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Effects of heat stress on ovarian development and the expression of *HSP* genes in mice

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ABSTRACT

Heat stress reduces oocyte competence, thereby causing lower fertility in animals. Chronic and acute heat stresses cause extensive morphological damage in animals, but few reports have focused on the effects of chronic and acute heat stresses on ovarian function and heat shock protein (*HSP*) gene expression during ovarian injury. In this study, we subjected female mice to chronic and acute heat stresses; we then calculated the ovary index, examined ovary microstructure, and measured the expression of multiple *HSP* family genes. Chronic heat stress reduced whole-body and ovarian growth but had little effect on the ovarian index; acute heat stress did not alter whole-body or ovarian weight. Both chronic and acute heat stresses impaired ovary function by causing the dysfunction of granular cells. Small *HSP* genes increased rapidly after heat treatment, and members of the *HSP40*, *HSP70,* and *HSP90* families were co-expressed to function in the regulation of the heat stress response. We suggest that the HSP chaperone machinery may regulate the response to heat stress in the mouse ovary.

1. Introduction

Global average temperatures have risen in recent decades due to the effect of increased atmospheric carbon dioxide (Schuur et al., 2015; Takahashi, 2012); resultant increases in ambient temperatures can cause heat stress. In animals, heat stress has deleterious effects on reproductive functions, including follicular development, oocyte quality, sperm vitality, and embryonic development (Boni, 2019; Gao et al., 2016). Exposure to a high-temperature environment causes increased body temperature in animals (Cooper and Washburn, 1998), and they have evolved numerous mechanisms to respond to its harmful effects. The specific detrimental effects of heat stress are dependent on the duration of exposure (Xie et al., 2014). Chronic heat stress (long-term heat) provokes injury to organs and induces the expression of heat shock proteins (HSPs) (Horowitz, 2007). It induces adaptations to high temperature, and the damage accumulates gradually. By contrast, acute heat stress (short-term, sub-lethal heat stress) leads to significant tissue damage and the rapid synthesis of HSPs through dramatic upregulation of their mRNA transcripts (Feder and Hofmann, 1999). Therefore, HSPs play critical roles in the regulation of both acute and chronic heat stresses.

The functions of HSPs in heat stress have been extensively studied. HSPs, whose molecular masses range from 15 to 110 kDa, are categorized into several groups based on their size and function (Kregel, 2002). They are found in various locations (Kregel, 2002) and include the HSP27 family (small HSPs, HSPB), the HSP40 family (DNAJ), the HSP60 family, the HSP70 family (HSPA), the HSP90 family (HSPC), and the HSP110/104 family (HSPH). Most HSPs function as chaperones, interacting with other proteins to influence their folding, trafficking, and degradation and thereby regulate the development of the reproductive system (Feder and Hofmann, 1999).

Small HSPs are comprised of eleven small molecular weight proteins (15–40 kDa). They are transiently up-regulated in response to heat stress (Dubińska-Magiera et al., 2014) and are important for cellular homeostasis under high temperatures and oxidative conditions (Takahashi et al., 2010). HSPBs, a group of ATP-independent molecular chaperones, bind to unfolded proteins after heat stress to prevent their irreversible aggregation before they are transferred to the ATP-dependent chaperone network for refolding. HSPBs are expressed in ovarian cells, including follicles, nurse cells, and oocytes (Velázquez et al., 2011). However, the loss-of-function of small HSPs does not severely affect female fertility, possibly because of the functional redundancy between different HSP

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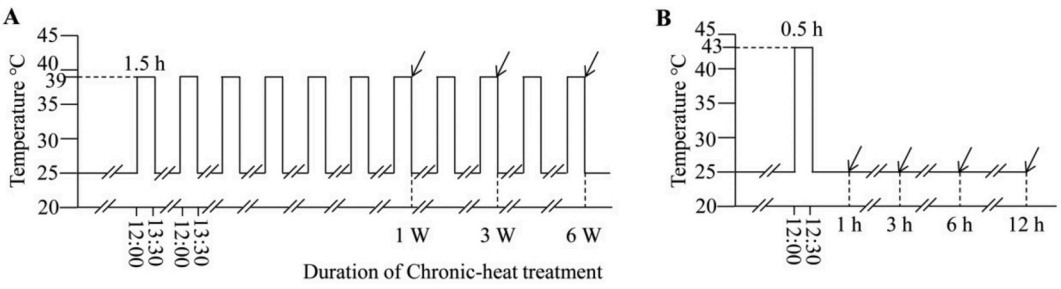

Fig. 1. Illustration of thermal application in the chronic and acute heat stress treatments. A, Chronic heat stress treatment; B, Acute heat stress treatment. The arrows indicate the sampling point.

families (Christians and Benjamin, 2006).

The HSP40 family plays a fundamental role in the regulation of HSP70 activity (Fan et al., 2003). Complexes of HSP40 and HSP70 can engage HSP90s to receive substrates for further folding or degradation (Cintron and Toft, 2006; Tsuboyama et al., 2018). Therefore, the HSP70 family has a major regulatory role in animal development. The functions of HSP70 proteins include protection against heat stress, as well as house-keeping roles in non-stressed cells (Daugaard et al., 2007). Heat stress induces HSP70 expression and reduces aromatase proteins in antral follicles; atretic follicles and apoptotic granulosa cells, which can decrease fertility, are increased in heat-stressed animals (Santos et al., 2008). Induced HSP70s may reduce the apoptosis of granulosa cells caused by heat stress (Ravagnan et al., 2001). Moreover, HSP70s can downregulate autophagy to reduce premature ovarian failure (Sisti et al., 2015). The 78 kDa glucose-regulated protein GRP78 (also called HSPA5) belongs to the HSP70 family and localizes to the ER lumen. GRP78, as a receptor, is presented on the cell surface to regulate cellular proliferation and survival. It is mainly expressed in granulosa cells in healthy follicles (Yang et al., 2013), although its expression has been linked to follicular atresia in goats (Lin et al., 2012).

HSP90 is a highly expressed chaperone and house-keeping gene. It plays a role in early embryonic development and is mainly expressed in the ovary, especially in the oocytes and the early embryo (Neuer et al., 1998). Anti-HSP90 antibodies can inhibit embryo development and cause ovarian failure (Neuer et al., 1998; Petríková and Lazúrová, 2012). More HSP90 is present on the cell surface, and more autoantibodies can therefore bind to HSP90. The HSP90-bound autoantibodies are easily internalized into the cell and disrupt the interactions between HSP90 and cytoskeletal proteins, leading to a collapse of ovarian cytoarchitecture (Neuer et al., 1998; Petríková and Lazúrová, 2012). HSP90 and HSP70 can also interact with one another to enhance glucocorticoid receptor (GR) ligand affinity, demonstrating that coordinated chaperone interactions can increase stability, function, and regulation (Kirschke et al., 2014).

Some previous studies have shown that heat stress induces the expression of HSP genes and causes ovarian injury, but few reports have focused on the distinct effects of acute and chronic heat stress on ovarian development and *HSP* expression. Here, we analyze ovarian microstructure and *HSP* gene expression under acute and chronic heat stress to explore whether they are correlated.

2. Materials and methods

2.1. Ethics statement

All animal experimental procedures were reviewed and approved by the Institutional Ethics Committee of Zhejiang Agriculture and Forestry University to ensure compliance with international animal welfare guidelines.

2.2. Animals and treatments

Sixty-six 3-week-old female ZCK mice were obtained from the Zhejiang Medical Academy Experimental Animal Center (Hangzhou, China). The animals were raised in cages in an auto-controlled room (25 °C, 12-h light and 12-h dark, 50% humidity) at Zhejiang Agriculture and

Table 1
Primers used in this study.

Gene name	Forward	Reverse
HSP10	AGTTTCTTCCGCTCTTTGACAG	CAATCTCTCCACTCTTTCCTTTCC
HSP20	CAGGAACCCCAAACTCAAACTG	TGGCCTAGCACATCTGGAGAA
HSP22	CGTCTCGCAGTCTACAGTTG	CTGAGGTGAGCCAATGAGTC
HSP25	GAGCCAAGTAGAAGCCATCAG	CGCACAGATTGACAGAGAGG
HSP27	ACTGGCAAGCACGAAGAAAG	AATGGTGATCTCCGCTGACT
HSP30	GCAGGAGAAGATGGTGTATGTC	TGCGTATCAGTCAGGCTAGA
HSPB2	GAGTTCTGTCGCACCTATGTC	CTGGCTATCTCTTCCTCTTCCT
HSPB3	CACAGCACGGAACCAGAATG	ATGACAGAGGATGGCAGACAA
HSPB7	CGCCATTGTCCTATGCCTTA	CGCCAGTCTCCATTGATGAA
HSPB9	GACTTGGTGGTGCGGATA	GGTGAACACTCTGCTCCAT
HSPB10	AAGAAGGTGGACAGAGAATTAAGAC	TGGAGTAGCAGAGGCAGTAG
HSP32	CACGCATATACCCGCTACCT	GTGTCATCTCCAGAGTGTTCATTC
HSP40	GCGGCTGTACCAAGAAGATG	CGATGTCTGCTGGAATGTTGT
HSP47	CTACTATGACGACGAGAAGGAGAA	AGGATGATGAGGCTGGAGAG
HSP60	CATCGGAAGCCATTGGTCATAA	GACTGCCACAACCTGAAGAC
HSP65	CTGTGTTGAAGGTTGGAGGAA	TGTAGCATTGAGAGCATCAGTAAC
HSP70	TCGTGGAGGAGTTCAAGAGG	GCGTGATGGATGTGTAGAAGTC
HSP75	CTCAACATCCGCAGCATCTT	CCTCACTATCCACCACACCT
HSPA9	ACATCAACTTGCCATACCTTACC	ATGCCACCAACCAGAATCAC
HSP90B1	GAACCTCTGCTCAACTGGATG	CTGGTATGCTTGTGCCTTCA
HSPH1	GGTGATGGAGTGGATGAATAATGT	TCCTTGACCTTCGCTCTGAT
β-actin	AGATTACTGCTCTGGCTCCTA	TCGTACTCCTGCTTGCTGAT

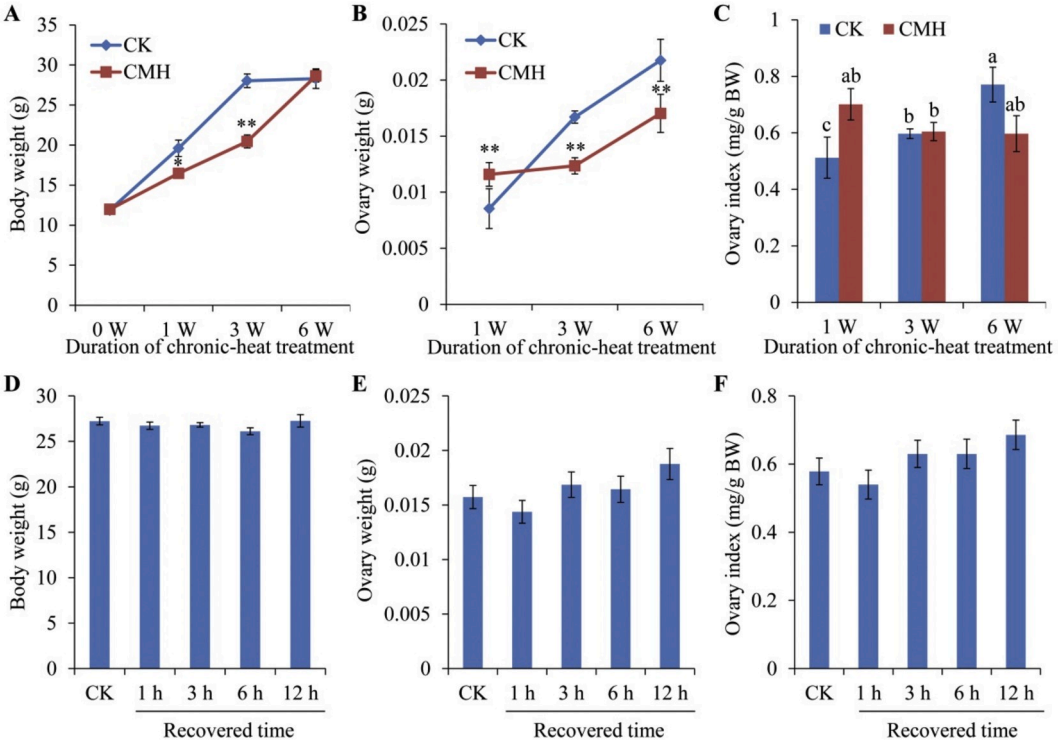

Fig. 2. Effect of heat stress on the body weight and ovary index. A, the body weight under normal and chronic heat stress conditions. Asterisks indicate significant difference from CK using Student's *t*-test (*$P < 0.05$, **$P < 0.01$, ±SE). B, the ovary weight under normal and chronic heat stress conditions. Asterisks indicate significant difference from CK using Student's *t*-test (**$P < 0.01$, ±SE). C, ovary index (ovary weight/body weight) under normal and chronic heat stress conditions. Compared with each other, different letters indicate significant difference between treatments using one-way ANOVA at $P < 0.05$ (±SE). D, the body weight (±SE) under normal and acute heat stress conditions. There was no significant difference among groups. E, the ovary weight (±SE) under normal and acute heat stress conditions. There was no significant difference among groups. F, ovary index (ovary wet weight (mg)/body weight (g) × 100%, ±SE) under normal and acute heat stress conditions. There was no significant difference among groups. CMH, chronic moderate heat group; CK, control check group; BW, body weight; SE, standard error.

Forestry University in Hangzhou, China.

For the chronic heat stress experiment, mice were randomly assigned to either the chronic moderate heat group (CMH) or the control check group (CK). Food and water were withheld during the heat treatment but were freely available at all other times. After exposure to a constant temperature of 25 °C at 50% humidity for 1, 3, or 6 weeks (CK, $n = 6$) or to daily 1.5 h (12:00–13:30) 39 °C treatments at 50% humidity for 1, 3, or 6 weeks (CMH, $n = 6$), the mice were euthanized. Their ovaries were weighed, and the ovary tissue was used to prepare tissue sections for histology and to measure *HSP* gene expression (Fig. 1A). Body weights were also measured weekly during the experiment.

For the acute heat stress experiment, 6-week-old mice (27 ± 2 g) were randomly assigned to either the acute transient heat group (ATH) or the control check group (CK). The ATH mice were exposed to a constant temperature of 43 °C at 50% humidity for 0.5 h and then permitted to recover for 1, 3, 6, or 12 h (ATH, $n = 6$) at room temperature (Fig. 1B). The CK and ATH mice were euthanized and their ovaries were processed as described above.

2.3. Histological analysis

Following euthanasia, the ovaries from each mouse were weighed, fixed in 10% formalin for 24 h at 4 °C, processed in paraffin, sectioned, and stained with haematoxylin and eosin as previously described (Li et al., 2016). The tissue sections were observed and photographed using an Olympus optical microscope.

2.4. Real-time PCR

Total RNA was extracted from mouse ovary tissue using the Trizol reagent (TaKaRa). First-strand cDNA was synthesized using the TaKaRa PrimeScript™ RT reagent kit with gDNA Eraser. Real-time qPCR was performed using the StepOnePlus Real-Time PCR System with the TaKaRa SYBR® *Premix Ex Taq*™ II reagent kit, following the manufacturer's instructions. Specific primers for the *HSP* genes were designed using Beacon Designer 7 software and are presented in Table 1. The β-actin gene was selected as an internal control. The relative expression of genes was calculated using the $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen, 2001). All experiments consisted of at least three biological replicates.

2.5. Statistics

Statistical analysis was performed with Excel and SPSS software. The statistical significance of treatment differences was assessed with Student's *t*-test or a one-way analysis of variance (ANOVA).

3. Results

3.1. Heat stress inhibits whole-body and ovarian growth

Heat stress inhibits the animal growth (Takahashi, 2012). At the beginning of the chronic heat stress experiment, there were no differences in body weight between the CK and CMH groups (Fig. 2A). At the conclusion of the 1-week heat treatment, there was a significant difference in average body weight between the CK group (19.6 g) and the CMH group (16.5 g) ($P < 0.05$, Fig. 2A). After the 3-week heat treatment, the significant difference in body weight between the groups was greater (28.0 g CK versus 20.5 g CMH, $P < 0.01$, Fig. 2A). The average body weight of the 6-week CK group (28.3 g) was the same as that of the 3-week CK group (28.0 g) and did not differ significantly from that of the

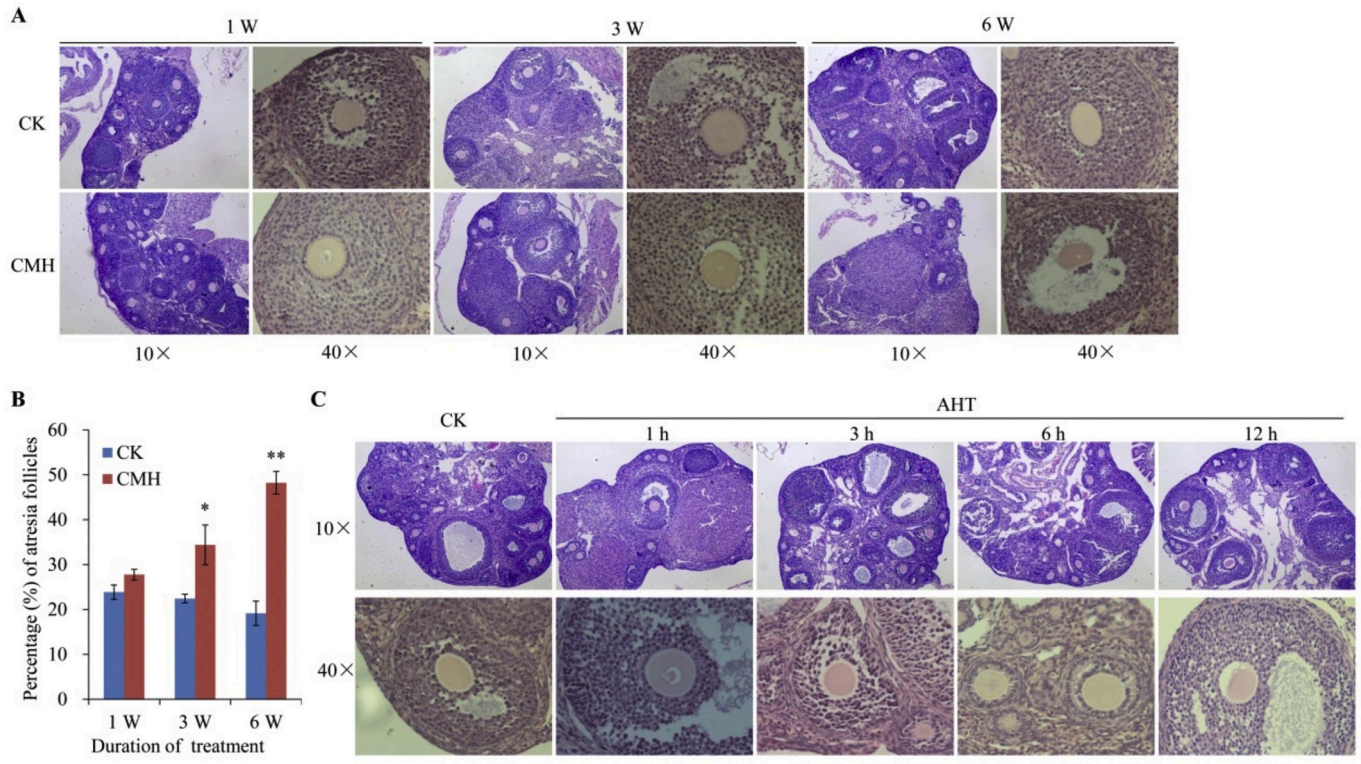

Fig. 3. Morphology of ovaries stained with haematoxylin and eosin from ovaries of control and heat-stressed mice. A, the phenotypes of ovaries under normal and chronic heat stress conditions at 10× and 40× folds. B, the percentage of atretic follicles in the total population of antral follicles on the ovaries after chronic heat stress treatment. Values are mean ± SD of two biological replicates. * indicates $P < 0.05$ and ** indicates $P < 0.01$ compared with the CK groups. C, the phenotypes of ovaries under normal and acute heat stress conditions at 10× and 40× folds.

six-week CMH group (28.6 g) ($P > 0.05$, Fig. 2A).

Average ovary weight increased with age in the CK group (Fig. 2B). After 1 week of heat treatment, the average ovary weight of the CMH group was significantly higher than that of the CK group (Fig. 2B), suggesting that heat stress may have caused bloating of the ovaries. At 3 and 6 weeks, ovary weights had increased in the CMH groups, but they were significantly lower than those of the CK groups (Fig. 2B). To further analyze the relationship between body mass and ovary mass, we calculated the ovary index (ovary wet weight (mg)/body weight (g) × 100%) and found that it increased with age in the CK groups (Fig. 2C). By contrast, there were no significant differences in ovary index with age in the three heat-treated groups (Fig. 2C). Moreover, the ovary index of the 1-week CMH groups was greater than that of the CK group (Fig. 2C). These results suggest that chronic heat stress inhibited both whole-body and ovarian growth and transiently affected ovary index in the first week of treatment.

In the acute heat stress experiment, the average body weight (approximately 27 g in all groups) and the ovary weight (approximately 0.015 g in all groups) did not differ between CK and ATH mice and were not affected by the length of recovery time at room temperature (Fig. 2D and E). Acute heat stress also had no effect on the ovary index (Fig. 2F).

3.2. Heat stress impairs ovary function

Exposure of animals to high temperatures resulted in a significant decrease in fertility. However, in this study, the mouse ovary index did not differ consistently between normal and heat-stressed animals. Therefore, we reasoned that heat stress may have damaged the ovary's microstructures, thereby reducing fertility. To test this hypothesis, we performed a histological analysis of ovarian sections from mice exposed to normal and heat-stressed conditions.

In the chronic heat stress experiment, the ovaries of CK mice had many primary follicles and granular cell layers; the granulosa cells were round and their nuclei were darkly stained. As the mice reached sexual maturity, the ovary volume gradually increased, and the connective tissue of the medulla was intact and densely covered with arteries (Fig. 3A). By contrast, the ovaries of the 1-week CMH mice had more primary growing follicles than those of the CK group, and the staining of the granulosa cell nuclei was lighter (Fig. 3A). In the 3-week CK group, the secondary growing follicles were mainly contained in the cortex, and the follicles gradually matured and protruded on the cortical surface. Follicular cavity and cumulus cells were formed, and the granulosa cells became bigger and rounder (Fig. 3A). In the 6-week CK group, numerous primordial follicles and growing follicles were contained in the cortex. The granular cell layers were dense, and their structures were clearly visible (Fig. 3A). In the 3- and 6-week CMH groups, the ovaries were smaller, and the medullas were partially contracted with reduced numbers of blood vessels. Atresia follicles were more numerous, and the oocytes were detached from the granular cell layer. The granular cell layers were disordered and had partially fallen off. Their granular cells were reduced, irregular, and karyopyknotic (Fig. 3A). Statistics results showed that the percentage of atretic follicles of 3- and 6-week CMH groups was significantly greater than that of CK groups (Fig. 3B). These results indicated that chronic heat stress had impaired the function of the ovary by inhibiting its growth and causing dysfunction of the granular cells.

In the acute heat stress experiment, heat stress had a greater impact on the connections between granulosa cells (Fig. 3C). After 3 h of recovery, the granular cells were disordered, loosened, irregular and karyopyknotic (Fig. 3C). After 6 h of recovery, numerous vacuoles were observed in the granulosa cells of the primary growing follicles (Fig. 3C). However, after 12 h of recovery, there were no observable differences compared with the CK group (Fig. 3C). These results suggest that the ATH treatment primarily affected the function of the granulosa cells.

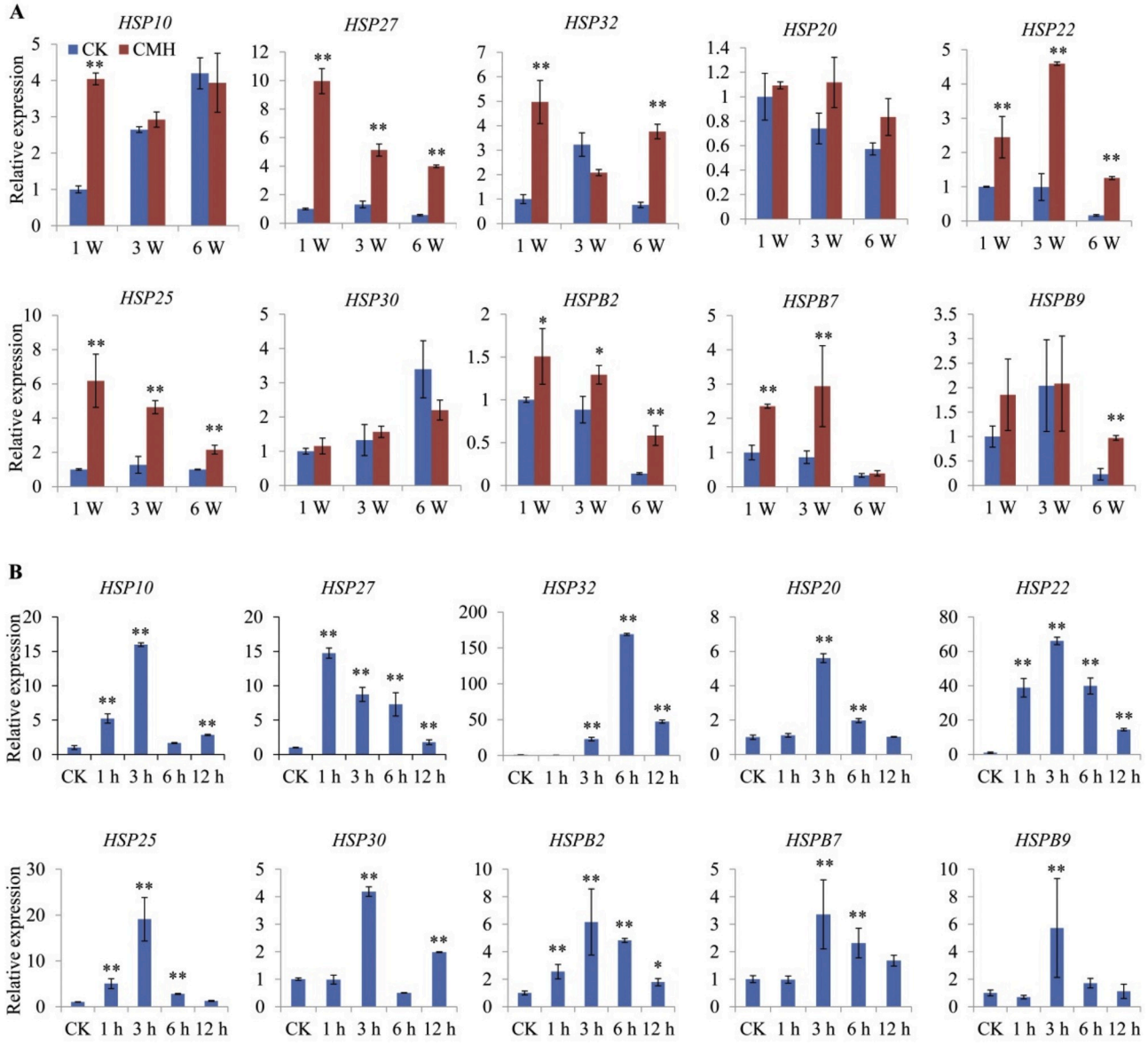

Fig. 4. Expression of small HSPs in ovaries of control and heat-stressed mice. A, under normal and chronic heat stress conditions; B, under normal and acute heat stress conditions; Asterisks indicate significant difference from CK groups using Student's *t*-test (**$P < 0.01$, *$P < 0.05$). Values are mean ± SD of three biological replicates.

3.3. Effect of heat stress on the expression of small HSP genes

Despite the extensive morphological damage caused by chronic and acute heat stresses, the expression changes of small *HSP* family genes were unclear. In the chronic heat stress experiment, the expression of most small *HSPs* including *HSP10*, *HSP27*, *HSP32*, *HSP22*, *HSP25*, *HSPB7*, *HSPB2*, and *HSPB*9, rapidly increased in the 1-week treatment group compared with the CK group (Fig. 4A). At 3 and 6 weeks, the expression of *HSP27*, *HSP22*, *HSP25*, and *HSPB7* remained higher, but the expression of other small HSPs did not differ from that of the CK groups. The expression of *HSP20* and *HSP30* did not differ significantly between heat-treated and CK groups (Fig. 4A).

In the acute heat stress experiment, the expression of all detected small *HSP* genes was elevated after heat treatment. The highest expression levels of *HSP10*, *HSP20*, *HSP22*, *HSP25*, *HSP30*, *HSPB7*, *HSPB2*, and *HSPB*9 occurred after 3 h of recovery, while the highest expression levels of *HSP27* and *HSP32* occurred after 1 and 6 h of recovery, respectively (Fig. 4B).

3.4. Effect of heat stress on the expression of HSP40, HSP70 and HSP90 family genes

HSP40, HSP70, and HSP90 proteins function in a complex to prepare substrates for folding or degradation and to control animal development (Cintron and Toft, 2006). In the chronic heat stress group, the expression of *HSP40* increased from 1 week and reached its highest level at 3 weeks compared with CK. Its expression then decreased to the 1-week level in the 6-week group (Fig. 5A). The expression of *HSP47* and *HSP60* reached its highest level at 1 week compared with CK and thereafter decreased to the basal level of CK (Fig. 5A). Members of the HSP70 family, including *HSPA1B*, *HSP75,* and *HSPA9*, exhibited the highest expression after the 1-week treatment, similar to *HSP90B1* (Fig. 5A). In addition, the expression of *HSPH1* was high throughout the experiment (Fig. 5A).

The expression of *HSP40* and *HSPA9* was higher in the acute heat stress group than in the CK group throughout the experiment (Fig. 5B). *HSP47*, *HSP65*, *HSPA1B*, *HSP90B1,* and *HSPH1* reached their highest expression levels compared with CK following 1 or 3 h of recovery and thereafter returned to basal levels (Fig. 5B). *HSP75* expression

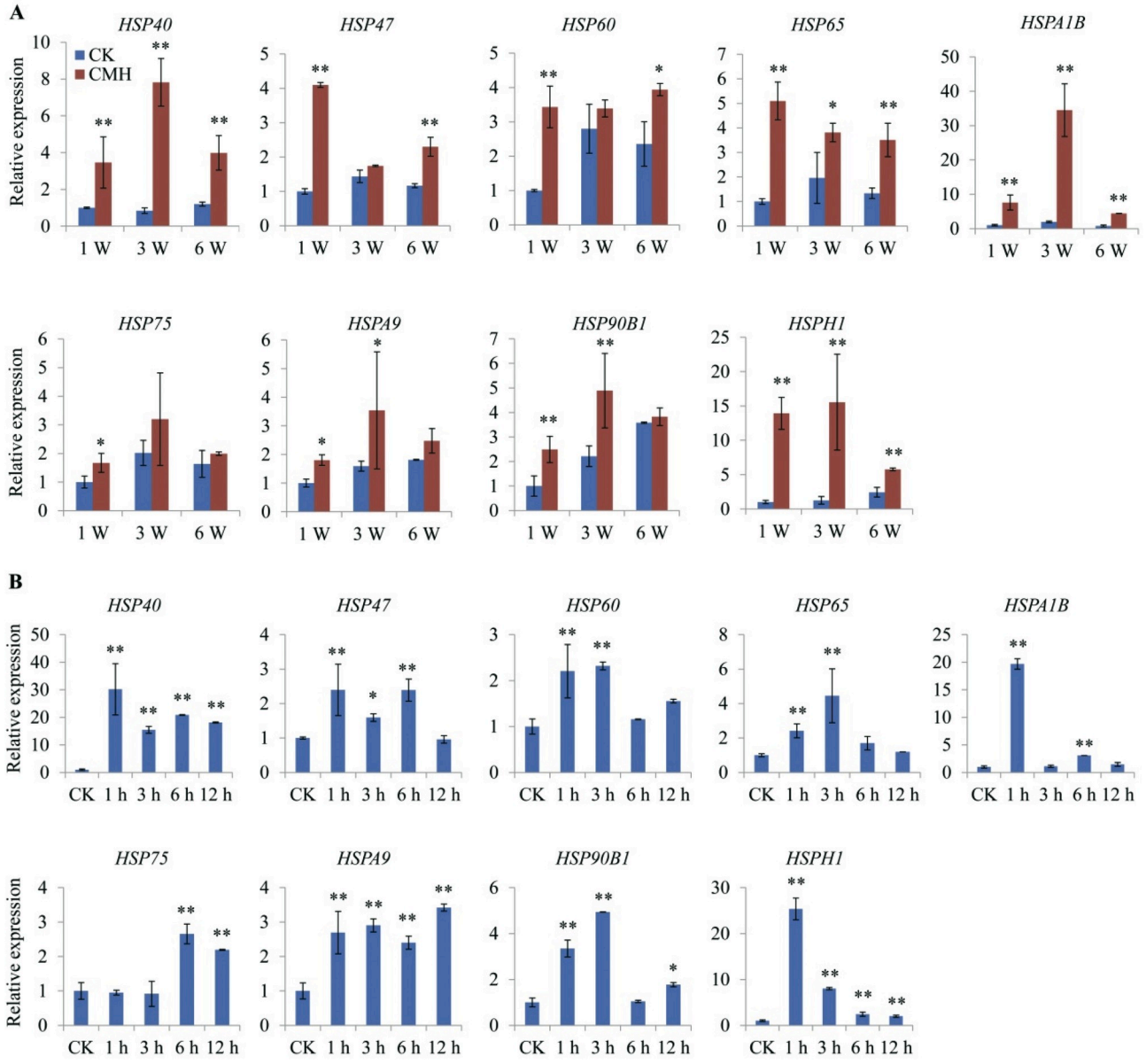

Fig. 5. Expression of HSP40, HSP60, HSP70, HSP90 families in ovaries of control and heat-stressed mice. A, under normal and chronic heat stress conditions; B, under normal and acute heat stress conditions; Asterisks indicate significant difference from CK groups using Student's *t*-test (**$P < 0.01$, *$P < 0.05$). Values are mean ± SD of three biological replicates.

responded more slowly and increased following 6 and 12 h of recovery (Fig. 5B).

4. Discussion

High environmental temperatures cause reduced fertility by changing animal physiology and performance (Takahashi, 2012). Numerous studies have focused on the harmful effects of heat stress following different durations of exposure, i.e. chronic heat stress and acute heat stress. In this study, we observed a significant reduction in body weight and ovary weight in mice after 1 and 3 weeks of chronic heat treatment, and the ovary index differed significantly between the CMH and CK groups after 1 week of chronic heat treatment. In the CK groups, the ovary index increased with age, but chronic heat exposure prevented this increase. Moreover, the significant enlargement of the ovaries observed after 1 week of chronic heat treatment may have led to pathological changes in the ovaries. Rats under heat stress must take in more water to maintain their whole-body water balance, minimize heat gain, and increase heat loss. Heat stress increases rectal, scrotal surface, and body surface temperatures, possibly by inhibition of digestive enzyme activity (Li et al., 2013). Reductions in body weight may be caused by reduced nutrition intake and nutritional imbalances under high temperatures (Evans and Anderson, 2012). Most importantly, this unbalanced nutrition may lead to reproductive dysfunction (Li et al., 2016). Therefore, alterations in the ovary index may have affected fertility and there may have been functional injury to the ovaries of heat-stressed mice. Histological observations of ovary sections showed that CMH and ATH both affected the function of the granulosa cells by producing irregular and karyopyknotic granular cells and causing the granular cell layers to become disordered and separated. These changes may be the main cause of reduced fertility under heat stress.

We observed more primary growing follicles and fewer secondary growing follicles in the CMH group than in the CK group. This result may have been due to increased follicular atresia and elevated vacuoles that inhibited the connection between the primary growing follicles. In pig, heat stress induced autophagy in ovaries during follicular development

but had no effect on follicle size or number (Hale et al., 2017). Previous studies have shown that chronic heat stress can reduce estradiol levels and increase granulosa cell apoptosis and follicular atresia in mice (Li et al., 2016). The occurrence of apoptosis and atresia were probably related to nutritional imbalance (Li et al., 2016). In livestock, such as dairy cows, heat stress decreased the production of estradiol and reduced the viability of granulosa cells by changing glucose and non-esterified fatty acid levels in the blood (Miller-Cushon et al., 2019). Moreover, the duration of heat stress appeared to affect the response to dietary protein in birds (Gonzalez-Esquerra and Leeson, 2005). These studies indicate that heat stress alters the distribution of nutrition to follicles for oocyte growth.

Heat stress can be classified as either short- or long-term. Many studies have demonstrated that HSPs participate in acclimation to stressors (Takahashi, 2012). Here, we analyzed the expression of genes from the small *HSP*, *HSP40*, *HSP60*, *HSP70*, *HSP90,* and *HSP110* families in mice ovaries that had been exposed to acute and chronic heat stresses. We found that small HSP genes were induced more quickly than other groups and that their expression declined at 3 and 6 weeks, suggesting that they regulated early responses to heat stress. Newly synthesized small HSPs are thought to maintain cellular homeostasis by enhancing correct protein folding. HSP10 acts as a growth factor in the cell, and elevated HSP10 levels under heat stress may decrease growth inhibition. Our results indicated that the small HSP family probably played an important role in early protection of the ovary under heat stress.

HSP40 is the co-chaperone of HSP70 (Qiu et al., 2006). Newly synthesized HSPs are recruited to chaperone the denaturing protein pool for cellular restoration and cytoprotection (Wang et al., 2014). In the present study, *HSP40* and *HSP7*0 mRNAs were inducible and remained at a high level during heat treatment. Increased levels of the DnaJ protein HSP40 have been shown to stimulate ATPase activity to promote protein binding (Qiu et al., 2006). The induced HSP40s and HSP70s may protect cells from apoptosis, autophagy, and follicular atresia in the ovary. In this study, it appeared that the HSP40 and HSP70 families were important for the continual protection of cells.

HSP60 can interact with HSP10, forming a chaperone complex that promotes the folding of a subset of client proteins (Richardson et al., 2001). Previous studies have shown that HSP10 is involved as a co-factor in HSP60-mediated protein folding and sorting (Carra et al., 2019). Moreover, the biogenesis of HSP60 depends on the interaction between HSP70 and HSP10, and HSP70 promotes the import of HSP60 (Böttinger et al., 2015). Here, we found that gene expression levels of most *HSP* families were elevated under heat treatment, suggesting that the HSPs collaborated with one another to respond to stress by refolding heat-damaged proteins.

In conclusion, chronic heat stress reduced body weight and ovary weight, and affected the ovary index; it also inhibited the development of primary growing follicles. Chronic and acute heat stresses increased the number of dysfunctional granulosa cells. Small HSPs played roles in the early stages of heat stress, and the HSP40 and HSP70 families functioned in all development processes under heat stress. Further studies are needed to interpret the relationship between phenotype and *HSP* expression.

Declaration of competing interest

The authors declare no conflicts of interest.

CRediT authorship contribution statement

Mingyan Bei: Formal analysis, Writing - review & editing. **Qian Wang:** Formal analysis, Writing - review & editing. **Wensai Yu:** Formal analysis, Writing - review & editing. **Lu Han:** Writing - review & editing. **Jing Yu:** Formal analysis, Writing - original draft, Writing - review & editing.

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