Surface Ultrastructural Characteristics of Bovine Oocytes Following Heat Shock

Hiroyuki SUZUKI1)*, Jyh-Cherng JU1,2), John E. PARKS2) and Xiangzhong YANG1)

1)Department of Animal Science, University of Connecticut, Storrs, CT 06269, and 2)Department of Animal Science, Cornell University, Ithaca, NY 14853, *Present address: Faculty of Agriculture and Life Sciences, Hirosaki University, Hirosaki 036-8561, Japan

Abstract. Surface morphology of the zona pellucida (ZP) and the vitellus of bovine oocytes treated by heat shock were evaluated by scanning electron microscopy (SEM). Bovine oocytes were recovered from slaughterhouse ovaries and matured in vitro for 24 h (young oocytes) or 40 h (aged oocytes). After maturation incubation, they were heat-shocked at 42 C for 30 min and then incubated at 39 C for 1, 2, 4, 8 and 16 h. The ZP was characterized by a mesh-like structure in untreated young oocytes, whereas in untreated aged oocytes the ZP meshwork became thinner. After heat shock, partial breakage (16/69) or coalescence (53/69) of the ZP meshwork was noted in young oocytes, and the altered surface of the ZP was not restored during incubations for up to 16 h. In aged oocytes, however, partial peeling off of the outer ZP surface was noted frequently after heat shock (6/10 at 1 h, 10/10 at 2 h, 7/10 at 4 h and 6/6 at 8 h). The vitelline surface of untreated young oocytes was predominantly covered by well-developed microvilli (MV). In aged oocytes, a mixed distribution pattern of MV and cytoplasmic protrusions (CP) was frequently noted (8/9). After heat shock, the observance of the mixed MV and CP increased in the young and aged oocytes and enlarged CP were observed more frequently in the aged oocytes. These observations suggest that a brief heat shock (42 C, 30 min) facilitates a breakage of the ZP surface and changes the vitelline surface from a MV-predominant to a CP-predominant pattern; furthermore its effect may manifest itself more quickly and to a greater degree in aged oocytes than in the young oocytes.

Key words: Ultrastructure, SEM, Microvilli, Cytoplasmic protrusion, Cattle.

(F. Reprod. Dev. 44: 345–351, 1998)

Female animals exposed to heat stress experience increased embryonic mortality [1–4]. Maternal heat stress seems to be a critical factor during the first few cleavage divisions of early embryos [1–4], partially due to the direct effects of elevated temperature on gametes and early embryos. The embryo itself is susceptible to heat shock, which can disrupt embryonic development and viability [5–11]. It has been well known that transient hyperthermia in vitro (heat shock) can induce parthenogenetic development of oocytes in mice [12, 13] and rabbits [14], but similar effects have been demonstrated in cattle (Ju, Suzuki, Parks, Yang, in preparation).

The objectives of this study were to examine surface ultrastructural characteristics of bovine oocytes following heat shock treatment and aging. Our previous studies on the surface structure of bovine oocytes have demonstrated that the cumulus cells and the vitelline membrane manifested profound changes during maturation in vitro or in vivo [15, 16], suggesting an intimate relationship between the surface characteristics and the functional mat-
urational status of the oocytes. Understanding the ultrastructural changes following heat shock at different ages may help explaining the oocyte contribution to the reduced fertility in the heat stressed females.

Materials and Methods

Oocyte collection and in vitro maturation

Cumulus-oocyte complexes (COCs) were collected by aspiration from antral follicles (2–8 mm) of slaughterhouse ovaries. They were matured in TCM 199 (Earl’s salts, Gibco, Grand Island, NY) and 7.5% fetal bovine serum (FBS) plus hormones (oFSH 0.5 µg/ml, oLH 5 µg/ml and E2 1 µg/ml) under 5% CO2 and 95% humidified air at 39 C [17] for 24 or 40 h.

Remove of cumulus cells and zona pellucida

Cumulus cells were removed with 0.1% hyaluronidase in calcium free Dulbecco’s phosphate-buffered saline (DPBS, Gibco) containing 0.1% polyvinyl alcohol (PVA, Sigma, St. Louis, MO). The ZP was removed by exposure to prewarmed acidified DPBS-PVA (pH=2.5) for 1 min and 0.5% pronase (Sigma) for 3–5 min.

Heat shock treatment

After 24 h- or 40 h-maturation incubation, the oocytes were heat-shocked at 42 C for 30 min and then incubated at 39 C for 1, 2, 4, 8 or 16 h. Oocytes before and just after heat shock as well as those incubated for 8 or 16 h without heat shock were also evaluated at 24 h and 40 h of maturation. Twenty oocytes (10 each for observation of the ZP and vitelline surfaces) at each time point were processed for SEM. Since cleaved or cleaving aged oocytes were noted after 8 and 16 h of incubation with or without heat shock, they were processed for SEM as a separate group.

SEM observations

According to the methods reported previously [15, 16], the samples were fixed for 1 h in 3% glutaraldehyde and 0.5% paraformaldehyde in Hanks’ balanced salt solution (HBSS, Gibco) with 0.1% PVA. They were rinsed in three changes of HBSS and placed on small coverslips (6 × 6 mm) pre-coated with 0.1% poly-L-lysine solution (Sigma). The oocytes on the coverslips were postfixed in 1% osmium tetroxide in HBSS for 1 h. After rinse, the samples were rendered electrically conductive by immersing in 2% tannic acid solution (2 h) and then in 1% osmium tetroxide solution (1 h). The specimens were then dehydrated in a series of increasing concentrations of ethanol, critical point dried, and sputter-coated with gold. Observations were performed with a Zeiss DSM-982 or a JOEL JSM-5300 scanning electron microscope at an accelerating voltage of 2–20 kV.

Results

Zona pellucida

The ZP surface of the young oocytes matured in vitro was characterized by a meshwork structure of thin fibers spattered with droplets of various sizes (Fig. 1a). Some club-shaped cytoplasmic projections were found within the mesh holes. After heat shock, partial breakage of the meshwork was observed in young oocytes (Fig. 1b), and this altered ZP surface was not restored during incubation as long as 16 h. The ZP surface of the control oocytes (without heat shock) after 8 h and 16 h incubation remained undamaged. These observations suggest that heat shock may facilitate a breakage of the ZP meshwork in young oocytes and this damage is irreversible.

The ZP meshwork of aged oocytes became thinner compared to that of young oocytes (Fig. 1c), and the size of the mesh holes appeared to become slightly wider. At 1 h after heat shock, partial peeling off of the outer surface of the ZP was noted more frequently in aged oocytes (6/10; Fig. 1d). Such features were not observed in the young oocyte. There was no correlation between the percentage of oocytes with an altered ZP surface and time post-treatment. No remarkable differences were noted in the ZP surface ultrastructure for the 16 h group of 24 h matured oocytes and the 0 h group of 40 h-matured oocytes.

Vitelline surface

Prior to the heat shock, the vitelline surface of young oocytes was characterized by well-developed MV which were interspersed with small cytoplasmic protrusions (CP; Fig. 2a). In aged oocytes, a mixed distribution pattern of MV and CP was frequently noted (3/10 for young vs. 8/9 for aged oocytes, Fig. 2c). Just after heat shock, there...
were no remarkable changes in young oocytes, whereas most (8/10) aged oocytes showed enlarged CP. At 1 or more h after heat shock, the enlarged CP were observed in both young and aged oocytes, but the enlargement of the CP was more prominent in aged oocytes (Figs. 2b and 2d). The percentage of young oocytes exhibiting enlarged CP did not vary with time post-heat shock, the range being 50–70% (6/10, 5/10, 4/6, 7/10 and 7/10 for 1, 2, 4, 8 and 16 h after heat shock, respectively). In contrast, the percentage of aged oocytes having enlarged CP was relatively higher at 1–8 h after heat shock, the range being 60–100% (8/9, 8/8, 6/10 and 7/7 for 1, 2, 4 and 8 h, respectively). At 16 h after heat shock, 7 of 10 aged oocytes had an altered surface; 4 of them were covered primarily with enlarged CP; the other 3 were covered sparsely with very short and thick MV. These observations suggest that there may be differences in the response of the vitelline surface of young and aged oocytes to heat shock and the effect of heat shock may be sustained throughout 16 h post-treatment.
Cleaving and cleaved oocytes

No cleavage oocyte was found during 8 and 16 h incubation in both control (n=19 and 20, respectively) and heat-shocked (n=20) young oocytes. For the aged oocytes, fourteen cleaving oocytes obtained after 8 h of incubation (9 heat-shocked; 5 non-heat-shocked), and eight 2- to 3-cell eggs obtained after 16 h of incubation (6 heat-shocked; 2 non-heat-shocked) in the aged group were evaluated by SEM. The majority of the cleaving oocytes (11/14) had a mixed pattern of low density MV and CP; some exhibited partial losses of MV and CP on the surface of blastomeres (Figs. 3a and 3b). The other 3 oocytes were covered primarily with MV. In cleaved oocytes obtained after 16 h, it was generally noted that short MV were distributed sparsely on the surface of blastomeres (Figs. 3c and 3d). It should be noted that the incidence of fragmentation also increased around 16 h of incubation.

Discussion

To our knowledge, this is the first report describing the three-dimensional surface...
characteristics of heat-shocked bovine oocytes. The surface characteristics of the ZP and vitelline membrane of bovine oocytes after 24 h of maturation were similar to what we had observed previously [15, 16]. The present study demonstrated clearly that heat shock treatment induced much more deterioration of the ZP surface of the bovine oocyte than did oocyte aging; and the vitelline surface changed from a MV-predominant to an enlarged CP-predominant one. A large CP-predominant pattern has been observed frequently in immature bovine oocytes [15, 16]. Interestingly, the altered surface morphology shortly after heat shock was sustained throughout a 16 h post-treatment. The present observations also suggest that effects of heat shock on the bovine vitelline surface appear more quickly and occur at a higher incidence in aged oocytes than in young oocytes.

Cleaving or cleavage of aged oocytes was observed at 8–16 h of incubation with or without heat shock. Our observations were in agreement with the previous report [18], in which an average of approximately 60% of aged oocytes (≥40 h) developed parthenogenetically in vitro regardless of the

Fig. 3. Surface characteristics of vitelline membrane of cleaving/cleaved eggs. Bar represents 10 µm in a and c and 1 µm in b and d. a) An aged oocyte 8 h after heat shock. The cleaving furrow was obviously distinguished. Some MV/CP of the vitelline surface were lost partially. b) A higher magnification of Fig. 3a. The vitelline surface was covered with a sparse distribution of MV and small CP. Note very short and thick, mushroom-like MV (arrows). c) An aged, 3 cell-egg after 16 h of incubation without heat shock, showing spontaneous activation. The vitelline surface was covered with a sparse distribution of short MV. d) A higher magnification of Fig. 3c. The vitelline surface was covered with a mixed distribution of relatively long MV and very short, mushroom-like MV.
activation stimulus. Although no cleavage was observed, the chromosome separation might have occurred in some of the heat-shocked young oocytes during 8- and 16-h incubation. Previously, we found that around 10–40% of heat-shocked young oocytes had chromosome separation without cleavage during 1–16 h incubation period (Ju, Suzuki, Parks, Yang, in preparation). There were no specific differences in the vitelline surfaces between oocytes activated spontaneously or treated by heat shock in this study. Cleaved oocytes were characterized by a sparse distribution of short MV with occasional small CP. These surface features were very close to those of 2- to 8-cell bovine embryos derived from in vitro fertilization [19]. Interestingly, some “additional” MV structures were found on the surface of the cleavage furrow or the membrane.

In conclusion, a brief heat shock changed the ZP and vitelline surfaces of bovine oocytes, which were distinguished from those observed during oocyte aging. Further, the effect of heat shock was manifested more quickly and to a greater degree in aged oocytes than in young oocytes.

Acknowledgments

This research was supported in part by the Cooperative State Research Education, and Extension Service, U.S. Department of Agriculture, under Agreement No. 96–35203–3268 and by Genex Inc. Ovine FSH and LH used throughout our research were kindly provided by the National Institute of Diabetes and Digestive and Kidney Diseases, the National Institute of Child Health and Human Development, the U.S. Department of Agriculture, and the National Science Council, Taiwan, R.O.C. H. Suzuki was supported by Hiroasaki University. This work was also partially supported by the Ito Foundation and Mishima Kaiun Memorial Foundation, Japan. The authors thank Marina Julian for critical reading of this manuscript. The authors also thank Wendy West for the help in all aspects.

This manuscript is a scientific contribution (number 1761) of the Storrs Agricultural Experiment Station at the University of Connecticut.

References

205–231.


