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Coculture with good-quality COCs enhances the maturation and development rates of poor-quality COCs

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ABSTRACT

In pigs, more than half of the recovered cumulus cell–oocyte complexes (COCs) have one or two layers of cumulus cells and are considered morphologically poor. If we could take full advantage of these poor-quality COCs, we could potentially improve the efficiency of *in vitro* embryo production. During IVM, although some maturation factors are transmitted bidirectionally between the oocyte and the cumulus cells of the same COC, transmission also occurs between different COCs. We hypothesized that morphologically poor COCs fail to undergo complete oocyte maturation because of their insufficient secretion of maturation factors. Here, we investigated whether coculture with morphologically good COCs (having three or more layers of cumulus cells) could improve the maturation and utilization rates of morphologically poor COCs. Our results revealed that the oocyte maturation rate, glutathione level, embryo development capacity, blastocyst quality, and cumulus cell gene expression levels of *BCL-2* and proliferating cell nuclear antigen were similar in the coculture and good-quality groups and that these levels were all significantly higher than those in the poor-quality group. Our results strongly suggest that the coculture strategy greatly improved the utilization rate of morphologically poor COCs without reducing their capacity for maturation and subsequent development.

1. Introduction

Considering the great anatomic and physiological similarities between pigs and humans, transgenic pigs derived from *in vitro* production (IVP) are regarded as the most likely sources in biomedical animal fields [1]. One of the major barriers in producing transgenic pigs, however, is that IVP embryos have a low developmental capacity compared to their *in vivo*–derived counterparts. Successful IVP generally requires numerous cumulus–oocyte complexes (COCs) of very high quality. It is generally believed that the use of high-quality oocytes can improve oocyte maturation and developmental competence *in vitro*. Conversely, low-quality oocytes are often associated with low rates of maturation, embryo development, and birth.

Many laboratories have tried supplementing the IVM medium with various chemical agents to improve oocyte quality and subsequent developmental potential [2–6], whereas others have sought to increase the recovery of high-quality oocytes via the IVM preantral follicle strategy [7–10] and improved oocyte recovery methods [11–13]. However, the practical use of such strategies may be limited by the high costs of the chemical agents and the use of complicated methods (i.e., preantral follicle isolation and so forth).

In terms of defining high- and low-quality oocytes, the relationship between follicle diameter and oocyte developmental competence has been widely studied in several species [14–19]. These reports have consistently shown

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that oocytes derived from relatively small follicles possess lower maturation and developmental competence than those derived from relatively large follicles. In addition, the size of the actual oocyte has been reported to affect its maturation and developmental competence [20,21]. However, although it may be very effective to use such measurements to select oocytes, the necessary techniques are complicated and time-consuming.

In addition to follicle size and oocyte diameter, oocyte quality may also be predicted from morphologic criteria of the COC, such as the number and compactness of the cumulus cell layers surrounding the oocyte [22,23]. Several studies comparing the morphologies of COCs with their maturation and/or developmental abilities have shown that morphologically poor oocytes experienced decreases in meiotic resumption, cytoplasmic maturation [13,20], and developmental competence [24]. However, little information is currently available on how to improve the utilization rate of morphologically poor oocytes.

At present, researchers typically select morphologically good oocytes with three or more layers of cumulus cells and discard morphologically poor oocytes with one or two layers of cumulus cells. Porcine COCs are usually recovered from antral follicles (diameter, 3–6 mm) found on the ovary surface; in practice, however, fluid is probably aspirated from follicles of 2 to 8 mm in diameter because of unavoidable personal artifacts. Thus, it is not always practical to ensure oocyte quality by measuring and controlling the follicle size. In our laboratory, we intentionally recovered as many COCs as possible during each oocyte collection and have found that as many as half (50.1%) of the recovered COCs are morphologically poor. As it is a waste of money and resources to discard such a high proportion of recovered oocytes, we set out to improve their chances of maturing into proper embryos.

Here, we hypothesized that poor-quality oocytes fail to undergo complete maturation because they do not secrete sufficient maturation factors. Thus, we tested whether the coculture of poor-quality oocytes and good-quality oocytes could improve the maturation of the former and enhance their subsequent developmental potential.

2. Materials and methods

2.1. Animal care and ethics statement

All of the experimental procedures used in this study were approved by the Institutional Animal Care and Use Committee of Chungnam National University.

2.2. Chemicals

All used chemicals were purchased from Sigma (St. Louis, MO, USA) except as otherwise indicated.

2.3. Porcine COC collection, classification, and maturation

The general procedures for the collection and maturation of oocytes were as described previously [25]. In brief, fresh ovaries of prepubertal gilts were obtained from a local abattoir and transported to the laboratory within 2 hours in physiological saline maintained at 30 °C to 35 °C. Cumulus–oocyte complexes were harvested from follicles (3–6 mm diameter) and cultured in four-well multidishes, with each well containing 50 COCs and 500-μL tissue culture medium supplemented with 10% porcine follicular fluid, 10 ng/mL of EGF, 10 IU/mL of pregnant mare's serum gonadotropin, and 10 IU/mL of hCG. After 22 hours, the COCs were further cultured for 22 hours in the same medium without pregnant mare's serum gonadotropin or hCG.

Before maturation, each COC was graded according to its number of cumulus cell layers (Fig. 1A, B) as follows: grade I, five or more layers of cumulus cells; grade II, three to four layers of cumulus cells; grade III, one or two layers of cumulus cells; and grade IV, partial cumulus cells or denuded oocytes (DOs).

Three experimental groups were subjected to IVM: a normal group (or traditional group) consisting of 50 grade I + II COCs (the proportion was randomly selected); a coculture group consisting of 25 grade I + II COCs plus 25 grade III COCs (1:1 ratio; high vs. low quality); and a small group consisting of 50 grade III COCs (Fig. 1C). The COCs of each group were cultured in 500-μL IVM media.

2.4. Assessment of nuclear status

To observe the nuclear status, we denuded oocytes after 0, 22, and 44 hours of IVM, stained them with 4′,6-diamidino-2-phenylindole (DAPI), and classified them into the following groups: germinal vesicle (GV) stage; metaphase I (MI) stage; telophase I/metaphase II (TI/MII) stage; and atypical. The GV chromatin configurations were grouped into three classes: surrounded nucleolus (SN), nonsurrounded nucleolus (NSN), and intermediary (SN/NSN; Fig. 2A).

2.5. Transcription labeling and immunocytochemistry of GV-stage oocytes

Cumulus–oocyte complexes (grades I, II, and III) were denuded of cumulus cells by vortexing in PBS-PVA (PBS supplemented with 0.1% [wt/vol] polyvinyl alcohol) containing 0.5% hyaluronidase. The DOs were incubated in 5-mM 5-fluorouridine (FU) at 38.5 °C under a humidified 5% CO_2 atmosphere for 1 hour, washed with PBS-PVA, fixed with 4% paraformaldehyde solution, and permeabilized with 0.5% Triton X-100 in PBS-PVA for 30 minutes. The embryos were then washed in PBS containing 100-mM glycine and blocked with 3% BSA in PBS for 30 minutes. The blocked oocytes were washed in PBS containing 0.5% (wt/vol) BSA and 0.1% (wt/vol) fish skin gelatin (PBG) and then incubated overnight at 4 °C in the same medium containing monoclonal antibromodeoxyuridine (B2531, diluted to 1:200). A fluorescein isothiocyanate–conjugated goat antimouse IgG secondary antibody (1:200; Santa Cruz Biotechnologies, Santa Cruz, CA, USA) was applied for 1 hour at room temperature. The oocytes were washed twice with PBG solution for 10 minutes each, stained with DAPI in VECTASHIELD mounting medium, and mounted on slides (Fig. 2B).