and without P4 implant treatment was noted. Besides, the number of oocytes aspirated from pregnant cows was lower than the amount obtained from non-pregnant cows with CL [12].

Some studies indicate that plasma P4 affects oocyte quality [1,13-15], oocytes collected in late diestrus phase are more competent than oocytes collected in early diestrus or follicular phase where P4 levels are lower [14]. Progesterone allows the follicle be exposed for a long period to low amplitude LH pulses, resulting in better quality of the oocyte [14,16].

In crossbred cows (*Bostaurustaurus* x *Bostaurusindicus*) treatment with P4 implants could increase the availability of follicles for follicular puncture, the amount of total oocytes, the number of good quality oocytes, the overall cleavage rate and blastocysts production [14]. However, Ramos et al. [17] found no advantages for embryo production in synchronizing follicular wave of crossbred heifer donors with P4 implants associated with administration of estradiol benzoate.

It is possible that in addition to the effects on LH pulsatility, CL and P4 can exert a paracrine effect on follicular development, which could explain the differences in quality of collected oocytes between the ovary with a CL and the contralateral in the same female [18].

A local effect of the CL is related to a high vascularization of the ovary. After ovulation, CL develops quickly from rupture of the follicle wall, and within a few days, gradually increases the P4 secretion [19]. The intensity of luteal angiogenic process reaches a peak in two to three days after ovulation [20], which causes an increase in blood flow [21], which, in turn, can propitiate a proper hormonal and nutritional environment for developing follicles.

It is accepted that a CL with larger diameter synthesizes higher progesterone concentrations [22,23]. Besides CL diameter positively influence probability to obtain good quality oocytes [8] (Fig. 1) as well as it is related to higher pregnancy rates [24].

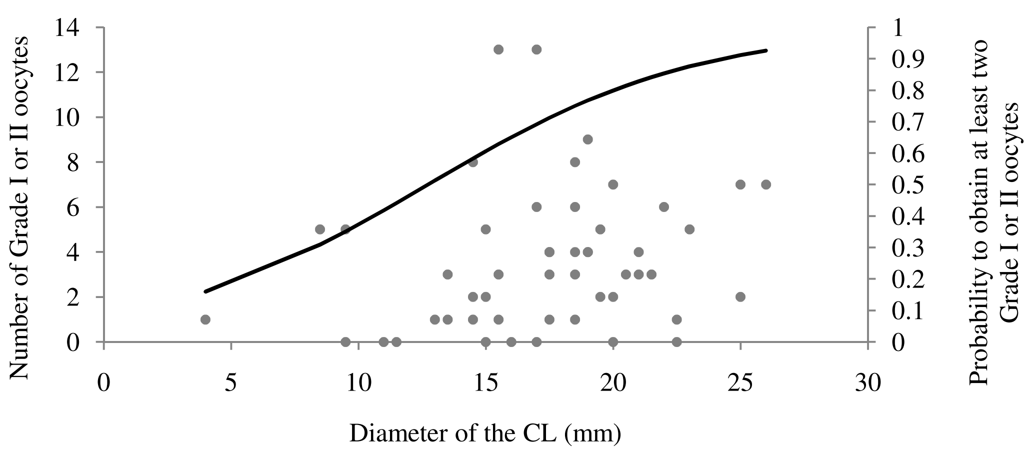

Fig. 1. Dark line: Probability to obtain at least two Grade I or II oocytes according to CL diameter. Gray points: number of recovered Grade I or II oocytes

$Probability\ value = \frac{e^{(-2.4161)+(0.1897\times diameter)}}{1+e^{(-2.4161)+(0.1897\times diameter)}}$. *Data were from Penitente-Filho et al. [8]*

Nevertheless, the absence of CL at the time of follicle aspiration may show favorable aspects such as improved ease of location and puncture of the follicles as well as lower blood aspiration [25].

3. DOMINANT FOLLICLE

Studies have demonstrated that follicular development during estrous cycle of the cow usually occurs in two or three waves [26,27] and for each wave, one follicle becomes dominant while others follicles in the same wave regressed [28]. Thus, the dominant follicle (DF) exerts an inhibitory effect on the growth and development of other follicles [26] (Fig. 2), called subordinate follicles, due to the estradiol and inhibin production, which decrease FSH levels, leading subordinate follicles to atresia [29-31].

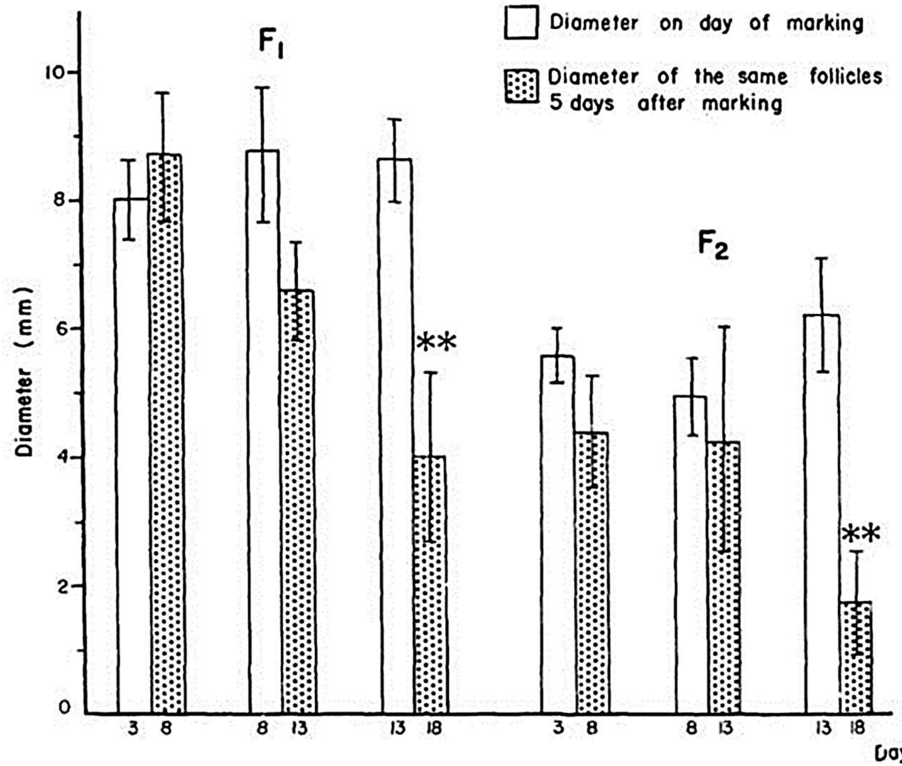

Fig. 2. Changes in size of the largest (F1) and second largest (F2) follicles on each ovary over a period of five days during the cycle (five animals)

***P<0.01 (Wilcoxon matched pair test). Data were from Matton et al. [26]*

The developmental capacity of oocytes from small antral follicles of Holstein heifers was not affected by the presence of a DF [32]. Furthermore, for crossbred cows, there was no difference in the proportion of either healthy or degenerated oocytes collected from the ovary containing the DF or contralateral, although less denuded oocytes were obtained from the ovary containing the CL than the contralateral ovary [18]. However, Varisanga et al. [27] found that the presence of a DF has a negative effect on IVP in Holstein cows.

Some studies suggest that better results for IVP of Holstein cows come from the aspiration of oocytes at the final stages of follicular growth, during the regression of DF [33]; however, according to Machatkova et al. [34], the initial growth phase was more effective for collection of oocytes; besides, the number of oocytes from medium follicles and the developmental capacity of oocytes from small follicles decreased in the dominant phase in Holstein cows (Table 1).

Table 1. Mean (± S.E.M.) numbers of medium follicles, collected oocytes and developed embryos in the growth and dominant phases

Donors	Growthphase (n = 25)	Dominantphase (n = 14)
Total numberofmediumfollicles	288	105
Meannumberofmediumfollicles/donor	11.5±1.8	7.5±0.7
Total numberofisolatedoocytes	259	74
Meannumberofisolatedoocytes/donor	10.4±1.6	5.3±0.6
Total numberofuseableoocytes	240	54
Meannumberofuseableoocytes/donor	9.6±1.4[a]	3.9±0.6[b]
Total numberofembryos	88	15
Meannumberofembryos/donor	3.5±0.6[a]	1.1±0.3[b]
Development rate ofoocytesintoblastocysts (%)	36.7	27.8

Within the same row, values with different superscript letters (a, b) are different (P<0.01). Data were from Machatkova et al. [34]

4. CONCLUSIONS

It seems clear that the question regarding to the effects of the CL presence on follicular development, oocyte recovery and IVP efficiency still deserves attention, requiring further studies [8]. Moreover, the developmental ability of oocytes from small follicles can be influenced by DF. Therefore, the ovarian condition of regularly cyclic females needs to be a concern in the collection of oocytes for industrial production of bovine embryos.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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