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# Association of heat shock proteins, heat shock factors and male infertility

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ABSTRACT

It has been well established that heat shock proteins (HSP) and heat shock factors (HSF) are involved in wide varieties of physiological regulation process and signal pathway. Numerous members of heat shock family exhibit a cell-type-specific expression pattern during spermatogenesis and play crucial roles in germ cell development. This led to the emerging studies to reveal the association between heat shock family and male infertility. Aberrant expressions of HSP/HSFs observed in sterile men and animal models indicate the two opposite effects, both protective and harmful, of heat shock family on male fertility. Moreover, HSP/HSFs are also involved in the two major causes of male infertility. It seems that different behaviors of HSP/HSFs patients with varicocele and *Chlamydia trachomatis* infection lead to distinct outcomes of male fertility. In addition, emerging evidence has demonstrated that the altered expression of HSP/HSFs may be responsible for the abnormal germ cell apoptosis and subsequently results in impaired spermatogenesis. Therefore, heat shock family may play an important role in the quality-control of germ cells during spermatogenesis, raising the prospect of their utility for novel treatment targets in male infertility.

## 1. Introduction

The precise three-dimensional conformation is essential for proteins to fulfill their physiologic functions. One of the most important components that play a critical role in proteins folding is the heat shock protein (HSP), which was first identified in 1974 by Tissiere *et al.* in salivary glands of *Drosophila melanogaster*[1]. Over the past four decades, more than 80 proteins have been identified as HSPs[2,3]. The human HSPs have been classified into six subgroups according to their molecular weight, namely, HSPH (HSP110), HSPC (HSP90), HSPA (HSP70), HSPD/HSPE (HSP60/HSP10), DNAJ (HSP40) and HSPB (small HSP)[2]. HSPs are highly conserved among species, they were first found to be induced by cytotoxic stresses like hyperthermia, oxidizing conditions, toxic compounds, but emerging evidence has demonstrated that some of HSPs are constitutively expressed in the absence of stress in a variety of tissues and cells in human. HSPs are originally identified as molecular chaperone to assist proteins in folding into correct structure, transport, assembling into complexes, and preventing proteins from aggregation, while synaptic transmission, autophagy, endoplasmic reticulum stress response, protein kinase and cell death signaling are also involved[3].

Heat shock response in organism is a sophisticated regulation procedure and heat shock factors (HSFs) are the principal regulators. So far, four different HSFs (HSF1, HSF2, HSF3 and HSF4) have been identified in mammals[4]. By modulating the HSPs expression, HSFs possess the ability to promote cells survival against environment stresses[5]. Furthermore, they are also essential for cell differentiation and development[4,6].

Recently, the repertoire of HSPs/HSFs functions has expanded well beyond molecular chaperone. Growing evidence demonstrates that HSPs/HSFs are involved in the spermatogenesis and may affect male fertility. This review

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aims to bring up to date information of HSPs and HSFs, try to elucidate the association between HSPs, HSFs and male infertility.

## 2. Expression pattern of HSPs/HSFs during spermatogenesis

Spermatogenesis is a unique and meticulous process of cell differentiation that starts with the division of stem cells and ends up with the formation of mature sperms, which are capable of fertilizing an ovum. Spermatogenesis process can be divided into several stages, including mitotic proliferation and differentiation of spermatogonia, meiosis of spermatocytes, transformation of spermatids into testicular sperm, and release into the lumen of the seminiferous tubules. It has become increasingly clear that the expression of HSPs is a highly regulated event in the process of spermatogenesis. The expression patterns of numerous HSPs during differentiation of germ cells have been discovered in previous studies (Figure 1[7-23]). In mouse testis, HSF1 was transiently expressed in spermatocytes and round spermatids but disappeared since elongated spermatids[7]. HSPA2 used to be considered a testis-specific protein. In human testis, expression of HSPA2 began in meiotic spermatocytes, and was prominent during elongating spermatids and mature sperms[24]. However, Scieglinska *et al.* have revealed that HSPA2 was detected in ten other tissues in addition to testis, such as adrenal gland, bronchus, cerebellum and colon, *etc*[25]. More information about expression patterns of HSPs and HSFs (including human HSPC1, HSPA5, HSPA2, HSPD1, HSPB1 and mouse HSPH3, HSPH2, HSPA5, HSPA2, HSPC4, HSPD1, DNAJA1, DNAJA2, HSPE1, HSF1, HSF2) is presented in Figure 1. Since the heat shock family contains various members and their complicated expression patterns and molecular mechanisms in human tissues remain largely unclear, more research need to be performed in order to gain further insight into HSPs/HSFs.

## 3. General properties of HSPs/HSFs and their roles in male infertility

Approximately 9% to 15% couples suffer from infertility, while male factor infertility is responsible for about 50% of the cases[26]. A significant decline in semen quality (particularly in sperm count) has been reported for many years[27]. While the number of sterile men keeps growing up, there is increasing evidence that HSPs/HSFs may be involved in male factor infertility. In this section, we will briefly introduce the general properties of six HSP and two HSF families, then concentrate on their impacts on male infertility (Table 1[10-12,22,28-35]).

### *3.1. HSPA (HSP70)*

The HSPA is currently the most widely studied family among HSPs, it contains 13 genes in human which encode 13 highly similar members[2,3]. Each member contains an amino-terminal ATPase domain and a carboxy-terminal substrate binding domain, both of which are highly conserved among species. They perform a typical heat inducible chaperone function, but most of which are also found with cellular housekeeping function[3]. The chaperone function of HSPA mainly depends on ATP hydrolysis and nucleotide exchange in cooperation with the co-chaperone including HSPH (HSP110) and DNAJ (HSP40), which will be discussed below.

In 1996, Dix *et al.* reported for the first time that Hspa2 *(Hsp70-2)* knock-out (KO) male mice resulted in failed

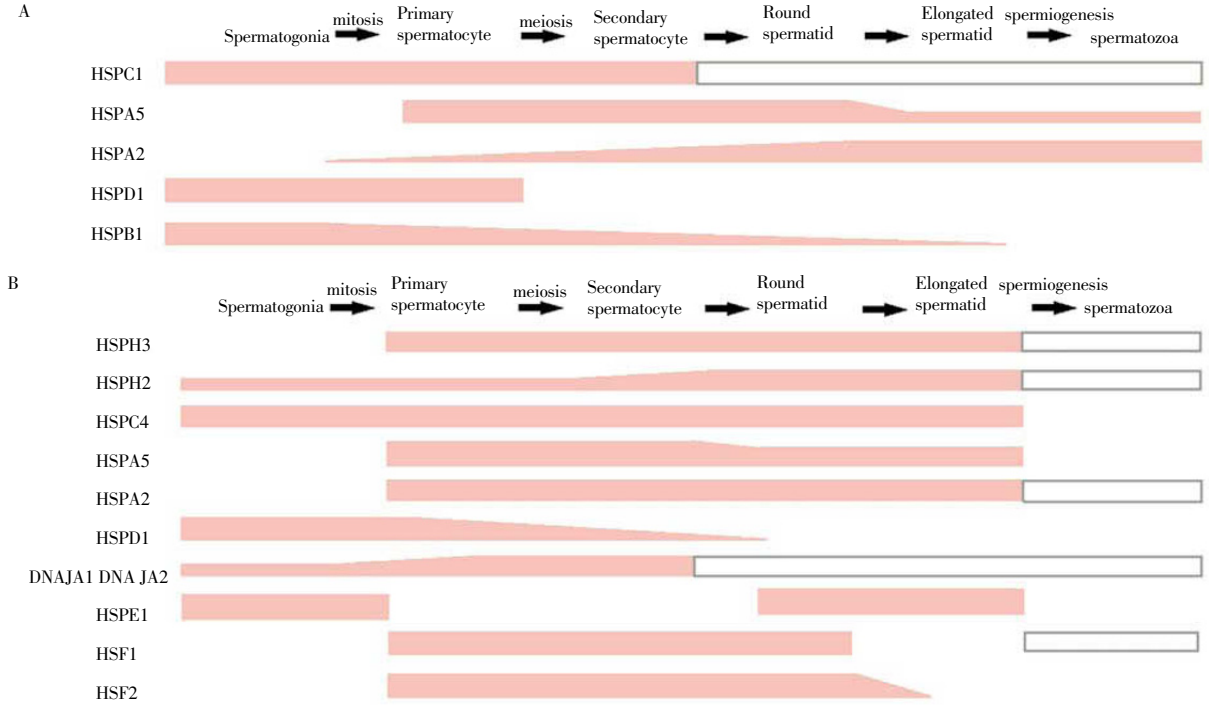


**Figure 1.** Expression patterns of HSPs/HSFs during spermatogenesis in human (A) and mouse (B).
Pink boxes indicate the expression of HSPs/HSFs, absence of boxes indicates lack of expression in those cell types, and white boxes indicate lack of analysis for those cell types[7-23].

meiosis, pachytene spermatocytes undergoing massive apoptosis and male infertility, while *Hspa2* KO female mice showed totally normal fertility[30]. Their research provides a valuable novel insight into male infertility associated with HSPs and also suggests that HSPs participate in male germ cell development. Furthermore, HSPA2 appears to play a crucial role in desynapsis of synaptonemal complexes and in the assembly of the Cdc2-cyclin B1 complex during meiotic phase[30,36]. These findings concur with the results that *Hspa2* KO mice exhibited failure in meiotic phage. HSPA2 is also the first chaperone that was involved in the process of post-meiotic genome-wide reorganization due to its ability to associate with major spermatid DNA-packaging proteins, transition proteins 1, 2, and 4[37]. More recently, accumulating evidence has demonstrated that aberrant expression of HSPA can disturb male fertility[38]. There were significantly different expression levels of HSPA2 among normal, maturation arrest and Sertoli cell-only testis tissues. Low expression of HSPA2 was detected in maturation arrest testes compared to normal testes and HSPA2 could not be detected in Sertoli cell-only testes[8]. It seems that HSPA2 is essential for spermatogenesis and low expression of HSPA2 can cause male infertility. To further examine the effects of HSPA, Western blot and TUNEL assay were performed by Erata *et al*. Increase expression of HSPA was found in spermatozoa from men with asthenozoospermia and oligoasthenozoospermia, whereas HSPA could not be detected in azoospermic group. The TUNEL assay demonstrated that spermatozoa DNA fragment was higher in infertile men than the fertile ones. Both of the TUNEL-positive cells and HSPA expression had a notable inverse correlation with concentration of spermatozoa[15]. These studies support the assumption that HSPA up-regulates in response to adverse conditions and acts as germ cells protective factors. The lack of HSPA in testis may be associated with spermatogenic failure and thus impair male fertility.

### 3.2. HSPC (HSP90)

HSPC contains five highly homologous members and it is the most highly abundant constitutively expressed HSP that comprises 1% to 2% of total cell proteins even under non-stress conditions[3,5,29]. HSPC contains an amino-terminal ATP binding domain which can be competitively blocked by its inhibitor geldanamycin. HSPC and HSPA have many similarities in localization and expression, and they collaborate on many activities through the co-chaperone including HSPH (HSP110) and DNAJ (HSP40). Disturbance of HSPC resulted in up-regulated HSPA expression and enhanced function[3]. More importantly, HSPC is one of the most promising anti-cancer targets. Latest studies showed that HSPC inhibitor could suspend the growth of e migration inhibitory factor (MIF)-expressed breast tumor by destabilizing the MIF[39], and inhibited the growth of leukemia by promoting the degradation of both wild-

**Table 1**
Impacts of *Hsp/Hsf* knock-out/over-express on fertility of male mouse.

| Gene | Reproductive organs | Sperm quality | Testis tissue histopathological analysis | Tunel analysis | Fertility status |
|---|---|---|---|---|---|
| *Hsph3*$^{-/-}$[29] | Lacked data | Reduced sperm count and motility | Dramatic reduction of Sd, multinucleated giant cells, premature release of germ cells into the lumen | Increased apoptosis germ cells | Hypo-fertility |
| *Hsph2*$^{-/-}$[30] | Smaller testis | Reduced sperm count and motility | Reduced diameter of ST, ST vacuolization, reduced Sc and Sd, multinucleated giant cells | Increased apoptosis germ cells (mostly Sc) | Mostly infertile |
| *Hspc4*$^{-/-}$ (*Hsp90b1*$^{-/-}$)[26] | No abnormality | Large and globular sperm head | No abnormality | Lacked data | Infertile |
| *Hspc1*$^{-/-}$ (*Hsp90 alpha*$^{-/-}$)[17] | Smaller testis | Azoospermia | Lack of post-meiotic germ cell, arrest at pachytene Sc | Increased apoptosis germ cells | Infertile |
| *Hspa2*$^{-/-}$[12] | Smaller testis | Lacked spermatozoa in epididymis | ST vacuolization, lack of post-meiotic germ cell, arrest at pachytene Sc | Increased apoptosis germ cells (mostly Sc) | Infertile |
| *Dnaja1*$^{-/-}$[27] | Smaller testis | Reduced sperm count and motility | Reduced diameter of ST, intraepithelial vacuoles, reduced Sc and Sd | Increased apoptosis germ cells (mostly Sc) | Mostly infertile |
| *Hsf1* overexpress[42] | Smaller testis (50%) | Lacked data | Reduced diameter of ST, increased LC, arrest at pachytene Sc | Increased apoptosis germ cells (mostly Sc) | Infertile |
| *Hsf1*$^{-/-}$[39,48] | No abnormality | No abnormality | No abnormality | No abnormality | Fertile |
| *Hsf1*$^{-/-}$[41] | No abnormality | Reduced sperm count | Disorganized/missing layers of germ cells in ST | No abnormality | Fertile |
| *Hsf1*$^{-/-}$[7] | No abnormality | Reduced sperm count, increased abnormal sperm heads | Disorganized/missing layers of germ cells ST, aberrant level of chromatin packing proteins | Lacked data | Fertile |
| *Hsf2*$^{-/-}$[47,48,74] | Smaller testis and epididymis | Lower sperm count | Reduced Sc and Sd, reduced diameter of ST, ST vacuolization, arrest at pachytene Sc, defect in synaptonemal complex | Increased apoptosis germ cells (mostly Sc) | Hypo-fertility |
| *Hsf1*$^{-/-}$*Hsf2*$^{-/-}$[48] | Dramatic reduction of testis size | Complete lack of spermatozoa | Sg and Sc significantly reduced, multinuclear giant cells and large vacuolar in ST | Large number of apoptosis cells | Infertile |

ST: Seminiferous tubules; LC: Leydig cells; Sg: Spermatogonia; Sc: Spermatocyte; Sd: Spermatid.

type and mutant Janus kinase 2 (JAK2)[40]. Studies have also demonstrated that HSPC played a central role in the formation of functional forms of steroid hormone receptors including androgen receptor[41].

The association between HSPC and fertility was first found in *Drosophila melanogaster*, where *Hspc* mutant male exhibited sterility[42]. Inhibiting the function of HSPC by geldanamycin can lead to decline of porcine sperm motility and increase of newt spermatogonia apoptosis[43,44]. HSPC1 (HSP90 alpha) was highly expressed in spermatogonia and spermatocyte in human testes (Figure 1A). The level of HSPC1 was significantly increased in patients with spermatogenetic arrest compared with control group[9]. Studies have also revealed that the decline of human sperm motility after cryopreservation may be due to the significant decrease of HSPC[45]. Moreover, *Hspc4* (*Hsp90b1*) KO mice displayed sterility because of the large and globular heads of sperm, which resembles human globozoospermia[28]. Several abnormalities were observed in *Hspc1*-deficiency mice, such as atrophic testis, azoospermia and infertility. Spermatogenesis was arrested at pachytene spermatocyte, while the spermatocytes suffered from abnormal apoptosis[29]. The authors suggested that these abnormalities may result from the failure in synaptonemal complex disassemble[29], which needs further study to confirm.

### 3.3. HSPH (HSP110) and DNAJ (HSP40)

We put HSPH and DNAJ together in this section due to that both of them act as co-chaperones in HSPA/HSPC-mediated heat shock response. There are four members in HSPH which are highly homologous to HSPA[2]. In fact, two members of HSPH family, HSPH2 and HSPH3, were previously classified into HSPA family which named HSPA4 and HSPA4L. To date, DNAJ is the largest HSP family and that 50 members have been discovered containing the typical J-domain unique to the DNAJ family[2]. The J-domain enables DNAJ to interplay with HSPA/HSPC. ATP hydrolysis of HSPA/HSPC is highly accelerated by DNAJ, leading to the ADP-bound state of HSPA/HSPC which is able to bind to their substrates stably[10,46]. Then HSPH acts as nucleotide exchange factors of HSPA/HSPC, catalyzes the ADP-ATP exchange and results in substrates release[3,46].

In recent studies, HSPH2 was detected in both somatic and germ cells in the mouse testis during prenatal and postnatal development. *Hsph2* or *Hsph3* deficiency in male mice resulted in drastic decrease in sperm count and motility accompanied by impaired fertility. Spermatogenesis was arrested at meiotic prophase stage[11,12]. This abnormity is similar to *Hspa2* and *Hspc1* deficiency mice which indicate that lack of HSPH2 may impair spermatogenesis via disrupting the function of HSPA/HSPC in testis. Besides, *Dnaja1* KO mice displayed severe defects in spermatogenesis and fertility[10]. Since DNAJA1 could repress the transactivation of androgen receptor and the amount of androgen receptor protein was significantly increased in *Dnaja1* KO mice, the authors presumed that aberrant androgen signal may be involved[10]. These studies indicate that the co-chaperones, HSPH and DNAJ, are also indispensable for maintaining male fertility, although the mechanism remains largely unknown.

### 3.4. HSPD1/HSPE1 (HSP60/HSP10)

HSPD1 (HSP60) and its co-chaperone HSPE1 (HSP10) mainly localize in mitochondria, and they are essential for maintenance of mitochondria function. In eukaryotes, HSPD1 is synthesized in the cytoplasm and then transfers into the mitochondrial matrix, in which it is assembled into a single toroidal structure of seven subunits[13,47]. Similar to HSPA/HSPC, the formation of functional structure of HSPD1/HSPE1 is ATP dependent. In addition to localization in mitochondria, approximately 15% to 20% of cellular HSPD1/HSPE1 exists in extra-mitochondria sites and may be involved in tissue-specific function[3,13].

In human testis, HSPD1 was detected in germinal cells lining the basal epithelium, spermatogonia and probably in primary spermatocyte, but disappeared in other differentiation stage of spermatogenesis (Figure 1B)[13,14]. Furthermore, the HSPD1 positive spermatogonia significantly reduced in human testes showing spermatogenesis arrest at the stage of primary spermatocyte compared with testes exhibiting normal spermatogenesis[13]. These observations suggest that down-regulation of HSPD1 in germ cells may be associated with low spermatogenic efficiency and male infertility. Studies also have showed that HSPE1 was expressed in spermatogonia, round spermatids and elongated spermatids in mouse testes[15]. Moreover, both HSPD1 and HSPE1 proteins was detected in ejaculated spermatozoa, and disruption of HSPD1/HSPE1 in ejaculated spermatozoa resulted in abortive capacitation and sperm-zona pellucida interaction, which will be discussed below[14-16].

### 3.5. HSPB

The small HSP (HSPB) family is the latest discovered HSP, which possesses some unique characteristics. HSPB does not contain an ATPase domain and thus it performs the chaperone function independent of ATP. Instead, HSPB family members become oligomers through a conserved crystallin domain in the C-terminus, which often forms a beta sandwich structure, and then the oligomers are able to act as chaperone of proteins[3].

As a typical member of HSPB family, HSPB1 (HSP27) was expressed in a cell type specific pattern in human testis, which was highly expressed in cytoplasms of Sertoli cells and spermatogonia, moderately expressed in cytoplasms of spermatocytes, and was absent in spermatozoa (Figure 1A)[17]. The expression of HSPB1 significantly reduced in human testes with maturation arrest, indicating that HSPB1 may be associated with male germ cell development. Furthermore, HSPB10 mainly constituted the proteins of sperm tail and it played a critical role in sperm motility. Disruption of HSPB10 resulted in lower sperm motility and male infertility[48].

### 3.6. HSF1

The heat shock response and expression of HSPs are modulated mainly by HSFs and HSF1 is the most important and ubiquitous one, which displays a typical characteristic of heat-inducible, DNA binding and oligomerization. By binding to the conserved sequences heat shock elements (HSE) found in all *HSP* gene promoters, HSF1 triggers the heat shock response and up-regulates the expression of several HSP families. Under non-stressful circumstance,

HSPA and HSPC bind to the monomeric HSF1, tether it in the cytosol as an inactive form. After exposed to deleterious stresses, HSPA and HSPC are recruited to bind to the denatured proteins and HSF1 is released. Subsequently, HSF1 translocates into nucleus, undergoes trimerization and phosphorylation, then binds to *HSE* and activates HSP genes. On the other hand, HSPA and HSPC have feedback suppression on HSF1 activity[2,49]. Moreover, HSF1 interplays with HSF4 in the development of sensory organs such as lens and olfactory epithelium. While HSF4 plays a crucial role in sensory organs development, HSF1 seems to have opposing effects by competing for the same target with HSF4[4,49]. Intriguingly, while HSF1 can activate stress-induced *HSP (HSPi)* genes, such as *Hspa1a, Hsph2, Hspb1,* in response to stress in the somatic cells, studies have revealed that the major *HSPi* genes could not be activated by HSF1 in male germ cells and Sertoli cells[32,50], highlighting the need to elucidate the function of HSF1 in spermatogenesis.

In mice, HSF1 acts as a maternal factor that is essential for oogenesis and early post-fertilization development[4,6]. Contradictory results have been reported on *Hsf1*-deficient male mice. In Izu's study, *Hsf1*-null mice displayed normal testis weight, histology and fertility[32]. Nevertheless, two recent studies demonstrated that *Hsf1*-null mice produced less sperm, exhibited an increase in disorganized or missing layers of germ cells in the seminiferous tubules while the *Hsf1*-null mice still displayed normal fertility[7,34]. In addition, an increase of sperm with slightly abnormal head structures and aberrant levels of chromatin packing proteins, such as transition protein 2 and protamine 1, were observed in *Hsf1*-null mice[7]. Surprisingly, transgenic male mice with a continually expression of active form of HSF1 in the testes exhibited significant reductions in testis size (50%), diameter of seminiferous tubule, amount of round and elongated spermatids. The transgenic mice were sterile and spermatogenesis was blocked at the pachytene phage[31]. The authors demonstrated that HSF1-induced cell death occurred mainly in the late pachytene spermatocyte. Moreover, the active HSF1 could activate both mitochondria-dependent and death receptor-dependent pathway to induce germ cells apoptosis[50]. This is coincident with Izu's study, which has demonstrated that heat-induced cell death of spermatocyte was inhibited in *Hsf1*-null mice and the magnitude of spermatocyte apoptosis mainly depended on the activity of HSF1[32]. Furthermore, active HSF1 in mice somatic cells resulted in the induction of HSPi under non-stress conditions[50]. Different from somatic cells, expression of constitutively active HSF1 in germ cells could not induce HSPi[50]. Instead, active HSF1 down-regulated HSPA2 mRNA and proteins expression and altered the endogenous HSPA2 subcellular distribution in testis[51], suggesting that active HSF1 may also induce germ cells apoptosis by disrupting the normal expression of HSPs during spermatogenesis. In addition to promoting cell death in spermatogenesis, HSF1 also played a crucial role in protection of more immature germ cells including spermatogonia in response to testis hyperthermia[32]. Taken collectively, HSF1 appears to have two opposite effects in spermatogenesis and may be involved in quality control of male germ cells.

### *3.7. HSF2 and male infertility*

Similar to HSF1, HSF2 contains highly conserved DNA binding and oligomerization domains. Both of them acquire the DNA-binding activity only in the state of trimer. They also co-express in most tissues and cell lines. Whereas, HSF2 was initially found to be involved in the development and differentiation-related processes rather than hear shock response. However, HSF2 protein that existed in a constitutively active DNA-binding state has been found to localize in the nuclei of spermatocytes and round spermatids[18]. The active HSF2 was able to bind to the promoter of *Hspa2* gene[18], suggesting that HSF2 may regulate some heat shock protein genes during spermatogenesis. More recently, there is growing evidence that HSF2 may also participate in the heat shock response via the interplay with HSF1[52,53]. Studies showed that HSF2 can bind to the promoters of *HSP* genes only in the presence of HSF1. HSF1 and HSF2 colocalize into nuclear stress bodies in response to heat stress[54]. Moreover, by forming a heterocomplex with HSF1, HSF2 modulates, both positively and negatively, the HSF1-mediated expression of HSPs, including HSPH, HSPC, DNAJ, and HSPB[53].

HSF2 exhibits a stage-specific expression pattern during the cycle of the seminiferous epithelium in mouse testes (Figure 1B). HSF2 expression level significantly increased in adult mice compared with the juvenile mice, because of the highest expression levels in spermatocytes and round spermatids[18]. Moreover, *Hsf2* deficiency in male mice led to reduction of testis size, sperm count, and vacuolization of the seminiferous tubules[4,35]. Synaptonemal complex of spermatocytes was disorganized and up to 90% of spermatocytes suffered from apoptosis[35]. In contrast, disruption of both *Hsf1* and *Hsf2* resulted in complete arrest of spermatogenesis. In addition to failure in meiotic stage, earlier defects were observed during spermatogenesis[33]. These studies indicate that HSF1 and HSF2 are tightly intertwined during germ cell development and their function may be at least partially compensated by each other.

## 4. Impact of HSPs/HSFs on varicocele-associated male infertility

Varicocele, which is abnormal dilated veins in the pampiniform plexus with a reflux of blood. It is the most common cause of male infertility. Approximately 15% of general population has a varicocele, with 40% of them having fertility problems[54]. Varicocele, either palpable or subclinical, can cause testicular and epididymal malfunction, impairment of semen quality. The impact of varicocele on spermatogenesis varies from person to person, which could be normozoospermia, oligozoospermia, asthenozoospermia or even azoospermia. Despite numerous potential mechanisms have been put forward, such as an increase in scrotal temperature, reactive oxygen species, hormone disturbance, reduced perfusion, autoimmune response and backflow of toxic substances of renal origin, actually by what mechanisms varicocele influences fertility still under debate. Beyond these, the reasons why varicocele affects fertility in just parts of men have not yet received an adequate answer.

Recently, several studies of HSPs/HSFs expression in sperm of varicocele patients shed a new light on varicocele-associated male infertility. In order to clarify the abnormal expression levels of HSPs/HSFs in varicocele clearly, here

we define varicocele oligozoospermia (group VO), varicocele normozoospermia (group VN), oligozoospermia without varicocele (group ON) and the normozoospermia men without varicocele (group control). According to Ferlin's research, HSPA4, HSF1, and HSF2 had the similar behavior, which were increased in the sperm of group VN and ON patients but were at the highest level in group VO[55]. It seems that the expression of HSPA4, HSF1 and HSF2 are stimulated in response to spermatogenic damage as well as varicocele. In contrast, HSPC just elevated in oligozoospermia patients (group VO, ON) (Table 2)[55-58]. However, it still remains unknown whether varicocele impairs spermatogenesis via these factors or up-regulation of these factors act as a compensatory procedure to protect germ cells from damage. More studies concentrate on HSPA2 in varicocele because it's highly expressed in human testis in a cell-type-specific expression pattern. There was a significantly lower expression of HSPA2 in the sperm of group VO and a slightly higher expression (not significant) in group VN[56,57]. These results indicate that HSPA2 plays a crucial role in maintaining male fertility. The high level of HSPA2 observed in group VN may be interpreted as a compensatory effect to protect germ cells under the condition of varicocele, and it can at least partially explain the reason why some varicocele patients are fertile. This finding is further supported by the observation that sperm HSPA2 activities increased significantly after varicocelectomy compared with preoperatively[57,58]. Furthermore, a significant negative correlation was observed between HSPA2 expression and sperm DNA fragmentation in varicocele patients[57], indicating that individuals with higher levels of HSPA2 was less susceptible to DNA damage. Taken collectively, although the versatile functions of HSPs/HSFs in varicocele remain largely unclear, HSPA2 is a vital protective factor for male fertility.

## 5. HSPs may be involved in *Chlamydia trachomatis(CT)*-related infertility

Except for varicocele, genital tract infection is the most common aetiological factors for male infertility and CT is the most prevalent bacterial of sexually transmitted infections[59]. Several studies have strongly implicated that CT may impair fertility via damaging the function of HSPs on the surface of spermatozoa[60-62]. These findings may give a proper answer to the mechanisms of pathogens-induced infertility.

The ejaculated spermatozoa must undergo a series of physiological changes collectively termed capacitation while moving through the female genital tract before being capable of penetrating the ovum. Recently, numerous studies have confirmed the existence of HSPD1, HSPE1, HSPA5 and HSPC4 proteins in the ejaculated spermatozoa (Table 3)[14-16,63]. To further the functions of these chaperones in the capacitation and sperm-zona pellucida interaction, two studies have demonstrated that both HSPD1 and HSPE1 appeared on the surface of spermatozoa during capacitation[15,16], and HSPE1 antibodies suppressed sperm-zona pellucida interaction in a significant dose-dependent manner, whereas no abnormality was observed in sperm viability and motility[15]. The molecular mechanisms that HSPs participate in the capacitation and sperm-zona pellucida interaction remain poorly understood. However, emerging evidence suggests that the antibodies induced by CT infection may be responsible for infection-related reproductive failure[60,62].

As mentioned above, HSPs exist in organisms ranging from bacteria to human and it is highly conserved among species. The CT HSP60 (ctHSPD1) shares almost 50% amino acid sequence homology with the human HSPD1[60]. As a consequence, the presence of anti-ctHSPD1 immunoglobulin induced by CT infection can also interfere the function of HSPD1 during fertilization. Through this molecular mimicry between bacteria and sperm, anti-sperm antibodies are formed and induce anti-sperm immunological reactions. Although it has been well demonstrated that the anti-ctHSPD1 antibody is a major cause of CT-related female infertility and it is correlated with adverse *in-vitro* fertilization treatment outcome[62], the role of CT infection in male infertility is still under debate. Intriguingly, CT serum antibodies in men significantly correlated with lower pregnancy rate but there was no correlation between ctHSPD1 IgG and pregnancy rate[60]. Furthermore, contradictory results have been reported on the impact of HSPD1 antibodies on semen quality. Some studies

**Table 2**
Abnormal expression of HSPs/HSFs in ejaculated spermatozoa of patients with varicocele and/or oligozoospermia[56-59].

| HSPs/HSFs | Varicocele oligozoospermia (group VO) | Varicocele normozoospermia (group VN) | Oligozoospermia without varicocele (group ON) |
|---|---|---|---|
| HSPA2 | ↓ | ↑ (non-significant) | Lacked data |
| HSPA4 | ↑ ↑ | ↑ | ↑ |
| HSP90 | ↑ | No change | ↑ |
| HSF1 | ↑ ↑ | ↑ | ↑ |
| HSF2 | ↑ ↑ | ↑ | ↑ |

↑: Increase; ↓: Decrease.

**Table 3**
Localization of HSPs in mature sperm of human and mouse.

| Species | Acrosome | Neck | Mid piece | Principal piece | End piece |
|---|---|---|---|---|---|
| Human[33] | ND | HSPA5 | HSPD1 | ND | ND |
| Mouse[34,35] | HSPC4, HSPD1, HSPE1 | ND | HSPD1, HSPE1 | HSPE1 | ND |

ND: Not detected.

demonstrated that HSPD1 IgG/IgA was correlated with sperm motility and concentration[60,61], while others suggested that no association of seminal HSPD1 IgA with semen quality[64]. Collectively, although plenty of evidence contributes to the connection between HSP antibodies and CT-related infertility, the effects of HSP antibodies on sperm quality and fertilizing capacity need to be well elucidated.

## 6. Roles of HSPs/HSFs in the regulation of germ cells apoptosis in male infertility

Apoptosis is a precise process of programmed cell death in multicellular organisms that does not initiate an inflammatory reaction, which differs from necrosis. This process is modulated by different kinds of genes to maintain a proper amount of cells in balance with proliferation. Apoptosis is common in spermatogenesis and up to 75% of sperm may be lost in spermatogenesis due to apoptosis[65]. It can occur in every phase during spermatogenesis while the primary spermatocytes are most sensitive to apoptosis[50]. Apoptosis plays a critical role in eliminating germ cells with genetic defects during spermatogenesis. Recently, several lines of evidence suggest potential roles of HSPs/HSFs in the regulation of apoptosis, including the intrinsic pathway and extrinsic pathway. In fact, different HSPs can exert either anti-apoptotic or pro-apoptotic effects.

HSPs have the ability to provide survival-promoting and anti-apoptotic effects in response to a variety of detrimental stimulus including heat and oxidative stress mainly depend upon their chaperoning ability[66-72]. Recent studies have suggested that HSPA, DNAJ, HSPB2 can inhibit the Bcl-2 family pro-apoptosis members, such as Bid and Bax, translocate to mitochondria and consequently suppress the pro-apoptotic factors such as cytochrome c release from mitochondria, which is a critical point within the intrinsic pathway[70-72]. HSPC, HSPA, HSPB2 are also reported to disrupt the apoptosome assembly by directly associating with Apaf-1 and prohibit its oligomerization, which will consequently prevent the recruitment and activation of the initiator caspase pro-caspase-9[66,69]. Furthermore, HSPs have the ability to regulate the extrinsic apoptosis pathway signaling events engaged by the death receptors Fas, tumor necrosis factor (TNF) and TNF-related apoptosis inducing ligand, and also stabilize elements of the NF-$\kappa$B pathway, such as Akt, to promote cellular survival[67,68]. To the contrary, HSPs/HSFs have also been implicated in enhancing apoptosis according to several studies[73,74]. HSPD1 can form a complex with HSPE1 and pro-caspase-3 in mitochondria to promote activation of pro-caspase-3 and over-expression of HSPA can significantly augment T-cell receptor-mediated apoptosis by directly promoting the activity of caspase-activated DNase[73,74]. More importantly, Vydra *et al* have revealed that both intrinsic and extrinsic pathway were involved in the HSF1-dependent induction of apoptosis in male germ cells. The constitutively HSF1-expressed transgenic mice exhibited higher levels of Bcl-2 family proteins, p53 proteins, death receptor proteins and active caspase-3 compared with non-transgenic control[50]. This is the direct evidence to prove that HSPs/HSFs are involved in the regulation of apoptosis in germ cells.

As we reviewed above, the most common aberrations in *Hsp/Hsf* KO mice are meiosis failure and germ cells apoptosis. It has been well established that altered regulation of apoptosis during spermatogenesis is a crucial problem in male infertility. Since heat shock family has the complicated functions in regulation of apoptosis, it raises the high possibility that apoptosis modulated by HSPs and HSFs may play a crucial role in spermatogenesis. The heat shock family participates in the regulation of apoptosis during spermatogenesis in order to maintain a proper amount of germ cells to match the supportive capacity of Sertoli cells, and also to eliminate the abnormal sperms. The disturbance of heat shock family in germ cells, including *Hsp/Hsf* deficiency, may interfere their anti-apoptosis function and ultimately result in male infertility. The increased expressions of HSPs and HSFs (HSPA4, HSPC, and HSF2) that observed in infertile patients may be interpreted as the compensatory protection against apoptosis under detrimental conditions, including testis hyperthermia, ROS. In some patients suffering from varicocele or other detrimental situations, the compensatory effects made by HSPs/HSFs succeed to counteract the adverse factors and consequently maintain fertility while others do not. This may at least partially explain that why varicocele have a harmful effects on spermatogenesis only in some men. In contrast, some members of heat shock family can activate the apoptosis pathway in order to control the quality of germ cells. Over-expression of HSPs/HSFs, such as HSPA and HSF1, excessively activated the apoptosis pathway in germ cells. As a consequence, male germ cells underwent massive apoptosis and therefore impaired male fertility[50,73,74].

Taken together, the heat shock family seems to have a vital impact on sperm quality. HSPs/HSFs are essential for spermatogenesis, which can promote the normal germ cells survival and inhibit apoptosis under physiological or pathological conditions. On the other hand, HSPs/HSFs may activate apoptosis pathway to eliminate the abnormal germ cells in order to prevent the defective development of the next generation.

## 7. Perspectives

As described in this review, HSPs/HSFs participate in the process of spermatogenesis and have a critical impact on male fertility. Although previous studies provided a valuable novel insight into molecular mechanisms of male infertility, several questions remain to be classified. Whether the increased level of HSF1, HSF2, and HSPA4 mRNA in the ejaculated spermatozoa of varicocele patients exert harmful or protective effects on fertility and whether HSPs/HSFs involved in other causes of male infertility including idiopathic infertility are still unclear. The mechanisms that HSF1 doesn't activate *HSPi* genes in male germ cells and *Hsp/Hsf* KO mice displayed infertility needs to be elucidated. If HSPs/HSFs are indeed able to control germ cells quality, how do they distinguish the abnormal germ cells from the normal ones and what signal pathway is involved in this process? Since recent study showed that miR-18 directly targeted HSF2 and regulated HSF2 activity in spermatogenesis[75], whether microRNAs are responsible for male infertility remains to be determined. Despite numerous questions have not been fully clarified, these findings would certainly broaden the current view of HSPs/HSFs and provide a new research direction for the cause and maybe the therapy of male infertility.

## Conflict of interest statement

We declare that we have no conflict of interest.

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