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Developmental competence of bovine oocytes: effects of follicle size and the phase of follicular wave on in vitro embryo production

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Abstract

Developmental competence of bovine oocytes collected from follicles of different size categories (in either the growth or the dominant phase of the first follicular wave) was studied, with the aim of improving in vitro embryo production. Estrus and ovulation of 39 cyclic Holstein dairy cows were synchronized by two prostaglandin $F_{2\alpha}$ treatments at 11-day intervals and one hCG treatment on the day of onset of estrus (Day 0). Cows with follicles in either the growth (Day 3, $n = 25$) or the dominant phase (Day 7, $n = 14$) were slaughtered, and follicles >5 mm were counted. Three oocyte populations were recovered separately from large (11–15 mm), medium (6–10 mm) and small (2–5 mm) follicles in both follicular phases. All collected cumulus–oocyte complexes (COC), except for markedly atretic oocytes without cumulus cells, were used in experiments. Oocytes were matured, fertilized and cultured by standard methods. There were no significant differences between the growth and the dominant phases for mean numbers of large follicles, useable oocytes and embryos per donor. Generally, those numbers were low, but the development rates of oocytes into blastocysts were high, particularly in the growth phase (60.0%). Mean (±S.E.M.) numbers of medium follicles, oocytes and embryos per donor were higher in the growth as compared with the dominant phase; in the useable oocytes and embryos, this difference was significant (9.6 ± 1.4 and 3.5 ± 0.6 versus 3.9 ± 0.6 and 1.1 ± 0.3; $P < 0.01$). The development rates of oocytes into blastocysts, however, did not differ significantly between the growth and the dominant phases (36.7% versus 27.8%). Mean numbers of useable oocytes and embryos per donor recovered from small follicles in both follicular wave phases were similar. The development rate of oocytes into blastocysts was generally low, but higher ($P < 0.01$) in the growth than in the dominant phase (24.5% versus 11.7%). Comparison between the two phases showed that mean number of all counted follicles and all useable oocytes collected per donor were similar, but the mean number of embryos per donor and the development rate of oocytes into blastocysts were higher in the growth phase than in the dominant phase (8.0 ± 1.2 versus 3.8 ± 2.4; $P = 0.012$ and 30.3% versus 14.9%; $P < 0.01$). The interaction between follicle size and the phase of follicular wave affected the efficiency of embryo

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production. The yield of embryos was primarily influenced by the number of oocytes collected from medium follicles and the developmental competence of oocytes from small follicles. The growth phase was more effective for oocyte collection; the number of oocytes from medium follicles and the developmental competence of oocytes from small follicles decreased in the dominant phase.

Keywords: Cattle; Follicular wave; Follicle size; Oocyte development

1. Introduction

The developmental potential of in vivo matured oocytes is known to be greater than that of in vitro matured oocytes. It has been demonstrated that the in vitro development of in vivo matured oocytes is twice as high as that of in vitro matured oocytes, because important molecular changes occur during in vivo capacitation [1–3]. The developmental competence of oocytes is acquired gradually and increases with follicular development. To improve developmental potential in vitro, oocytes that have received follicular instructions before they are collected and matured should be used [4].

The ability of an oocyte to develop into an embryo depends on it having enough specific information in the form of mRNA or proteins [5]. If this information is missing or is insufficient, defects in nuclear or cytoplasmic maturation, or in both processes, may arise and thus affect the in vitro development of fertilized oocytes.

There is general agreement that the origin of an oocyte plays an important role [6]. The in vitro meiotic and developmental competence of oocytes is related to follicle size, estrous cycle stage and the level of atresia influenced by other follicles, mainly the dominant follicle [7–10]. Oocytes will acquire an intrinsically greater in vitro developmental capacity if the follicles reach 7 mm in size. On the other hand, significant differences in oocyte developmental potential during the estrous cycle, in terms of presence or absence of a dominant follicle, have been observed irrespective of follicle size. Development to the blastocyst stage was greater when oocytes were obtained during follicular growth, as compared with follicular dominance [10,11].

In our previous work, we have also documented that the developmental competence of oocytes collected by transvaginal aspiration from living donors is related to the stage of follicular wave [12]. In this study we examined the interaction between the stage of follicular development and follicle size, in relation to the efficiency of embryo production. We evaluated not only qualitative parameters such as the developmental potential of oocytes, but also quantitative parameters such as yields of oocytes and embryos. Our experiments were designed to compare the efficiency of embryo production from oocytes collected from different size follicles in the growth phase with that in the dominant phase of the first follicular wave.

2. Materials and methods

2.1. Synchronization of donors

A total of 39 cyclic Holstein dairy cows, 4–6 years of age, were used as oocyte donors. Estrus and ovulation were synchronized by two prostaglandin $F_{2\alpha}$ treatments at 11-day

intervals and one hCG treatment (1500 IU, Organon Co, Holland) on the day of estrus onset (Day 0). Animals with follicles in either the growth (Day 3, $n = 25$) or the dominant phase (Day 7, $n = 14$) of the first follicular wave were slaughtered and both ovaries from each donor were transported at 27 °C to the laboratory. Evaluation criteria for the growth and the dominant phase included the presence of a hemorrhagic corpus luteum with signs of ovulation and no follicle larger than 11 mm in diameter, and the presence of an advanced corpus luteum and two large follicles, 14–15 and 8–11 mm in diameter, respectively. The experiment was carried out in seven replicates.

2.2. Oocyte isolation

All follicles >5 mm were counted, and three separate oocyte populations were collected from the donors in either the growth or the dominant phase. Oocytes from large (11–15 mm) and medium (6–10 mm) follicles were recovered by aspiration and those from small follicles (2–5 mm) were collected by slicing the ovarian cortex. All collected cumulus–oocyte complexes (COC), except for markedly atretic oocytes (without cumulus cells), were used in experiments.

2.3. Oocyte maturation and fertilization

Oocytes were matured in TCM-199 medium (Earle's salt), supplemented with 20 mM sodium pyruvate, 50 U/ml penicillin, 50 μg/ml streptomycin (Sigma Chemicals, Prague, Czech Republic), 5% estrus cow serum (ECS, Sevapharma, Prague, Czech Republic) and gonadotropins (P.G. 600 15 U/ml, Intervet, Boxmeer, Holland) in four-well plates (Nunclon Intermed, Roskilde, Denmark) for 24 h.

Mature oocytes were fertilized with spermatozoa from a bull previously shown to have good fertility in the IVF system. Spermatozoa were isolated from frozen–thawed semen by Percoll gradient, using modified Tyrode's medium (SP-TALP). Oocytes were fertilized in modified Tyrode's medium (IVF-TALP) that contained 1×10^6 spermatozoa and 10 μg/ml heparin. Cumulus cells were removed from oocytes by vortex 24 h after fertilization. Presumptive zygotes were transferred to a Buffalo rat liver cell line monolayer (ATCC, Rockville, MD, USA) and cultured for further 8 days in B2 Menezo medium with 10% ECS. All the cultures were incubated at 39 °C in a humidified atmosphere and 5% CO_2. The developmental competence of the oocytes was expressed as percentages of oocytes that developed to the blastocyst stage on Days 7–8 after fertilization.

2.4. Statistical analyses

Data were analyzed by the nonparametric exact test and Chi-square test, using the SPSS, Version 11.5 for Windows software (SPSS, Inc., Chicago, IL, USA). The results were presented as mean ± S.E.M. values.

3. Results

Only a small number of large follicles per donor were present on the ovaries in both the growth and the dominant phases (1.2 ± 0.4 and 1.4 ± 0.2, respectively); therefore the yield

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

Donors	Growth phase ($n = 25$)	Dominant phase ($n = 14$)
Total number of large follicles	29	19
Mean number of large follicles/donor	1.2 ± 0.4	1.4 ± 0.2
Total number of isolated oocytes	27	11
Mean number of isolated oocytes/donor	1.1 ± 0.4	0.8 ± 0.2
Total number of useable oocytes	25	11
Mean number of useable oocytes/donor	1.0 ± 0.3	0.8 ± 0.2
Total number of embryos	15	4
Mean number of embryos/donor	0.6 ± 0.2	0.3 ± 0.1
Development rate of oocytes into blastocysts (%)	60.0	36.4

There was no significant difference between phases for any of the means.

of useable oocytes and embryos per donor was also very low (1.0 ± 0.3, 0.6 ± 0.2 and 0.8 ± 0.2, 0.3 ± 0.1, respectively). The development rate of these oocytes into blastocysts, particularly of those collected in the growth phase, was generally high (Table 1).

Mean numbers of medium follicles, isolated oocytes and embryos per donor were higher in the growth as compared to the dominant phase; for useable oocytes and embryos, this difference was significant ($P < 0.01$). The development rates of oocytes into blastocysts, however, did not differ significantly between the growth and the dominant phases (36.7% versus 27.8%, Table 2).

Mean numbers of useable oocytes and embryos per donor collected from small follicles did not differ significantly between phases. Although the developmental competence of oocytes recovered from these small follicles in both follicular phases was generally low, it was greater ($P < 0.01$) in the growth than in the dominant phase (Table 3).

There was no significant difference in the mean numbers of all counted follicles and all useable oocytes per donor between the growth and the dominant phases (12.7 ± 2.1 and 26.2 ± 3.7 versus 8.9 ± 0.9 and 25.4 ± 2.3). However, the mean number of embryos per donor and the development rate of oocytes into blastocysts was higher in the growth than in

Table 2
Mean (±S.E.M.) numbers of medium follicles, collected oocytes and developed embryos in the growth and dominant phases

Donors	Growth phase ($n = 25$)	Dominant phase ($n = 14$)
Total number of medium follicles	288	105
Mean number of medium follicles/donor	11.5 ± 1.8	7.5 ± 0.7
Total number of isolated oocytes	259	74
Mean number of isolated oocytes/donor	10.4 ± 1.6	5.3 ± 0.6
Total number of useable oocytes	240	54
Mean number of useable oocytes/donor	9.6 ± 1.4[a]	3.9 ± 0.6[b]
Total number of embryos	88	15
Mean number of embryos/donor	3.5 ± 0.6[a]	1.1 ± 0.3[b]
Development rate of oocytes into blastocysts (%)	36.7	27.8

Within the same row, values with different superscript letters (a, b) are different ($P < 0.01$).

Table 3
Mean (±S.E.M.) numbers of collected oocytes and developed embryos from small follicles in the growth and dominant phases

Donors	Growth phase ($n = 25$)	Dominant phase ($n = 14$)
Total number of isolated oocytes	467	331
Mean number of isolated oocytes/donor	18.7 ± 3.3	23.6 ± 2.4
Total number of useable oocytes	391	290
Mean number of useable oocytes/donor	15.6 ± 2.6	20.7 ± 2.3
Total number of embryos	96	34
Mean number of embryos/donor	3.8 ± 0.8	2.4 ± 0.5
Development rate of oocytes into blastocysts (%)	24.5[a]	11.7[b]

Within the same row, values with different superscript letters (a, b) are different ($P < 0.01$).

the dominant phase (8.0 ± 1.2 versus 3.8 ± 2.4; $P = 0.012$ and 30.3% versus 14.9%; $P < 0.01$).

4. Discussion

A highly heterogeneous population of oocytes derived from slaughterhouse ovaries, regardless of follicular dynamics, is commonly used for in vitro embryo production. The proportion of embryos obtained from such oocytes is insufficient, reaching on average 20%. However, by selecting oocytes with a greater developmental potential, the rate of their development into blastocysts may be increased [13–15].

This study was designed to characterize, on the basis of embryo production efficiency, a population of oocytes defined by follicle size and follicular wave phase. Oocyte subpopulations collected from small, medium and large follicles in the growth phase of the first follicular wave were different from those recovered in the dominant phase.

It has been established that large follicles contain more oocytes capable of developing into blastocysts than do smaller follicles [7,8,16]. The greater developmental competence of oocytes aspirated from larger follicles, as compared with smaller follicles, both before and after dominant follicle selection, was described by Johnson et al. [17]. In our study, too, a greater developmental competence of bovine oocytes was associated with an increased follicle size, irrespective of follicular wave phase. The development rates of oocytes collected from small and medium follicles were lower in comparison with those of oocytes recovered from large follicles (19.1 and 35.0% versus 52.8%) and these differences were significant ($P < 0.01$ and $P < 0.05$).

In addition, in the present study, follicles of different diameters as well as populations of collected oocytes were selectively influenced by the dominant follicle, decreasing the developmental potential of oocytes collected from large and small follicles, but not from medium follicles. The development rate of oocytes collected from medium follicles in the dominant phase did not differ significantly from that of oocytes collected in the growth phase. Hagemann et al. [11] and Guilbault et al. [18] assumed that the inhibitory effect of the dominant follicle was related to the size of subordinate follicles, when they described the increased atresia of medium follicles during the dominant phase. It seems that atresia of

medium follicles during dominance is not necessarily detrimental to the developmental competence of oocytes collected from these follicles. Blondin and Sirard [16] demonstrated that developmental competences of oocytes from nonatretic, intermediate and slightly atretic follicles were the same. The percentage of embryos produced from oocytes with signs of increasing atresia was even higher, except for oocytes that were derived from heavily atretic follicles [19,20].

We concluded that the interaction between follicle size and the phase of follicular wave influenced the efficiency of embryo production, because an oocyte population varies in relation to the stage of follicular development in which it was collected. In the present study, mean numbers of useable oocytes per donor were similar in both follicular phases, but the mean number of embryos per donor was two-fold when oocytes were collected in the growth versus the dominant phase. In that regard, in the growth phase, the number of medium follicles on ovaries increased and therefore, in the whole oocyte population, a higher proportion of oocytes was derived from them. In addition, oocytes from small follicles had greater developmental competence than those from the dominant phase. On the contrary, in the dominant phase, the number of medium follicles decreased and embryos were derived predominantly from oocytes recovered from small follicles; these had lesser developmental potential than oocytes from small follicles in the growth phase.

Recently, the pretreatment of donors with FSH before collection of oocytes, by transvaginal aspiration or after slaughter, has been suggested as a method for enhancing oocyte developmental competence [21,22]. An alternative method may be the selection of ovaries with competent follicles after slaughter [14,15,23] or utilization of follicular waves synchronized by transvaginal aspiration in living donors [12,24,25].

We speculate that the results of this study will allow us to enhance maturation of oocytes with lesser developmental competence, as well as to exploit oocytes with a greater developmental potential, in order to increase the efficiency of in vitro embryo production, particularly in individual, high-performance donors. We presume that standard maturation conditions should be modified for oocytes with differing developmental competence with respect to the stage of follicular development. In oocytes with presumptively lesser developmental potential, the adjustment of maturation time, use of growth factor-supplemented media (EGF, IGF) or establishment of a two-step culture system that includes prematuration in the presence of nuclear maturation inhibitors, will be employed.

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