Cumulus cell luteinization is enhanced during aging of bovine oocytes matured in vitro

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INTRODUCTION

Maturing mammalian oocytes can prevent luteinization of cumulus cells (CCs) both in vivo and in vitro by regulating CCs steroidogenesis (Gilchrist et al. 2004 Anim. Reprod. Sci. 82-83, 431–446; Ramirez et al. 2016 Anim. Reprod. 14, 280). However, when the oocyte attains the M-II stage, aging processes are accelerated and may impair CCs protective abilities.

The aim of the present research was to study the luteinization of CCs surrounding aging bovine oocytes and its susceptibility to prolactin (PRL), which performs a luteotrophic function in mammals.

MATERIALS AND METHODS

Aging processes in bovine cumulus-oocyte complexes (CEOs) were studied using a model of the prolonged culture consisting of two steps:

1. CEOs were cultured for 22 h in the IVM medium (TCM 199 supplemented with 10% fetal calf serum, 10 μg/ml FSH, and 10 μg/ml LH).
2. CEOs were cultured for additional 12 or 24 h in the aging medium (TCM 199 containing 10% fetal calf serum) without (control group) or with bovine PRL (50 ng/ml).

At the end of culture:
- Progesterone (P4) and estradiol-17β (E2) levels in spent media were determined by ELISA.
- The expression of luteinization-related genes STAR (steroidogenic acute regulatory protein), HSD3B1 (3β-hydroxysteroid dehydrogenase type 1), CYP11A1 (P450scc), PGR (genomic P4 receptor), PGRMC1 and PGRMC2 (P4 receptor membrane components 1 and 2) was analyzed by real-time RT-PCR.
- The data from 6 replicates (at least 170 oocytes for each condition) were analyzed by ANOVA following by Tukey’s (steroidogenesis) or Dunn’s (gene expression) tests.

RESULTS

In the control group, the level of progesterone production exhibited by COCs during 24 h-aging was 5.5-fold higher (P<0.001) than the level observed during IVM (Fig. 1). Conversely, estradiol-17β production by aging COCs was 2.7 times lower (P<0.001) than that by maturing COCs (Fig. 2). The CCs expression of genes responsible for progesterone synthesis, STAR, HSD3B1, and CYP11A1, increased progressively (7.1-fold, 34.6-fold, and 2.5-fold respectively, at least P<0.05) by 24 h of aging (Fig. 3-5). Meanwhile, the steroid secretion and expression of STAR, HSD3B1, and CYP11A1 genes were unaffected by PRL. The level of PGR transcript decreased (P<0.01) 9.9-fold at 12 h and 18.3-fold at 24 h of PRL-treated COCs, whereas this fall was only 5-fold (P>0.05) at 24 h in Control (Fig. 6). In addition, the relative mRNA abundance of PGRMC1 increased 2.3-fold (P<0.05) in the control (at 24 h) and PRL groups (at 12 h), while that of PGRMC2 did not change significantly in both groups (Fig. 7 and 8).

CONCLUSIONS

- Aging of bovine oocytes is accompanied by increasing production of progesterone and reducing production of estradiol-17β by cumulus cells.
- Senescence of bovine oocytes is likely to result in a loss of their the ability to prevent luteinization of surrounding cumulus cells.
- Prolactin does not affect steroidogenesis, but may modulate the expression of progesterone receptors in cumulus cells.

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