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Changes of spontaneous parthenogenetic activation and development potential of golden hamster oocytes during the aging process

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ABSTRACT

The golden hamster is an excellent animal experimental model for oocyte research. The hamster oocytes are very useful in clinical examination of human spermatozoan activity. Non-fertile oocytes can lead to time-dependent processes of aging, which will affect the results of human spermatozoa examination. As a consequence there is a need to investigate the aging and anti-aging processes of golden hamster oocytes. In order to study the aging processes and parthenogenetic activation of golden hamster oocytes, *in vivo* oocytes, oocytes cultured with or without cumulus cells, and oocytes treated with Trichostatin A (TSA) or caffeine were collected and investigated. We found that: (1) spontaneous parthenogenetic activation, developmental potential (cleavage rate), and zona pellucida (ZP) hardening undergo age-dependent changes in *in vivo*, *in vitro*, and after TSA or caffeine treatment; (2) *in vivo*, oocytes became spontaneously parthenogenetic 25 h post-hCG treatment; (3) *in vitro*, cumulus cells did not significantly increase the parthenogenetic activation rate of cultured hamster oocytes; and (4) TSA or caffeine could delay spontaneous oocyte parthenogenetic activation and the aging processes by at least 5 h, but also accelerated the hardening of the ZP. These results define the conditions for the aging and anti-aging processes in golden hamster oocytes. TSA and caffeine play roles in controlling spontaneous activation, which could facilitate the storage and use of golden hamster oocytes for studying processes relevant to human reproduction.

Introduction

Some mammalian species, especially primates (including humans), can potentially engage in sexual activity throughout the menstrual cycle. Ovulation and insemination may not be synchronized. If fertilization does not occur, non-fertilized oocytes remaining in the oviduct (aging *in vivo*) or in a suitable medium (aging *in vitro*) may undergo time-dependent aging processes (Whittingham and Siracusa, 1978; Yanagimachi and Chang, 1961). Aging not only decreases the potential of oocytes for fertilization and embryo development, but also can lead to offspring suffering from delayed sensorimotor integration during pre-weaning development, increased spontaneous activation, higher emotionality (Tarin et al., 1999), and decreased reproductive fitness (Tarin et al., 2002). However, culture of second meiotic metaphase (MII) eggs, derived from both *in vivo* and *in vitro* maturation, is common in many research models and clinical procedures. Therefore, control of oocyte aging *in vitro* could provide potential advantages in recently developed embryo technologies.

Aging can result in activation responses (Suzuki et al., 1999), including spontaneous parthenogenetic activation (Sun et al., 2002) and exocytosis of cortical granules (CGs) (Merchant and Chang, 1971). The mechanisms of aging are still unclear. Mitochondrial dysfunction and reduction of maturation promoting factor (MPF) may be involved in such processes. In aged oocytes, morphological abnormalities of mitochondria and decreased MPF activity (Kikuchi et al., 2000) were observed (Tarin et al., 1999). It has been reported that cumulus cells (CCs) can accelerate the aging progression of both *in vivo*- and *in vitro*-matured mouse oocytes by releasing a factor known as aging-promoting factor (APF) into the culture medium

Abbreviations: APF, aging-promoting factor; CCs, cumulus cells; CGs, cortical granules; COCs, cumulus–oocyte complexes; 6-DMAP, 6-dimethylaminopurine; HATs, histone acetyltransferases; hCG, human chorionic gonadotropin; HDACs, histone deacetylases; IVF, *in vitro* fertilization; ICSI, intracytoplasmic injection; MII, second meiotic metaphase; MAPK, mitogen activated protein kinase; MPF, maturation promoting factor; PI, propidium iodide; PMSG, pregnant mare serum gonadotropin; RT, room temperature; TSA, Trichostatin A; ZP, zona pellucida.

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(Miao et al., 2005; Qiao et al., 2008). Acetylation of nuclear core histones is also thought to play important roles in various cellular functions (Kurdistani and Grunstein, 2003; Turner, 2002). The histone deacetylase (HDATs) inhibitor, Trichostatin A (TSA), can delay the aging of porcine oocytes (Jeseta et al., 2008). Caffeine, an inhibitor of Myt1/Wee1 activity, can increase MPF and mitogen activated protein kinase (MAPK) activities and delay the aging of ovine oocytes (Lee and Campbell, 2006).

For many years, golden hamster oocytes have been used to evaluate the fertility of human spermatozoa (Aitken, 1988; Rogers et al., 1979) and to examine human sperm chromosomes (Alvarez et al., 1997). Oocyte parthenogenetic activation is a major hurdle in the examination of human sperm. Thus, it is necessary to further our understanding of the mechanisms of the oocyte aging processes. The aim of this study, was to examine the effects of CCs, TSA and caffeine on golden hamster oocyte parthenogenetic activation and aging.

Materials and methods

Chemicals and animals

Six-week-old female golden hamsters ($n = 200$) were purchased from the Changchun Hi-Tech Laboratory Animal Research Center (Changchun, China) and housed with three animals per cage under controlled light cycle conditions (12L:12D; 6.00:18.00). All animal studies were conducted according to the experimental practices and standards approved by the Animal Welfare and Research Ethics Committee at Jilin University. All chemicals were purchased from Sigma–Aldrich Chemical Co., St. Louis, MO, USA) unless otherwise noted.

Collection of oocytes

Group 1: *In vivo* matured oocyte collected from superovulated females at different stages

The hamsters were superovulated with intraperitoneal (ip) treatment of pregnant mare serum gonadotropin (PMSG, 30 IU, ip) followed by human chorionic gonadotropin (hCG, 30 IU, ip) at 72 h intervals. Both PMSG and hCG used in this study were purchased from Ningbo Hormone Product Co., Ningbo, China. Three superovulated hamsters were euthanized at different times (15 h, 20 h, 25 h, 30 h or 35 h) after hCG injection, and the oviductal ampullae were collected and broken open to release the cumulus-oocyte complexes (COCs). CCs were removed from COCs by pipetting with a thin pipette in M2 medium (Nagy et al., 2003) containing 0.1% hyaluronidase. Then the oocytes were washed and kept in M199TE medium (TCM199 with Earle's salt, 26 mM sodium bicarbonate, and 25 mM HEPES, supplemented with 5% heat-inactivated fetal bovine serum, 5 mM taurine and 25 μM EDTA; Barnett and Bavister, 1992) for further studies. Culture conditions for hamster oocytes was 37.5° C with 5% CO_2. 15–20 oocytes were used in each treatment.

Group 2: COCs and cumulus-free oocytes cultured *in vitro* for different duration

The superovulated hamsters were euthanized at 15 h after hCG injection. The COCs and denuded oocytes were collected as described above. Then, the oocytes, with or without CCs, were cultured in M199TE medium for 5 h, 10 h or 20 h. After *in vitro* culture, the COCs were denuded with 0.1% hyaluronidase for further treatment.

Group 3: Cumulus-free oocytes treated with TSA *in vitro*

Cumulus-free oocytes collected from superovulated hamster oviducts at 15 h post- hCG were incubated with 50 ng/ml or 100 ng/ml of TSA in M199TE medium for 5 h, 10 h or 20 h.

Group 4: Cumulus-free oocytes treated with caffeine *in vitro*

Cumulus-free oocytes collected from superovulated hamster oviducts at 15 h post hCG were incubated with 5 mg/ml or 10 mg/ml of caffeine for 5 h, 10 h or 20 h in M199TE.

Examination of oocytes

Examination of spontaneous parthenogenetic activation

After each treatment, oocytes were collected and examined under a phase-contrast microscope (DM IL LED, Leica, Germany). Only the oocytes with at least one pronucleus were considered as spontaneous parthenogenetic activation.

Assessment of oocyte cleavage

A combination of electrical and chemical activation stimuli was used in an effort to maximize the number of oocytes cleavage (Wang et al., 2008). *Electrical activation*: hamster oocytes collected from different groups were transferred to activation medium (0.3 M mannitol, 0.1 mM $MgCl_2$, 0.1 mM $CaCl_2$, and 0.01%, [w/v] BSA) and placed between parallel electrodes (spacing, 1 mm) in the chamber of a BTX Electro-cell Manipulator 2001 (BTX, San Diego, CA, USA). The electrical stimulation duration was 10 μs, pulse was 300 V. Then the oocytes were transferred to the culture medium for chemical activation.

Chemical activation: after electrical activation treatment, oocytes were subjected to incubation in M199TE containing 2 mM of 6-dimethylaminopurine (6-DMAP; D-2629 Sigma–Aldrich) for 2 h. The oocytes were rinsed and cultured in M199TE at 37.5 °C under 5% CO_2 overnight. Then the oocytes were examined under a microscope (DM IL LED, Leica, Germany) to assess activation and cleavage. Only oocytes with two nucleated cells were considered cleaved.

Assay for zona pellucida (ZP) hardening of treated oocytes

ZP hardening was examined in oocytes collected *in vivo*, cultured *in vitro* (with or without CCs) or treated with TSA/caffeine. The assay for ZP hardening was performed as described by Ge et al. (2008). About 20 cumulus-free oocytes under different condition were treated with 1 mg/ml of α-chymotrypsin (type II, C-4129) contained in a 100 ml drop of PBS covered with mineral oil. The treatment was conducted at room temperature (RT) maintained at 25 °C. Oocytes were monitored under the phase contrast microscope. The time at which 75% of the ZP underwent a complete dissolution (with ooplasm stuck to the bottom of the dish) was assessed as t75 for ZP dissolution.

Staining and observation of CGs and chromosomes in oocytes

Cortical granules of oocytes collected 15 h and 25 h post-hCG administration, were stained with fluorescein isothiocyanate-labeled (FITC) lens culinaris agglutinin (LCA). The zonae pellucidae were removed by treating the oocytes with 0.5% pronase. Then oocytes were fixed with 4% paraformaldehyde for 40 min at RT. After permeabilization with 0.1% Triton X-100 for 5 min in PBS, oocytes were blocked three times for 5 min each in a blocking solution (PBS containing 0.3% BSA and 100 mM glycine). Then they were cultured in 100 mg/ml of FITC-LCA in blocking solution for 30 min in dark. Finally, the oocytes were washed three times in PBS. The DNA was stained with 10 mg/ml propidium iodide (PI) in the final incubation for 10 min in PBS. After extensive washing, oocytes were mounted on slides with anti-fade medium (1,4-diazobicyclo-[2,2,2]-octane, DABCO, Sigma, D-2522). Finally, the oocytes were observed under confocal laser scanning microscope (FV1000, Olympus, Japan).

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