

Effect of epidermal growth factor and prolactin on the quality of bovine oocytes aging in vitro

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INTRODUCTION

Functional changes associated with aging of matured mammalian oocytes can be delayed or reversed by various treatments, preventing a decline in the oocyte quality and developmental capacity.

The goal of the present research was to study dose-dependent effects of three potential regulators of oocyte quality, prolactin (PRL) and epidermal growth factor (EGF) on deleterious alterations of aging bovine oocytes matured in vitro.

Therefore, the following functional changes occurring during oocyte aging were studied in the course of the prolonged culture of bovine oocytes:

- abnormal modifications in the morphology of metaphase-II (M-II) chromosomes;
- the apoptotic degeneration of *in vitro* matured oocytes.

MATERIALS AND METHODS

Bovine cumulus-enclosed oocytes (CEOs) were matured for 20 h in TCM 199 containing 10% fetal calf serum (FCS), 10 µg/ml porcine FSH, and 10 µg/ml ovine LH (**maturation medium**). After IVM, CEOs were transferred to the **aging medium** consisting of TCM 199 supplemented with 10% FCS and cultured for 24 h in the absence (Control) and in the presence of either PRL (20 and 50 ng/ml) or EGF (10 and 50 ng/ml).

At the end of culture, the state of metaphase-II (M-II) chromosomes was evaluated by the cytogenetic method of Tarkowski. The following morphological abnormalities were ascribed to destructive changes of M-II chromosomes:

- (1) chromosome decondensation,
- (2) adherence between chromosomes,
- (3) clumping of chromosomes into a single mass.

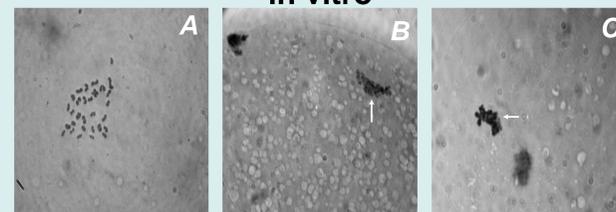
Apoptosis in oocytes was detected using the TUNEL method. The data from 3-4 replicates were analyzed by ANOVA.

CONCLUSIONS

- At physiological concentrations, PRL is able to decelerate abnormal modifications of metaphase-II chromosomes in bovine oocytes aging *in vitro*.
- PRL and EGF can exert *in vitro* inhibitory effects on the apoptotic degeneration of senescent bovine ova.
- PRL and EGF may be used during the prolonged culture of bovine oocytes for retarding age-related deleterious alterations of the cells.

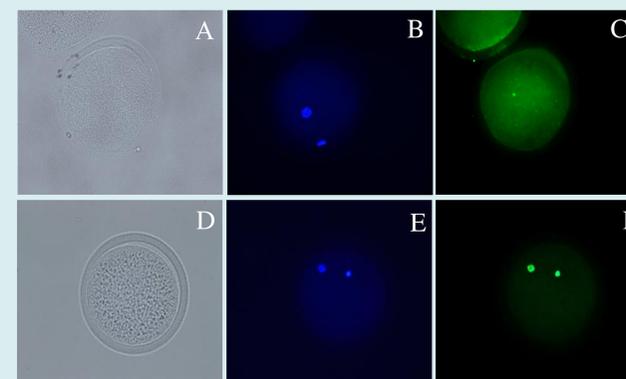
At the end of prolonged culture (24 h) PRL (not EGF) reduced the frequency of abnormal chromosome modifications (decondensation, adherence, clumping) (**Fig. 1**) at concentrations of 20–50 ng/ml from 58.7±2.1% (Control) to 41.2±1.9 and 45.6±2.7% respectively ($p < 0.01$) (**Fig. 2A**). The rate of apoptotic oocytes (**Fig. 3**) in the Control group was 47.4±8.5% (**Fig. 4**). EGF at concentration of 10 ng/ml (**Fig. 4B**) and PRL at both doses (**Fig. 4A**) decreased this rate to 15.0–22.1% ($p < 0.05$). respectively ($p < 0.01$).

Fig. 1. Morphology of M-II chromosomes in bovine oocytes aging in vitro



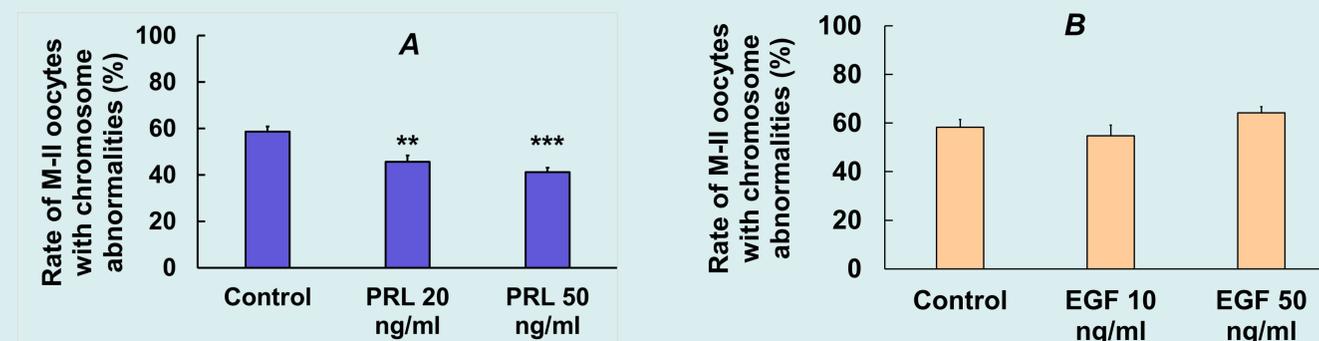
A: without abnormal changes, B: chromosome decondensation, C: chromosome decondensation plus adherence.

Fig. 3. Representative images illustrating apoptosis in aging bovine oocytes



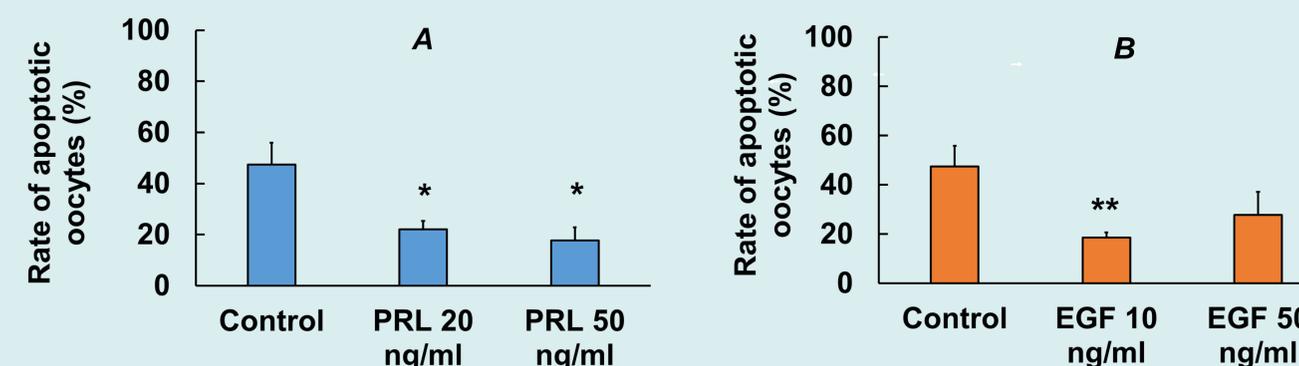
A, D: bright-field images. B, E: DAPI staining (blue). C, F: TUNEL staining (green). TUNEL-positive chromosomes are indicated by white arrows.

Fig. 2. Effects of different concentrations of PRL (A) and EGF (B) on destructive changes in M-II chromosomes during 24 h aging of bovine CEOs



** $p < 0.01$, *** $p < 0.001$ compared with the control group.

Fig. 4. Effects of different concentrations of PRL (A) and EGF (B) on the apoptotic degeneration of oocytes during 24 h aging of bovine CEOs



* $p < 0.05$, ** $p < 0.01$ compared with the control group.

ACKNOWLEDGMENTS

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