

The Role of Heat Shock Proteins 70 (HSP70) in Farm Animals Adaptation, A Review Paper

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Abstract

Heat shock proteins (HSPs) are a group of proteins produced by cells in response to stress conditions. One of the most important physiological responses to sudden environmental changes is HSPs. The genes of the HSPs are activated when cells are subjected to different stress conditions. The aim of this paper is to give a concise description of the heat shock proteins and highlighting of its action in the on the living organism at stress condition. A brief review of HSPs types and its functions were described with special topics on HSP70. In this review, we also present the relationship of the HSPs especially HSP70 and the adaptation process of the tissues against the stress factors. Furthermore, we discuss the correlation of high concentration of the HSP70 on improve the quality of semen in different farm animals. In conclusion, HSPs have significance values for maintaining the cells of the living organisms as the main factors in the adaptation process and have the important roles in protection of reproductive cells.

Keywords: Heat Shock Proteins, HSP70, Stress Factors, Adaptation

Heat Shock Proteins

General Overview

Heat shock proteins (HSPs) are a family of proteins produced by cells in response to stress conditions. It was initially thought to be related to temperature rise only in the first study by the Italian scientist Ritossa (1962) on the *Drosophila* insect. These proteins were described accurately in 1974, and this discovery eventually led to the identification of heat shock proteins (HSP) or stress proteins that were responsible for bulging in the chromosomes and these bulges that are formed in different places in the chromosome are responsible for Protein composition (Schlesinger 1990, Ritossa 1996 and Dai *et al.*, 2007). Subsequent studies have indicated that

these proteins are also produced as a result of other conditions on the organism such as ultraviolet radiation (Roh *et al.*, 2008), temperature fluctuation (Qiao *et al.*, 2015), as well as during wound healing and tissue reshaping (Peterson *et al.*, 2016)

Many members of this family act as molecular couplers between cells with some proteins in the membranes and prevent the formation of newly formed proteins to maintain their vital role. They also prevent aggregation and assembly from stress at their manufacturing sites, which means they do not reach their work sites (Mayer and Bukau 2005). Heat shock proteins play an important role when elevated temperatures have adverse effects on cell structure and cell physiology (Tiwari *et al.*, 2015).

The mechanism of action of HSPs is directly related to the heat shock factor (HSF), which occurs in almost all organisms starting from bacteria reaching to humans (Morimoto 1998). Both Pirkkala *et al.*, (2001) and Akerfelt *et al.*, (2010) point out that the HSF acts as a transcription factor with genes responsible for HSPs. When cells are exposed to different stress conditions, Genetic expression of HSPs is induced. This increase in gene expression is thought to be a response to stress through a variety of genetic and biochemical processes as referred as the HSR (Shamovsky and Nudler 2008). In the case of non-stressful natural conditions, the HSF is reduced in the cytoplasm (Zorzi and Bonvini 2011).

Heat shock proteins have different molecular weights measured by dalton. These proteins are called HSP60, HSP70 and HSP90 (Li and Srivastava 2004). Richter *et al.*, (2010) noted that HSPs are a wide range of proteins act to protect cells from different stress conditions and are classified according to their molecular weight.

The HSP70 family acts as molecular attachments with certain proteins in intracellular membranes, while HSP90 is associated with newly manufactured proteins at the moment of their release from their ribosome production centers and may prevent proteins from aggregating and condensing properly before they are damaged (Ritossa 1996).) Heat shock proteins are involved in the assembly or disassembly of proteins and antibiotics in the cell as each type of HSP reacts to a specific group of molecules. In addition, members of the HSP70 family are associated with heavy chains of immunoglobulin, Catherine chains and DNA multiplexes. Members of this family are also associated with ATP as well HSP90 that interact with steroid hormone receptors and modulate phosphorylation in the cell due to their association with cellular kinesis enzymes (O'Regan *et al.*, 2015).

Testori *et al.*, (2008) noted that HSPs are one of the oldest mechanisms of preservation of life, so they have been preserved during the evolution of living organisms. They have been shown to have played a role in the evolution process and are produced in response to stressful conditions including, and not just exposure to heat. These proteins help single cells to deal with it to keep

the work of cellular functions easily in the face of unfavorable conditions. One of the most important characteristics of the HSP70 is their stability and non-disintegration of the heat. The stability of its work and performance of functions is thought to be as the high conservation of its genetic structures. Consequently, there is consistency in the genetic expression of the catalysts for the production of these highly effective proteins under conditions unsuitable for the work of proteins (Kampinga and Craig 2010).

In humans, studies have indicated that HSP, specifically HSP70, play an important role in the functioning of the central nervous system as they play a protective role by repairing or removing proteins that have been damaged by various stress conditions and causing many diseases such as Parkinson's disease and other neurological diseases (Yenari 2013). On the other hand, increasing the genetic expression of HSP70 could increase the susceptibility of cells to oxidation resistance. Therefore, HSP have a protective role in the resistance to multiple sclerosis and increased autoimmunity, which is complementary to immunological defenses against cancer and pathogens. Heat shock proteins are of value in the development of multiple forms of new medicines and vaccines (Richardson *et al.*, 2006 and Boiocchi *et al.*, 2014).

On the other hand, Page *et al.*, (2010) pointed out that the association between the level of heat and maximum life-span proteins (MLSP) may be due to the mechanism of action of HSPs as molecular accompaniments by participating in the folding process proteins. In a study by Salway *et al.*, (2011), found that there was a positive correlation between the high level of gene expression of HSPs (especially HSP70) with aging, and increased genetic expression of the shock proteins. However, increasing the level of gene expression of shock proteins significantly reduced early aging and death in diseased mice (McLean *et al.*, 2002; McArdle *et al.*, 2004 and Salway *et al.*, 2011).

Srivastava (2002) stated that the HSPs are made up of many protein molecules as the HSP cage. Its internal branch is highly hydrophobic, so it attracts the hydrophobic amino acids of the uncoated proteins. Once the chain is attracted to the HSP cage, it will face hydrophilic medium, thus the amino acids that are hydrophobic try to move away and thus the molecule in the trap becomes forced to change its shape. This process may not take place at once. The HSP cage may be freed and then re-linked to the protein several times before it acquires the correct fold. Moreover, some members of the family of heat shock proteins work to remove protein folding as in the HSP100 (Srivastava 2002 and Dai *et al.*, 2007). This family works with HSP70 to break down damaged proteins or unwanted protein concentrations. The mechanism of action of HSPs are one of two types, either by inhibition the unwanted interactions or activate the desired interactions (Whitesell and Lindquist 2009) to form a stable and useful association between different proteins and to participate in the performance of a particular function

In addition, Enzolifesciences (2010) has documented that the importance of HSPs lays in their direct involvement in all vital protein-related processes such as protein secretion, synthesis and prevention of degradation, as well as the regulation of their replication processes. The types of the stress of the cell that stimulate the HSPs are represented in Table (1).

Table (1): Types of stress on the cell that trigger HSP (Enzolifesciences 2010)

Type of stress	Description
Physical	Hyperthermia (including fever), coldness, several types of radiation including UV and other causing physical stress
Oxygen	Deficiency of oxygen by insufficient blood supply to the organ or part of the body, especially the heart muscle
pH	Change in pH for several different reasons that lead to alkaline elevation or high acidity
Vital	Infections or various vital infections as well as various infections and fever
Psychological	Emotions, emotional conflicts, hormonal imbalance (pituitary axis, adrenal gland and autonomic nervous system)
Osmotic pressure	Changes in salts and glucose concentrations that lead to an osmosis (hyposmotic or hypoosmotic shock)
Nutrition	When there is a lack of essential food components needed by the body as a decrease in the various metal elements
Antibiotics	Various antibiotics when misused such as tetracycline, doxorubicin and others
Alcohol	Ethanol, methanol, butanol, propanol, octanol and various alcohol types
Minerals	Cadmium, copper, chromium, zinc, tin, aluminum, mercury, lead, nickel and others
Mechanical	Pressure, expansion and other effects
Others	Dehydration, carcinogens, toxic substances, nicotine, narcotic substances and pesticides

The ability of the organism for successfully adaptation to the surrounding environment is important for its survival. One of the most important physiological responses to sudden environmental changes is HSP. At high temperature for instance, organisms respond to this rise by working a set of HSPs to reduce the effect of heat in cells. The genes of heat shock proteins are activated when cells are subjected to different stress conditions (Christians *et al.*, 2003, Enzolifesciences 2010). It is important to note that although this group of proteins is called HSPs, all these proteins are synthesized by cells under normal conditions. However, the increase in gene expression of these proteins is under stressful conditions. Expression is what gives cells the ability to repair proteins as well as the secretion of new proteins to replace those that have been damaged due to stress (Bukau *et al.*, 2006).

The most important function of HSP is to play the role of molecular accompaniments and thus can be interpreted as important for normal growth as well as to give cells the ability to survive under stressful conditions. In particular, the primary role of HSP as molecular accompaniments as it helps to facilitate the early stages of folding and aggregation of cellular proteins, through the multiplication of multiple peptides, thereby reducing the probability of improper folding or assembly of proteins (Bukau *et al.*, 2006 and Enzolifesciences 2010).

The function of heat shock proteins

1. Up-regulation in stress

The production of high levels of HSP due to exposure to different environmental stress conditions such as infection, exposure to toxins, hunger, lack of oxygen and lack of water is sometimes called “the stress proteins” and sometimes described as up-regulation as the highest organization and form part of the response to different stress conditions (Santoro 2000). In general, the mechanism of action of HSPs is activated by the heat shock factor (HSF) and then affects the outer membrane of the cells to prevent protein damage (Walsh *et al.*, 2003). Therefore, some studies suggest that the increase in damaged or non-naturally proteins leads to an increase in the level and effectiveness of HSPs (Narberhaus 2010).

2. Role as molecular chaperone

Many HSPs act as molecular accompaniments within the cell to other proteins. They play a very important role in the protein-protein interactions as a protein folding, helping to give the correct form of protein and preventing proteins from unwanted assembly by helping stabilize in partially folded proteins. Thus, the action of HSPs can be summarized in helping to transport proteins across membranes within the cell. In addition, some members of the family of HSPs, despite their low levels in some organisms, have very important and essential role in maintenance of proteins (Walter and Buchner 2002 and Borges and Ramos 2005).

3. Management

The heat shock proteins under stressful conditions play the role of an observer of cell proteins as they carry the old proteins to the cell to recycle them from the proteasome complex. They also help the newly synthesized proteins to fold properly. These activities are part of the cellular system repair process. The cellular response is called pressure or shock response (Borges and Ramos 2005).

4. Direct effect on cardiovascular system

Heat shock proteins play an important role in helping the heart and blood vessels to act correctly. Several studies have indicated that HSP20, HSP27, HSP70, HSP84, and HSP90 have an important role in cardiovascular function (Willis and Patterson 2010). Antonova *et al.*, (2007) reported that HSPs work to form bonds between groups of hydrophilic proteins compounds that in turn participate in blood vessel relaxation. Small-block HSPs such as HSP20 share J protein by relaxing the smooth muscles (McLemore *et al.*, 2005). Fan *et al.*, (2005) also stated that the HSP20 play an important role in the development of the shape of the smooth muscles during growth and development. It also plays essentially in preventing platelet aggregation and apoptosis (Lamitina and Chevet 2012) especially during brain injury as well as help in muscle and skeletal function and insulin response. Moreover, HSP27 is a very important phosphorylation protein for uterine contractions in the female through its role in small muscle contraction (Salinthonne *et al.*, 2008).

5. Immunization

Extracellular HSPs (especially Hsp70) act to form links with the antibodies against antigens and thereby increase the efficiency of the immune system (Nishikawa *et al.*, 2008).

Heat shock proteins70 (HSP70)

The HSP70 exist in all animal and plant cells (Chang *et al.*, 2007 and Enzolifesciences 2010), and represent as the one of the largest and most important families of HSPs (Rynkowska *et al.*, 2011). The HSP70 are synthesized proteins (Basirico *et al.*, 2011). Gade *et al.*, (2010) and Pawar *et al.*, (2013) have reported that HSP70 consist of 641 amino acid in sheep and goats. The length of its fragments gene is approximately 1926bp nitrogen base as the size of the gene responsible for this protein in Cows and buffalo (Sodhi *et al.*, 2013), and this protein compound of 56-64 polypeptide and all polypeptide length of 10 amino acids. As well as the multiple polymorphisms of its gene (Habib *et al.*, 2017a, Habib *et al.*, 2018b)

On the other hand, the HSP40 act as adjunct agents and assist the HSP70 (Craig *et al.*, 2006, Jiang *et al.*, 2007 and Sajjadi *et al.*, 2013) by catalyzing of ATPase (Ahmad *et al.*, 2011). Bepperling *et al.*, (2012) have reported that the action of HSP70 by folding re-folding proteins correctly is requires the assistance of the HSP40. They act synergistically and this synergy requires an increase in the level of gene expression of both proteins (Haslbeck and Vierling, 2015).

The HSP70 is one of the most important molecules that represent an important indicator of animal adaptation to adverse environmental conditions, particularly higher temperatures that play a major role in the thermal tolerance (Hansen 2004). Gaughan *et al.*, 2013 and Deb *et al.*, (2014) have stated that higher temperatures and stress conditions lead to stimulate the HSP70. It plays as a vital factor in regulation of the folding and re-folding of proteins that have been mutated or damaged, and this is one of the main ways that the farm animals can be adapted against stress.

In the case of ADP, the HSP70 works more efficiently as molecular accompaniments with a lower likelihood of nucleotides becoming SBD (Bertelsen *et al.*, 2009). Daugaard *et al.*, (2007) have reported that HSP70 is one of the families of the most conserved HSPs (with less mutations) despite the evolution and the diversity in all living organisms ranging from the rudimentary to eukaryotic, and through the spread of HSP70 in different cells. They share or affect many of the structural and biochemical properties of these cells (Enzolifesciences 2010).

It is also important to note that the action of the family of HSPs, especially the HSP70, revolves around cell recovery after stress, as well as protecting the cell from stress conditions by preventing its degradation and damage to its proteins, often by increasing the genetic expression of HSPs, helps speed the manufacture of other proteins as well as increase glucose and oxidation of amino acids and reduce the metabolism of fatty acids and activate the response of endocrine stress conditions as well as the activation of the immune system by releasing extracellular HSPs.

If stress conditions continue, the process continues until it reaches the stage of adaptation, a process that is largely controlled by the endocrine glands (Collier *et al.*, 2008).

The action of HSP70 thermal shock proteins is highly dependent on the degradation of ATP because the bonds of HSP70 with ATP are converted into ADP bindings, followed by the action of the sub-nucleotides (SBD) by doing a cover-like work to cover the bonds. HSP70 then releases molecules (ADP) to be able to capture ATP again and as a result the protein released (Chang *et al.*, 2008).

The process of protein maturation and folding requires ATP energy. Here, the importance of the HSP70 as it stabilizes the new (nascent) proteins as they come out of the ribosomes through a process called “translocated” through the membranes of the endoplasmic or mitochondria. The interaction of HSP70 as molecular correlations with the target protein is induced by energy ATP (Bukau *et al.*, 2006 and Enzolifesciences 2010).

In general, the mechanism of action of HSP70 genes is focused on protecting cells from stress conditions such as oxidative stress by preventing or mitigating damage to essential proteins and facilitating the continuity of protein regeneration (He *et al.*, 2013). It also works in the same way to alleviate the effects of heat stress on cells (Miova *et al.*, 2015), as well as protecting cells by reducing the effect of cold stress (Maikova *et al.*, 2016).

The Relationship between HSP70 and Characteristics of semen

Semen assessment is the most critical instrument used to distinguish the regenerative wellbeing and execution of a creature (Umer *et al.*, 2017). The concentration of sperm and individual or group movements, as well as reduced deformities as well as membrane integrity and elevated live-to-dead ratio, are the most common measures of semen assessment and sperm fertility prediction in both bulls and sheep (David *et al.*, 2015). Beside the safety of DNA (Evenson 2016), recent studies have indicated that computer programs used in the analysis of semen (CASA) have increased the efficiency of dependence on these traits in sperm assessment (Sellem *et al.*, 2015, Habib *et al.*, 2017b and Habib *et al.*, 2018b).

Souza *et al.*, (2012) have reported that HSP70 are a component of sperm plasma in bulls and sheep, found particularly in the epididymis (Belleannée *et al.*, 2011) and in gonads excreted in bulls and in different stress situations (Chan *et al.*, 2013). In addition, Son *et al.*, (2005) have documented that the level of HSP70 is high in the brain and testicles, while it is low in most different tissues. It has an important role in protecting sperm by enhancing the immune response as its production to relieve the effects of different stress on cells (Fang and Zhang 2008). Although the production of these proteins is inside the cell, they can be released outside the cell and in different conditions with the surrounding cells (Speight *et al.*, 2012).

However, the HSP70 form part of the sperm membrane and are produced during sperm formation (Lippolis and Reinhardt 2010). These proteins bind to the fat in the cellular membranes of the membrane and can then act as catalysts for the adhesion of the chemites (Gaviraghi *et al.*, 2010). These proteins are excreted from the male reproductive system and the female reproductive system, helping to bind the ligament together with the enzyme Hyaluronidase to enhance the ability of the sperm for penetrating the egg's cumulus complex (D'amours *et al.*, 2010). Moreover, bulls sperms are complex mixture of secretions containing agents called HSPs, which are one of the cell's own molecular mechanisms, which protect them from the harmful effects of stress, such as heat, etc (Rajoriya *et al.*, 2014).

The HSP70 is present in the outer surface of sperm cells in mice, pigs, cattle, horses, dogs, cats and humans. Their actions are characterized by the high level of their expression in stress situations such as fever. This mechanism used in all organisms ranged from bacteria to humans. These proteins are very important for regulating the production of male genital girdles (Wang *et al.*, 2013). Thus, HSP70 spread in reproductive tissues and have important roles in protection of reproductive cells. It can be used for these proteins by modern techniques to improve the genetic traits of semen in farm animals, especially used in artificial insemination (Qing *et al.*, 2013).

The HSP70 has a close relationship with the quality of the semen, it has a protective role in the cell, helping them to cope with the negative effects of different stress conditions such as temperature rise and other stress conditions such as nutritional, respiratory, climatic and other stresses (Gullo and Teoh 2004), then consequently saving the DNA against damage. Additionally, several studies have pointed to the close correlation between DNA integrity and sperm characteristics, such as semen movement and semen concentration, as well as the percentage of live sperm to the dead (Erata *et al.*, 2008).

The increase in gene expression for HSP70 was positively correlated with reduced DNA damage, reduced damage to the plasma membrane, and damage to the chromosome (Rockett *et al.*, 2008). Studies have also shown that HSP70 in the head and the tail of the sperm in fresh and the frozen semen of pigs and bulls as well as rams (Volpe *et al.*, 2009). The HSP70 are also present in the testis and sperms at different stages of sperm formation in the lobules (Rathke *et al.*, 2014 and Rocha *et al.*, 2015), their high level is a fertility guide. However, when these proteins are low or almost non-existent, mammal's males will suffer from azoospermic absence and are therefore particularly important in the formation of sperm in the mammals (Widlak and Vydra 2017). Furthermore, a positive relationship between the level of HSP70 and the sperm motility and also indicated a positive relationship between semen quality and HSP70 in high temperature seasons (Erata *et al.*, 2008). On the other hand, the inverse relationship between the high level of HSP70 and the concentration of sperm, due to the high level of this protein prevented the folding and assembly of damaged proteins as a preventive immune measure (Wenhao *et al.*, 2015).

The abnormal and low expression of heat shock proteins can lead to disruptions in the defense system, which eventually leads to a decrease in sperm concentration and an increase in the rate of deformities. The low level of HSP70 leads to infertility or low fertility in males. The level of expression of these proteins with improved sperm characteristics can be considered as a protective mechanism for sperm against apoptosis in cells (Kotaja *et al.*, 2014 and Wenhao *et al.* 2015). The function of HSP70 as molecular accompaniment appears to play an important role in reducing the dangerous effects of high temperature at the stage of sperm formation. The natural sperm cells in the testis need a temperature of 4 ° to 5 ° below body temperature, so the function of proteins The HSP70 heat shock affects the specific characteristics of the seminal fluid of the organism, as it is associated with newly formed proteins and helps in the folding and synthesis of proteins during sperm formation (Dun *et al.*, 2012).

The Relationship of HSP70 with the characteristics of Semen after freezing and thawing

The HSP70 are a good indicator of sperm resistance to freezing (Paulo *et al.*, 2014). This can be attributed to increased sperm susceptibility to stress resistance caused by storage, as well as oxidation resistance. There is also a close correlation between the levels of HSP70 and semen in various species.

On the other hand, Lloyd *et al.*, (2012) have suggested that the addition of the HSP70 extract to the erythema diluent resulted in an increase in the longevity of the sperm and an increase in the vitality of the sperm during cooling and freezing. This is due to the cooling; solubility creates different stress conditions, whether physical or chemical, on sperm and thus will affect sperm fertility and decrease its quality. HSP70 reduce stress conditions (Dorado *et al.*, 2010). Between Kim *et al.*, (2011) The phenomenon of oxidation of sperm membranes when freezing is a reaction to exposure to different stress conditions because of the oxidation of unsaturated phosphatic fats in fatty acids, which leads to the formation of toxic fatty acids cause damage in the structure of a cell Sperm and DNA damage, as well as cellular membrane damage to sperm, and thus the negative effect on the sperm motility of buffalo spores (Fayya *et al.*, 2016). Here is the importance of a high level of HSP70 proteins to protect proteins Participate in the repair of nuclear acids and regenerative (Janssen *et al.*, 2016).

Conclusion

In conclusion, the HSPs are a wide range of proteins act to protect cells from different stress conditions and are classified according to their molecular weight. The HSP70 is one of the significant HSPs families and acts as molecular attachments with certain proteins in intracellular membranes. The importance of the HSP70 is represented by protecting cells from stress conditions such as, heat and cold climate, nutritional stress, oxidative stress by preventing or mitigating damage to essential proteins and facilitating the continuity of protein regeneration. It also works in the same way to alleviate the effects of heat stress on cells, as well as protecting cells by reducing the effect of cold stress. Furthermore, HSP70 spread in reproductive tissues and

have important roles in protection of reproductive cells. However, highlighting the significance of the HSPs is of value for maintaining the cells of the living organisms and deep molecular studies should be attempted for more understanding the effects of these vital proteins either on human or in farm animals.

Conflict of Interest

No conflict of interests is declared by authors for the contents in this manuscript.

Authors Contribution

Hassan Nima Habib and Wessam Monther Mohammed Saleh have designed and carried out and prepared the draft of this review.

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دور بروتينات الصدمة الحرارية 70 (HSP70) في التكيف مع حيوانات المزرعة ، ورقة مراجعة

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الخلاصة

بروتينات الصدمة الحرارية (HSPs) هي مجموعة من البروتينات التي تنتجها الخلايا استجابة لظروف الإجهاد. واحدة من أهم الاستجابات الفسيولوجية للتغيرات البيئية المفاجئة هي HSPs. يتم تنشيط جينات HSPs عندما تتعرض الخلايا لظروف إجهاد مختلفة. الهدف من هذه الورقة هو إعطاء وصف موجز لبروتينات صدمة الحرارة وتخليط الضوء على عملها في الكائن الحي في حالة الإجهاد. تم وصف مراجعة موجزة لأنواع HSPs ووظائفها بموضوعات خاصة على HSP70 في هذا الاستعراض ، نقدم أيضاً علاقة HSPs خاصة HSP70 وعملية التكيف للأنسجة مع عوامل الإجهاد. علاوة على ذلك ، نناقش العلاقة بين التركيز العالي لـ HSP70 على تحسين جودة السائل المنوي في حيوانات المزرعة المختلفة. في الختام ، فإن HSPs لها قيم مهمة للحفاظ على خلايا الكائنات الحية كعوامل رئيسية في عملية التكيف ولها أدوار مهمة في حماية الخلايا الإنجابية.