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Gene expression in bovine embryos derived from oocytes with different developmental competence collected at the defined follicular developmental stage

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Abstract

The aim of this study was to compare the expression of selected genes in bovine embryos developed from oocytes with different developmental competence. Four oocyte populations were collected, separately either from small (2–5 mm) or medium (6–10 mm) follicles, in the growth/stagnation (G/S) or dominance/regression (D/R) stage of the first follicular wave. They were matured, fertilized and cultured to D7 or D8 blastocysts by a standard protocol. Poly (A)+ mRNA was extracted from pooled blastocysts and the expression of bax-α (Bax), connexin 43 (Cx 43) and connexin 31 (Cx 31) was estimated using real-time RT-PCR. The cleavage rates were significantly higher in oocytes collected from both medium and small follicles, ($p \leq 0.05$ and $p \leq 0.01$, respectively) in the G/S than in the D/R stage. There were no significant differences in the D7 blastocyst rates between oocytes from both medium and small follicles in the G/S or D/R stage. But the D8 blastocyst rate was significantly higher in oocytes from small follicles in the G/S stage compared with those in the D/R stage. The relative abundance of Bax and Cx 31 made no significant difference in both D7 and D8 blastocysts developed from oocytes collected from medium or small follicles in the G/S or D/R stages. But the relative abundance of the Cx 43 transcript was significantly higher in D8 blastocysts developed from oocytes collected from both medium and small follicles in the G/S stage compared with those in the D/R stage.

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We conclude that the relative abundance of Cx 43 can be used as a marker of developmental potential for embryos derived from oocytes with different developmental competence because the level of Cx 43 transcript was greater in embryos derived from oocytes with greater developmental competence compared with those derived from oocytes with lesser developmental competence.

Keywords: Cattle; Follicular development; Oocyte development; Gene expression; Real-time RT-PCR

1. Introduction

Current in vitro production of bovine embryos includes a variety of applications. As such it has become useful not only for research but also in breeding programs and in the production of transgenic offspring. Until now this method has had lower efficiency in comparison to in vivo embryo development. One of the factors affecting the embryo yield, the implantation rate and the rate of healthy survivals, is the intrinsic quality of the oocyte [1–4]. Surgically retrieved oocytes from different follicles without regard to the follicular development stage represent the heterogeneous population in terms of their meiotic and developmental competence.

There is general agreement that with in vitro, the meiotic and developmental competence of oocytes are related to follicle size, the estrous cycle stage and the level of atresia influenced by other follicles, mainly the dominant follicle [5–12].

In cattle, two to three follicular waves emerge during every estrus cycle and they include follicular growth, stagnation, dominance and regression stages. Within each wave a group of follicles starts to grow over 3–4 mm in diameter. One of them is selected to become a dominant follicle and continues in growing, while other follicles reduce or terminate their growth [13–15]. The oocytes in growing follicles undergo a variety of changes (termed ''capacitation''), leading to the acquisition of the full developmental competence [16,17]. The further fate of the follicles depends on the extraovarian signals and intrafollicular microenviroment [13,15]. In the last (ovulatory) wave the dominant follicle ovulates. In contrast, dominant follicles of the first and the second (un-ovulatory) waves regress (via artresia or apoptosis), concurrently with all subordinate follicles located in the ovary [13,15].

In vivo, during its growth phase, the oocyte accumulates mRNAs and proteins important for maturation, fertilization and the early embryo cleavages. It plays a key role in supporting embryonic development until the switch from maternal to zygotic gene expression control occurs [18].

Once an oocyte is recovered from its follicle, it spontaneously resumes meiosis concomitantly with the chromatin decondensation. The transcription of this follicle rapidly decreases and its capacitation stops [14]. The source of oocytes and the stage of follicular development used for their collection have a very important impact on achieving both developmental competence of the oocytes, and blastocyst yield [18].

It has been described that development of early embryos to the blastocyst stage was greater when oocytes were obtained from medium rather than small follicles and during follicular growth/stagnation (G/S) than in the follicular dominance/regression (D/R) stage [8,19,20]. It has been accepted that greater developmental competence of