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ORIGINAL PAPER

Dynamics of HSPA1A and redox status in the spermatozoa and fluid from different segments of goat epididymis

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Abstract

The present study was attempted to investigate the dynamics of HSPA1A and redox status in the spermatozoa and fluid of different segments of buck epididymis. Testes were collected from sexually mature and healthy bucks aged between 2 and 3 years. The fluid and spermatozoa from different segments (caput, corpus and cauda) were harvested for further processing and analysis. The concentration of HSPA1A in spermatozoa lysate and epididymal fluid and its relative mRNA expression in spermatozoa from different segments of epididymis were studied. The HSPA1A concentration in epididymal fluid was significantly ($P < 0.01$) higher in the corpus as compared with caput and cauda, whereas, its concentration and relative mRNA expression decreased significantly ($P < 0.01$) in the spermatozoa from caput to cauda. The activities of SOD, GR, GST, and concentrations of manoldialdehyde and ROS decreased significantly ($P < 0.01$) in the spermatozoa from caput to cauda. The glutathione concentration and GPx activity decreased significantly ($P < 0.01$) in the spermatozoa of cauda as compared with the corpus. The SOD activity and ROS concentration were significantly ($P < 0.01$) higher in corpus, and GR and GST activity were significantly ($P < 0.01$) higher in caput fluid as compared with corpus and cauda. It may be concluded that HSPA1A concentration and its relative mRNA expression in spermatozoa decreased progressively, and redox status was altered during transit from caput to cauda.

Keywords HSPA1A · Epididymis · Goat · Redox status · Spermatozoa

Introduction

Heat shock proteins (HSPs) act as chaperones of cells and play an important "housekeeping" role. HSPs were first discovered in heat-shocked and stressed cells (Lindquist and Craig 1988). HSPs are required for various developmental stages of spermatogenesis, post-meiotic development of spermatids and maturation of spermatozoa (Purandhar et al. 2014). At least 20 HSPs or heat shock factors have been identified as being related to male spermatogenesis (Ferlin et al. 2010; Ji et al. 2012). In HSP families, germ cell-specific chaperones have been identified in the mature spermatozoa of mice (Asquith et al. 2004; Calvert et al. 2003), humans (Naaby-Hansen and Herr 2010), pigs (Spinaci et al. 2005), and bovine (Kawarsky and King 2001).

HSP70 has been identified in male germinal cells during spermatogenesis in mouse, rat, bull, boar, and human (Allen et al. 1988; Maekawa et al. 1989; Raab et al. 1995; Son et al. 1999; Huang et al. 2000; Kamaruddin et al. 2004). HSP70 and HSP70 family members have been detected and appeared to be abundant components of the sperm surface of the mouse, rat, bull, boar, and human sperm (Miller et al. 1992; Boulanger et al. 1995). Changes in HSP70 localization and concentration were reported during spermatogenesis and sperm maturation in epididymis and in ejaculated spermatozoa in bulls (Kamaruddin et al. 2004) and humans (Lachance et al. 2010; Naaby-Hansen and Herr 2010). A decrease in some variants of HSP70 (HSPA2 and HSPA5) was also reported in the mouse

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spermatozoa during transit from caput to cauda epididymis (Ijiri et al. 2011). On the other hand, HSPA5 was found to be increased in rat cauda epididymal sperm but decreased in hamster and mouse (Baker et al. 2005). Available literature suggests that species variation exists in HSP70 expression in spermatozoa of different segments of the epididymis. Ijiri et al. (2011) found that HSPA2 was present in the cytoplasmic droplet of caput epididymal spermatozoa, whereas it was absent from cauda epididymal spermatozoa. HSP70 was immunolocalized in spermatozoa acrosome of stallion and cat (Matwee et al. 2001; Spinaci et al. 2005). In bull spermatozoa, relocalization of HSP70 was found to be from acrosome to the equatorial segment, post-acrosomal region and mid piece during capacitation and acrosomal reaction (Kamaruddin et al. 2004). HSP70 has been proposed to cause sperm–zona pellucida (ZP) interaction, and antibodies against HSP70 have been reported to inhibit sperm–ZP interaction in boar and bovine studies (Matwee et al. 2001; Spinaci et al. 2005).

During the epididymal journey of a mammalian spermatozoon from caput to cauda, the level of polyunsaturated fatty acids (PUFA) in the sperm plasma membrane is increased, and therefore, it becomes more susceptible to oxidative damage (Vernet et al. 2004). Metabolic rate, morphology, and motility pattern of the spermatozoa vary during their transit in the epididymis, and therefore the redox status also changes (Noblanc et al. 2011). Multiple redox enzymes have been detected in the spermatozoa and fluid of different segments of the epididymis (Tramer et al. 1998; Jeulin et al. 1989; Bilodeau et al. 2000; Strzeżek et al. 2009; Koziorowska-Gilun et al. 2011). Rana et al. (2017) reported an increase in enzymatic antioxidant system in the epididymal fluid from the caput to cauda and a reverse trend in spermatozoa in goat epididymis. The antioxidative status of the spermatozoa is decreased when it undergoes cryopreservation and disturbs the post-thaw motility and fertilizing capacity (Reddy et al. 2018). The epididymal fluid also contributes to the semen volume which protects the spermatozoa from any oxidative insult.

Spermatozoa undergo morphological, cytoplasmic, and nuclear maturation, and eventually become motile and gain fertilizing ability during epididymal transit. The orchestrated maturation process of the spermatozoa in testes and epididymis indicate that gene transcription and translation play an important role (Steger 1999; Wu and Chu 2008). During this process, a number of new proteins are added to the spermatozoa either by protein synthesis or by absorption or adsorption from epididymal fluid (Gervasi and Visconti 2017). Changes in mRNA and protein levels have been reported in the developmental stages (Welch et al. 2006; Pandey et al. 2019; Grive et al. 2019) and capacitation states (Lee et al. 2011) of spermatozoa. Although variation in protein constituents has been documented, changes in mRNA expression during epididymal transit have not been reported.

The available research literature suggests that HSPA1A is an integral part of the spermatozoa and is necessary for its structural and functional competence. In our previous study (Reddy et al. 2018), we reported the transcriptional and translational changes in HSPA1A in mature spermatozoa during cryoprocessing which also correlated with post-thaw semen quality. In this study, we hypothesize that *HSPA1A* gene and its protein expression change during spermatozoa transit from caput to cauda. Although many studies have been carried out in different stages of spermatogonia and spermatids in the seminiferous tubule and in the ejaculated spermatozoa to characterize different heat shock family proteins, no study is available in goats to illustrate a modification in HSPA1A levels and its expression in the spermatozoa during its transit from caput to cauda. Similarly, the biochemical estimation of redox status in spermatozoa and fluid of different segments of goat epididymis has not been explored comprehensively. Therefore, the present study was undertaken to examine the changes in HSPA1A and redox status in spermatozoa and fluid of different segments of goat epididymis.

Materials and methods

Collection of testes/processing

The testes were collected from sexually mature and healthy bucks, between 2 to 3 years of age. Processing of testes for the removal of epididymis and the retrieval of spermatozoa was performed as per the procedure described by Rana et al. (2017) with slight modifications. A total of 20 testes were collected, and on the basis of gross examination, only 10 testes were further processed. Within 30 min after the slaughter, testes were transferred in pre-chilled phosphate buffer saline (PBS, pH 7.4) and brought to the laboratory. After removing the fascia, connective tissues, and blood vessels, epididymis was separated. Anatomically, three segments of epididymis, i.e., caput, corpus and cauda, were separated and immediately immersed in PBS (pH 7.4) in clean, separate sterile petri dishes. Each segment was incised in four to five pieces with a fine pair of scissors, and the luminal contents were allowed to exude from the cut ends in a known amount of PBS (pH 7.4). The residual content of each segment was gently squeezed out using forceps. The fluid content was transferred in micro-centrifuge tubes and incubated for 10 min at 37 °C to settle down the debris. A drop of fluid suspension from each segment of the epididymis was observed under a microscope at × 40 magnification to check gross features and observe progressive motility of the spermatozoa.

Sperm viability was examined using Eosin-Nigrosin stain. The epididymal fluid containing spermatozoa was

centrifuged twice at 10000×*g* for 15 min at 4 °C to produce clear, sperm-free epididymal fluid that was stored at − 20 °C until further analysis. The sperm pellets from each of the segments were washed in Sp-TALPH (pH 7.4) (sodium chloride, 100 mM; potassium chloride, 3.1 mM; sodium bicarbonate, 25 mM; sodium dihydrogen phosphate, 0.3 mM; sodium lactate, 21.6 mM; calcium chloride, 2.0 mM; magnesium chloride, 0.4 mM; HEPES, 100 mM; sodium pyruvate, 1.0 mM; and bovine serum albumin, 6.0 mM), by centrifugation at 1000×*g* for 5 min at room temperature. For the lysis of somatic cells, the sperm pellet was incubated in 1% Triton-X-100 at room temperature for 10 min, and then sperm suspension was centrifuged at 2000×*g* for 10 min, and the supernatant was discarded. The sperm pellet was washed thrice, and the pellet was resuspended in 200 μl of Sp-TALPH. The sperms were mechanically lysed using liquid nitrogen, and the lysate was centrifuged at 12000×*g* for 15 min at 4 °C. The supernatant was stored at − 20 °C till further analysis.

Estimation of redox status in spermatozoa and fluid

The antioxidant enzyme activity and concentration of prooxidants and antioxidants were measured in 10×10^8 spermatozoa and per mg protein in the fluid. Lipid peroxidation in sperm lysate and epididymal fluid was measured as per the method (Placer et al. 1966). Content of manoldialdehyde (MDA) was measured spectrophotometrically using a UV-VIS double beam spectrophotometer (Systronics-2203). The results were expressed as nM MDA/10×10^8 spermatozoa and nM MDA/mg protein in the epididymal fluid. Reactive oxygen species was estimated in terms of mM of H_2O_2 in test samples as developed by Alberti et al. (2000), and superoxide dismutase (SOD) activity was determined as described by Madesh and Balasubramanian (1998) in 96 well plate method.

Catalase (CAT) activity (Bergmeyer 1983), glutathione peroxidase (GPx) (Rotruck et al. 1973), glutathione-S-transferase (GST) (Habig et al. 1974), and glutathione reductase (GR) activities (Carlberg and Mannervik 1985) were also estimated. The amount of reduced glutathione (GSH) was estimated by the method described by Boyne and Ellman (1972). Protein content of the sperm lysate and epididymal fluid was estimated as per Bradford (1976).

Estimation of HSPA1A

Heat shock protein70A1A concentration was estimated in the sperm lysate and fluid of different segments of buck epididymis, using goat based heat shock protein 70 ELISA Kit (BlueGene Biotech, China) as per the manufacturer's instructions.

Isolation of total RNA

Total RNA was isolated from 50 million spermatozoa using RNA Purification kit (GeneJET, ThermoFischer Scientific, USA) as per manufacturer's instructions. The quantification and purity of total RNA were checked using Eppendorf BioPhotometer (Eppendorf, Germany). RNA samples with A260/A280 value approximately equal or more than 1.8 were used for cDNA synthesis. DNase treatment was carried out using RNase-free DNase I (SIGMA-ALDRICH, USA). The first strand cDNA was synthesized from 1 μg of total isolated RNA using Revert Aid First Strand cDNA Synthesis Kit (ThermoFischer Scientific, USA) in thermocycler (T100™ Bio-Rad, USA). Reaction mixture was mixed and incubated at 25 °C for 5 min followed by 42 °C for 60 min. Reaction was stopped by incubation for 5 min at 70 °C. The presence of cDNA was checked by amplifying *RPS15A* gene primers using Dream Taq PCR Master Mix (Thermo Fisher Scientific, USA), and the confirmation of amplification product was done by agarose gel electrophoresis. The cDNA was stored at − 20 °C.

Primer sequence

To amplify *HSPA1A* and *RPS15A* genes, specific primers were taken from the already published primer sequences (Dangi et al. 2012). These primers were further aligned by using PRIMER BLAST at NCBI, and the details have been given in Table 1.

Real-time PCR

Quantitative real-time PCR was performed using PowerUp SYBR Green qRT-PCR Master Mix (Applied Biosystems). The 20 μL reaction mixture contained 1.0 μL of cDNA, 1 μL each of forward primer (10 pmol), reverse primer, and cDNA template in addition to 10 μL PowerUp SYBR qPCR Mix and 7 μL of nuclease-free water. The thermal cycling profile was fixed as, one denaturing cycle for 3 min at 95 °C, and 35 cycles of PCR (95 °C for 10 s; 60 °C for 60 s for *RPS15A*; 62 °C for 60 s for *HSPA1A*). No template control (NTC) was placed for gene quantification for checking the contamination. Six biological samples in triplicate from each epididymal segment were used for real-time PCR measurements. For normalization, *RPS15A* was amplified for each sample as a housekeeping gene. Cycle thresholds (Ct) values for both the genes were obtained using Bio-Rad CFX96 Touch™ Real-Time PCR Detection System, USA. The analysis of resultant Ct values was performed using the relative comparative Ct method, commonly designated as $2^{-\Delta\Delta Ct}$ (Livak and Schmittgen 2001).

Table 1 Gene transcripts, primer sequences and resulting fragment size

Target gene	Sequence of nucleotide	Size (bp)	EMBL
HSP 70	For: 5'-GACGACGGCATCTTCAAG −3' Rev.: 5'-GTTCTGGCTGATGTCCTTC -3'	132	FJ975769.1
RPS15A	For: 5'- GAATGGTGCGCATGAATGTC -3' Rev.: 5′- GACTTTGGAGCACGGCCTAA −3'	101	BC108231

EMBL accession number or reference of published sequence

Statistical analysis

Analysis of variance was performed to determine the presence or absence of significant differences in the analytical variables among different segments using one-way ANOVA using SPSS 23.0 statistical software package, and differences between segments were tested using Tukey's-b test. P values < 0.05 were considered significant.

Results

Progressive motility and viability of spermatozoa

Progressive motility and viability of spermatozoa retrieved from different segments of the epididymis are presented in Table 2. Progressive motility and viability of spermatozoa were significantly ($P < 0.01$) higher in cauda as compared with corpus and caput epididymis; however, both the parameters were similar in caput and corpus epididymis.

Concentration of HSPA1A in spermatozoa and epididymal fluid

The mean ± SE of the concentration of HSPA1A in spermatozoa lysate and epididymal fluids in different segments of goat epididymis is presented in Table 3. The HSP70 concentration in epididymal fluid was significantly ($P < 0.05$) higher in the corpus as compared with caput and cauda. The HSPA1A concentration was found to be significantly ($P < 0.01$) higher in caput as compared with corpus and cauda spermatozoa lysate.

Relative expression of HSPA1A mRNA

The relative expression *HSPA1A* mRNA in the sperm of different segments of epididymis is presented in Fig. 1. The relative expression of *HSPA1A* mRNA significantly ($P < 0.05$) decreased in corpus and cauda epididymis as compared with caput; however, expression did not change significantly ($P > 0.05$) in the cauda as compared with corpus epididymis.

Redox status in fluid and spermatozoa lysate

The mean ± SE of the activity of GPx, SOD, GR, and GST, and the concentration of MDA, ROS, and GSH in epididymal fluid and spermatozoa lysate are presented in Tables 4 and 5, respectively. In the epididymal fluid, SOD activity and ROS concentration were significantly ($P < 0.01$) higher in corpus and cauda as compared with caput. GR and GST activities were found to be significantly ($P < 0.01$) higher in caput as compared with corpus and cauda epididymis. GPx activity, MDA, and GSH levels did not change significantly ($P > 0.01$) among different segments of the epididymis.

The GR and GST activity and MDA concentration were significantly ($P < 0.01$) higher in the spermatozoa lysate of caput as compared with corpus and cauda. GSH concentration and GPx activity were found to be significantly ($P < 0.01$) higher in corpus as compared with caput and cauda spermatozoa lysate. The CAT activity could not be estimated spectrophotometrically either in spermatozoa lysate or in epididymal fluid.

Discussion

HSPs are chaperone molecules, a prerequisite for spermatogenesis, capacitation, acrosomal reaction, sperm-oocyte interaction, and early embryonic development, and protect spermatozoa from environmental hazards. One of the most studied HSPs is HSP70A1A, and its presence is well established in different germ cells during spermatogenesis and ejaculated spermatozoa (Kamaruddin et al. 2004; Huang et al. 2005). In domestic animals, artificial insemination using cryopreserved spermatozoa is most effectively used assisted

Table 2 Progressive motility and viability of spermatozoa from different segments of goat epididymis

Parameters	Caput	Corpus	Cauda	P value
Progressive motility (%)	3.32 ± 0.78^{a}	4.83 ± 1.00^{a}	54.09 ± 0.65^{b}	0.000
Viability (%)	91.66 ± 2.20^{a}	91.63 ± 1.82^{a}	97.32 ± 2.31^{b}	0.01

Values in rows with different superscripts differ significantly ($P < 0.01$)

Table 3 Heat shock protein 70 status of the spermatozoa lysate and epididymal fluid of different segments of goat epididymis

Parameter	Caput epididymis	Corpus epididymis	Cauda epididymis	*P* value
HSP70 (ng/10^8 spermatozoa) in lysate	10.45 ± 1.45 [a]	7.87 ± 0.98 [b]	1.80 ± 0.23 [c]	0.000
HSP70 (ng/mg of protein) in fluid	0.43 ± 0.04 [a]	0.71 ± 0.08 [b]	0.56 ± 0.03 [ba]	0.015

Values in rows with different superscripts differ significantly ($P < 0.05$)

reproductive technology. The dilution of semen for cryopreservation causes dilution of seminal plasma containing essential milieu for optimum functional competence of spermatozoa. Recent proteomic and genomic studies revealed, down-regulation of *HSPA1A* mRNA and its protein expression in the cryopreserved spermatozoa (Reddy et al. 2018; Pini et al. 2018) besides deterioration of antioxidant potential (Reddy et al. 2018). However, changes in the HSPA1A in spermatozoa have not been studied in the maturational process during its transit from caput to cauda. The present study underlined the changes in HSPA1A concentration and mRNA expression, and redox status in the spermatozoa and fluid during epididymal transit from caput to cauda.

In the present study, the progressive motility and viability of the spermatozoa were significantly higher in cauda as compared with caput and corpus. The increase in progressive motility in spermatozoa has been attributed to differential gene expression in different epididymal segments which results in difference in luminal ionic concentration and addition of several proteins, lipids, and sugars to the spermatozoa (Gervasi and Visconti 2017). Similarly, higher spermatozoa viability in the cauda epididymis may be due to more macrophage activity in the cauda. HSPA1A level and redox status has been correlated with progressive motility in the ejaculated and post-thaw spermatozoa; however, in the present study, a correlation between redox status, HSPA1A expression, and progressive motility and viability could not be established in the epididymal spermatozoa.

In the present study, HSPA1A concentration progressively decreased in spermatozoa from caput to cauda, while in fluid, it increased from caput to corpus and decreased in cauda. Scanty literature is available on the concentration of HSP70A1A in the spermatozoa of different epididymal segments in goats. However, Ijiri et al. (2011) reported that some variants of HSP70 (HSPA2 and HSPA5) decreased in the mouse spermatozoa during transit from caput to cauda. On the other hand, Baker et al. (2005) reported that HSPA5 increased in the spermatozoa of cauda epididymis of rats but decreased in hamster and mouse. Ijiri et al. (2011) found that HSPA2 was present in the cytoplasmic droplet of caput epididymal spermatozoa, whereas it was absent in cytoplasmic droplet of cauda epididymal spermatozoa. To negate the role of HSPA1A in cytoplasmic droplets, in the present study, the relative expression of *HSPA1A* mRNA was evaluated in the spermatozoa which revealed that relative expression of *HSPA1A* mRNA was highest in caput epididymis which subsequently decreased in corpus and cauda. The spermatozoa is said to be transcriptionally inactive; however, the morphological, cytoplasmic, and nuclear alterations during epididymal transit require the process of transcription and translation in spermatozoa which makes it capable of movement with the potential for fertilization (Ren et al. 2017). Present study also

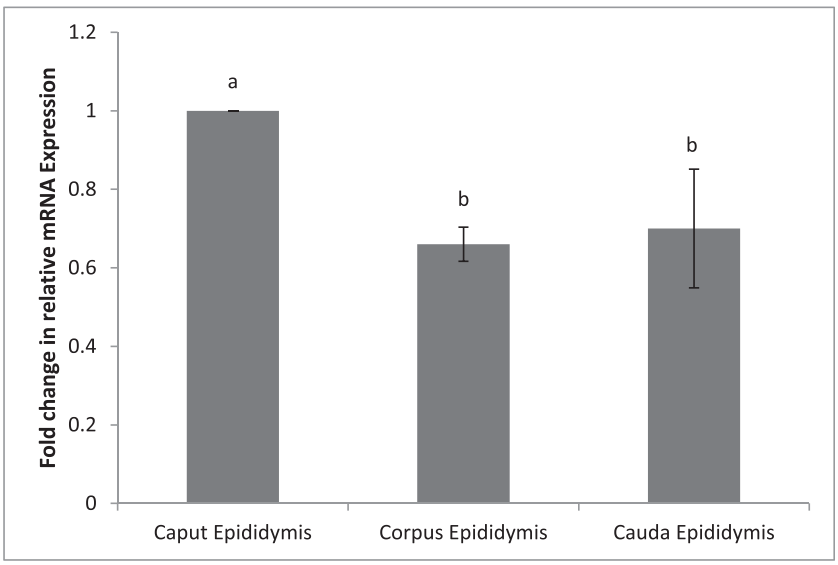

Fig. 1 Relative HSPA1A mRNA expression in spermatozoa derived from different segments of buck epididymis. The data is indicated in the form of bars indicating mean ± standard error. The bars with different superscripts differ significantly ($P < 0.05$). The superscripts indicate that fold change in relative *HSPA1A* mRNA expression in spermatozoa was significantly ($P < 0.05$) higher in caput epididymis as compared with corpus and cauda epididymis

Table 4 Redox status of the epididymal fluid from different segments of buck epididymis

Parameter	Caput epididymis	Corpus epididymis	Cauda epididymis	*P* value
Protein	2502.74 ± 72.87	2061.59 ± 204.22	2085.74 ± 174.97	0.110
GPx	0.47 ± 0.02	0.48 ± 0.02	0.49 ± 0.03	0.903
SOD	127.54 ± 0.98 ^{a}	170.39 ± 2.80 ^{b}	169.77 ± 1.75^{b}	0.000
GR	3.84 ± 0.16 ^{b}	3.20 ± 0.073^{a}	3.36 ± 0.13^{a}	0.000
GST	0.29 ± 0.010 ^{b}	0.17 ± 0.01^{a}	0.19 ± 0.003^{a}	0.000
MDA	3.15 ± 0.45	3.23 ± 0.20	2.93 ± 0.13	0.760
ROS	0.15 ± 0.002 ^{a}	0.19 ± 0.010^{b}	0.19 ± 0. 001^{b}	0.000
GSH	1.81 ± 0.08	1.59 ± 0.16	1.44 ± 0.10	0.103

Protein (mg/ml); *GPx* glutathione peroxidase (unit GPx/mg of proteins); *SOD* superoxide dismutase (unit/mg of protein); *GR* glutathione reductase (unit GR/mg of protein); *GST* glutathione S-transferase (GST unit/mg of protein); *MDA* malondialdehyde (mg of MDA/mg protein); *ROS* reactive oxygen species (mg of H_2O_2/mg of protein); and *GSH* reduced glutathione (μg GSH/mg protein)

Values in rows with different superscripts differ significantly ($P < 0.05$)

confirmed that the sperm cells are transcriptionally active. On the other hand, it was also observed that HSPA1A level increased in corpus epididymal fluid and subsequently decreased in cauda. In bulls, Kamaruddin et al. (2004) also reported that localization and quantity of HSPA1A change during different stages of spermatogenesis and sperm maturation in epididymis and in ejaculated spermatozoa. In stallion-ejaculated spermatozoa, HSPA1A localization was observed in the post-acrosomal region (Meyers and Rosenberger 1999). HSPA1A has been detected on the surface of mouse, rat, bull, boar, and human-ejaculated sperm (Allen et al. 1988; Miller et al. 1992; Boulanger et al. 1995; Raab et al. 1995; Kamaruddin et al. 2004; Huang et al. 2005).

In the present study, SOD activity and ROS concentration were higher in the fluid of corpus and cauda as compared with caput. More SOD activity in corpus and cauda fluid may be for the protection of spermatozoa from oxidative stress as more amounts of ROS are produced due to a higher concentration of unsaturated fatty acids in the cauda spermatozoa (Tramer et al. 2004). Further, glutathione and glutathione-based antioxidant enzyme activity decreased in corpus and cauda fluid, making spermatozoa more liable to oxidative damage; therefore SOD activity, as an alternate, needs to be increased in order to balance antioxidative defense. Similar results were reported in European bison (Koziorowska-Gilun et al. 2013; Park et al. 2012), goat (Rana et al. 2017) and miniature breed stallions (Bustamante-Filho et al. 2014). Rana et al. (2017) reported that GPx activity was higher in the cauda fluid as compared with caput and corpus in goat, whereas no change in GPx activity was observed in the present study. In contrast to our findings, the GPx activity of stallion epididymal fluid has been reported to decrease from caput to cauda (Bustamante-Filho et al. 2014).

Angrimani et al. (2013) found that SOD activity did not change in the canine spermatozoa in different

Table 5 Redox status of the spermatozoa lysate from different segments of buck epididymis

Parameter	Caput epididymis	Corpus epididymis	Cauda epididymis	*P* value
Protein	1474.70 ± 197.22^{b}	1209.10 ± 92.34^{b}	319.06 ± 34.53^{a}	0.000
GPx	2.77 ± 0.10^{b}	3.27 ± 0.19^{c}	0.60 ± 0.021^{a}	0.000
SOD	638.79 ± 20.47^{b}	647.30 ± 18.60^{b}	129.60 ± 2.81^{a}	0.000
GR	99.87 ± 3.75^{c}	58.69 ± 2.021^{b}	23.06 ± 1.34^{a}	0.000
GST	0.10 ± 0.005^{c}	0.08 ± 0.006^{b}	0.01 ± 0.001^{a}	0.000
MDA	30.48 ± 1.20^{c}	23.22 ± 0.90^{b}	6.69 ± 0.32^{a}	0.000
ROS	0.73 ± 0.025^{b}	0.74 ± 0.028^{b}	0.16 ± 0.011^{a}	0.000
GSH	0.05 ± 0.004 ^{b}	0.20 ± 0.008^{c}	0.01 ± 0.001^{a}	0.000

Protein (mg/10^8 spermatozoa); *GPx* glutathione peroxidase (unit GPx/10^8 spermatozoa); *SOD* superoxide dismutase (unit/10^8 spermatozoa); *GR* glutathione reductase (unit GR/10^8 spermatozoa); *GST* glutathione S-transferase (GST unit/10^8 spermatozoa), *MDA* malondialdehyde (mg of MDA/10^8 spermatozoa); *ROS* reactive oxygen species (mg of H_2O_2/10^8 spermatozoa); and *GSH* reduced glutathione (μg GSH/10^8 spermatozoa)

Values in columns with different superscripts differ significantly ($P < 0.05$)

epididymal segments. A decrease in SOD activity was reported in goat (Rana et al. 2017) and boar (Park et al. 2012) spermatozoa from caput to cauda which were also confirmed in the present study where nearly fivefold decrease in SOD activity was recorded in the spermatozoa of cauda as compared with caput and corpus. The variability in the results observed by various workers may be attributed to species variation. In the present study, GPx activity decreased in spermatozoa of the cauda and being highest in corpus spermatozoa. Higher activity of GPx in the spermatozoa of corpus region may be due to the higher concentration of GSH in the corpus spermatozoa as revealed from the present study. GPx activity has been reported to be higher in corpus epididymal tissues of the European bison (Koziorowska-Gilun et al. 2013). Other glutathione-based antioxidant enzymes, GR and GST, exhibited a declining activity in epididymal fluid and spermatozoa of different segments of the epididymis in the present study. The decrease in GR and GST activity in the spermatozoa of successive segments of the epididymis may be attributed to a specific requirement of spermatozoa against the excessive ROS and other oxidizing agents produced in different segments of the epididymis. Catalase in spermatozoa has been reported in both human and rat (Jeulin et al. 1989; Tramer et al. 1998; Lenzi et al. 1994) albeit at extremely low levels (Alvarez et al. 1987). Catalase was reported to be absent in rabbit (Holland and Storey 1981), mouse (Alvarez and Storey 1984), canine (Strzeżek et al. 2009; Angrimani et al. 2013), boar (Koziorowska-Gilun et al. 2011), and bull spermatozoa (Bilodeau et al. 2000) despite a high level in bovine seminal fluid (Bilodeau et al. 2000; Kar et al. 2015). Rana et al. (2017) reported presence of CAT activity, in both fluid and spermatozoa retrieved from different segments of the epididymis in goats. Contrary to these findings, the CAT activity could not be detected either in epididymal spermatozoa or in epididymal fluid in the present study.

Conclusion

From present study, it may be concluded that the concentration and expression of *HSPA1A* in the spermatozoa decreased progressively during its maturation and transit in the epididymis. Further, redox status was found to be altered during transit from caput to cauda. This may be due to remodeling and maturational processes occurring in spermatozoa passing through the epididymis. A decrease in HSPA1A concentration and *HSPA1A* mRNA expression and progressive decrease in antioxidative status during epididymal transit is a major concern as it is further downregulated during cryopreservation of spermatozoa, and therefore, the functional competence of spermatozoa may be compromised.

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References

Alberti A, Bolognini L, Macciantelli D, Carratelli M (2000) The radical cation of N, N-diethyl-para-phenylendiamine: a possible indicator of oxidative stress in biological samples. Res Chem Intermediat 26(3): 253–267

Allen RL, O'Brien DA, Jones CC, Rockett DL, Eddy EM (1988) Expression of heat shock proteins by isolated mouse spermatogenic cells. Mol Cell Biol 8(8):3260–3266

Alvarez JG, Storey BT (1984) Lipid peroxidation and the reactions of superoxide and hydrogen peroxide in mouse spermatozoa. Biol Reprod 30(4):833–841

Alvarez JG, Touchstone JC, Blasco L, Storey BT (1987) Spontaneous lipid peroxidation and production of hydrogen peroxide and superoxide in human spermatozoa: superoxide dismutase as major enzyme protectant against oxygen toxicity. J Androl 8(5):338–348

Angrimani DSR, Lucio CF, Veiga GAL, Silva LCG, Regazzi FM, Nichi M, Vannucchi CI (2013) Sperm maturation in dogs: sperm profile and enzymatic antioxidant status in ejaculated and epididymal spermatozoa. Andrologia 46(7):814–819

Asquith KL, Baleato RM, McLaughlin EA, Nixon B, Aitken RJ (2004) Tyrosine phosphorylation activates surface chaperones facilitating sperm-zona recognition. J Cell Sci 117(16):3645–3657

Baker MA, Witherdin R, Hetherington L, Cunningham-Smith K, Aitken RJ (2005) Identification of post-translational modifications that occur during sperm maturation using difference in two-dimensional gel electrophoresis. Proteomics 5(4):1003–1012

Bergmeyer HA (1983) Methods of enzymatic analysis: metabolites I, C005Aarbohydrates: Vol. 6, 3rd edition, New York, academic press

Bilodeau JF, Chatterjee S, Sirard MA, Gagnon C (2000) Levels of antioxidant defenses are decreased in bovine spermatozoa after a cycle of freezing and thawing. Mol Reprod Develop 55(3):282–288

Boulanger J, Faulds D, Eddy EM, Lingwood CA (1995) Members of the 70 kDa heat shock protein family specifically recognize sulfoglycolipids: role in gamete recognition and mycoplasma related infertility. J Cell Physiol 165(1):7–17

Boyne AF, Ellman GL (1972) A methodology for analysis of tissue sulfhydryl components. Anal Biochem 46(2):639–653

Bradford MM (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein: utilizing the principle of protein-dye binding. Anal Biochem 72(1–2):248–254

Bustamante-Filho IC, Rosa AP, Vander Linden LS, Pederzolli CD, Neves AP, Dutra-Filho CS, MIM J, Mattos RC (2014) Enzymatic scavengers in the epididymal fluid: comparison between pony and miniature breed stallions. Anim Reprod Sci 151(3–4):164–168

Calvert ME, Digilio LC, Herr JC, Coonrod SA (2003) Oolemmal proteomics–identification of highly abundant heat shock proteins and molecular chaperones in the mature mouse egg and their localization on the plasma membrane. Reprod Biol Endocrinol 1(1):27

Carlberg I, Mannervik B (1985) Glutathione reductase. In Methods in enzymology (Vol. 113, pp. 484–490). Academic Press

Dangi SS, Gupta M, Maurya D (2012) Expression profile of HSP genes: during different seasons in goats (*Capra hircus*). Trop Anim Health Prod 44(8):1905–1912

Ferlin A, Speltra E, Patassini C, Pati MA, Garolla A, Caretta N, Foresta C (2010) Heat shock protein and heat shock factor expression in sperm: relation to oligozoospermia and varicocele. J Urol 183(3): 1248–1252

Gervasi MG, Visconti PE (2017) Molecular changes and signaling events occurring in spermatozoa during epididymal maturation. Andrology 5(2):204–218

Grive KJ, Hu Y, Shu E, Grimson A, Elemento O, Grenier JK, Cohen PE (2019) Dynamic transcriptome profiles within spermatogonial and spermatocyte populations during postnatal testis maturation revealed by single-cell sequencing. PLoS Genet 15(3):e1007810

Habig WH, Pabst MJ, Jakoby WB (1974) Glutathione S- transferases the first enzymatic step in mercapturic acid formation. J Biol Chem 249(22):7130–7139

Holland MK, Storey BT (1981) Oxygen metabolism of mammalian spermatozoa: generation of hydrogen peroxide by rabbit epididymal spermatozoa. Biochem J 198(2):273–280

Huang SY, Kuo YH, Lee YP, Tsou HL, Lin EC, Ju CC, Lee WC (2000) Association of heat shock protein 70 with semen quality in boars. Anim Reprod Sci 63(3–4):231–240

Huang X, Guo Y, Shui Y, Gao S, Yu H, Cheng H, Zhou R (2005) Multiple alternative splicing and differential expression of dmrt1 during gonad transformation of the rice field eel. Biol Reprod 73(5):1017–1024

Ijiri TW, Merdiushev T, Cao W, Gerton GL (2011) Identification and validation of mouse sperm proteins correlated with epididymal maturation. Proteomics 11(20):4047–4062

Jeulin C, Soufir JC, Weber P, Laval Martin D, Calvayrac R (1989) Catalase activity: in human spermatozoa and seminal plasma. Gamete Res 24(2):185–196

Ji ZL, Duan YG, Mou LS, Allam JP, Haidl G, Cai ZM (2012) Association of heat shock proteins, heat shock factors and male infertility. Asian Pac J Reprod 1(1):76–84

Kamaruddin M, Kroetsch T, Basrur PK (2004) Immunolocalization of heat shock protein 70: in bovine spermatozoa. Andrologia 36(5):327–334

Kar S, Divyashree BC, Roy SC (2015) Temporal leakage of cu, Zn superoxide dismutase and loss of two low-molecular-weight forms of glutathione peroxidase-1 from buffalo (Bubalus bubalis) sperm after freezing and thawing. Theriogenology 83(4):512–519

Kawarsky SJ, King WA (2001) Expression and localisation of heat shock protein 70 in cultured bovine oocytes and embryos. Zygote 9(1):39–50

Koziorowska-Gilun M, Koziorowski M, Frase L, Strzeżek J (2011) Antioxidant defence system of boar cauda epididymidal spermatozoa and reproductive tract fluids. Reprod Domest Anim 46(3):527–533

Koziorowska-Gilun M, Gilun P, Fraser L, Koziorowski M, Kordan W, Stefanczyk-Krzymowska S (2013) Antioxidant enzyme activity and mRNA expression in reproductive tract of adult male: European Bison (Bison bonasus, Linnaeus 1758). Reprod Domest Anim 48(1):7–14

Lachance C, Fortier M, Thimon V, Sullivan R, Bailey JL, Leclerc P (2010) Localization of Hsp60 and Grp78: in the human testis, epididymis and mature spermatozoa. Int J Androl 33(1):33–44

Lee CK, Hwang JY, Mulligan BP, Kim HM, Ka HH (2011) Analysis of messenger RNA level changes for selected genes during capacitation in ejaculated boar spermatozoa. Biol Reprod 85(1):806

Lenzi A, Picardo M, Gandini L, Lombardo F, Terminali O, Passi S, Dondero F (1994) Andrology: glutathione treatment of dyspermia: effect on the lipoperoxidation process. Hum Reprod 9(11):2044–2050

Lindquist S, Craig EA (1988) The heat-shock proteins. Annu Rev Genet 22(1):631–677

Livak KJ, Schmittgen TD (2001) Analysis of relative gene expression data using real-time qPCR and the 2− ΔΔCT method. Methods 25(4):402–408

Madesh M, Balasubramanian KA (1998) Microtiter plate assay for superoxide dismutase, using MTT reduction by superoxide. Indian J Biochem Biophys 35(3):184–188

Maekawa M, O'Brien DA, Allen RL, Eddy EM (1989) Heat- shock cognate protein (hsc7l) and related proteins in mouse spermatogenic cells. Biol Reprod 40(4):843–852

Matwee C, Kamaruddin M, Betts DH, Basrur PK, King WA (2001) The effects of antibodies to heat shock protein 70 in fertilization and embryo development. Mol Hum Reprod 7(9):829–837

Meyers SA, Rosenberger AE (1999) A plasma membrane-associated hyaluronidase is localized to the posterior acrosomal region of stallion sperm and is associated with spermatozoal function. Biol Reprod 61(2):444−4512

Miller D, Brough S, Al-Harbi O (1992) Characterization and cellular distribution of human spermatozoal heat shock protein. Hum Reprod 7:637–645

Naaby-Hansen S, Herr JC (2010) Heat shock proteins on the human sperm surface. J Reprod Immunol 84(1):32–40

Noblanc A, Kocer A, Chabory E, Vernet P, Saez F, Cadet R, Conrad M, Drevet JR (2011) Glutathione peroxidases at work on epididymal spermatozoa: an example of the dual effect of reactive oxygen species on mammalian male fertilizing ability. J Androl 32(6):641–650

Pandey A, Yadav SK, Vishvkarma R, Singh B, Maikhuri JP, Rajender S, Gupta G (2019) The dynamics of gene expression during and post meiosis sets the sperm agenda. Mol Reprod Dev. https://doi.org/10.1002/mrd.23278

Park K, Jeon S, Song YJ, Lee SH (2012) Proteomic analysis of boar spermatozoa and quantity changes of superoxide dismutase 1, glutathione peroxidase and peroxiredoxin 5 during epididymal maturation. Anim Reprod Sci 135(1–4):53–61

Pini T, Rickard JP, Leahy T, Crossett B, Druart X, de Graaf SP (2018) Cryopreservation and egg yolk medium alter the proteome of ram spermatozoa. J Proteome 181:73–82

Placer ZA, Cushman LL, Johnson BC (1966) Estimation of product of lipid peroxidation (malonyl dialdehyde): in biochemical systems. Anal Biochem 16(2):359–364

Purandhar K, Jena PK, Prajapati B, Rajput P, Seshadri S (2014) Understanding the role of heat shock protein isoforms in male fertility, aging and apoptosis. World J Men's Health 32(3):123–132

Raab LS, Polakoski KL, Hancock LW, Hamilton DW (1995) Characterization of the heat shock protein P70 in rat spermatogenic cells. Mol Reprod Dev 40(2):186–195

Rana M, Roy SC, Divyashree BC (2017) Sperm antioxidant defences decrease during epididymal transit from caput to cauda in parallel with increases in epididymal fluid in the goat (*Capra hircus*). Reprod Fertil Dev 29(9):1708–1719

Reddy VS, Yadav B, Yadav CL, Anand M, Swain DK, Kumar D, Kritania D, Madan AK, Kumar J, Yadav S (2018) Effect of sericin supplementation on heat shock protein 70 (HSP70) expressions redox status and post thaw semen quality in goat. Cryobiology 84:33–39

Ren X, Chen X, Wang Z, Wang D (2017) Is transcription in sperm stationary or dynamic? J Reprod Dev 63(5):439–443

Rotruck JT, Pope AL, Ganther HE, Swanson AB, Hafeman DG, Hoekstra W (1973) Selenium: biochemical role as a component of glutathione peroxidase. Science 179(4073):588–590

Son WY, Hwang SH, Han CT, Lee JH, Kim S, Kim YC (1999) Specific expression of heat shock protein HSPA2 in human male germ cells. Mol Hum Reprod 5(12):1122–1126

Spinaci M, Volpe S, Bernardini C, De Ambrogi M, Tamanini C, Seren E, Galeati G (2005) Immunolocalization of heat shock protein 70 (Hsp70) in boar spermatozoa and its role during fertilization. Mol Reprod Develop 72(4):534–541

Steger K (1999) Transcriptional and translational regulation of gene expression in haploid spermatids. Anat Embryol (Berl) 199:471–487

Strzeżek R, Koziorowska-Gilun M, Kowalówka M, Strzeżek J (2009) Characteristics of antioxidant system in dog semen. Pol J Vet Sci 12(1):55–60

Tramer F, Rocco F, Micali F, Sandri G, Panfili E (1998) Antioxidant systems in rat epididymal spermatozoa. Biol Reprod 59(4):753–758

Tramer F, Caponecchia L, Sgro P, Martinelli M, Sandri G, Panfili E, Lenzi A, Gandini L (2004) Native specific activity of glutathione peroxidase (GPx-1), phospholipid hydroperoxide glutathione peroxidase (PHGPx) and glutathione reductase (GR) does not differ between normo and hypomotile human sperm samples. Int J Androl 27(2): 88–93

Vernet P, Aitken RJ, Drevet JR (2004) Antioxidant strategies: in the epididymis. Mol Cell Endocrinol 216(1–2):31–39

Welch JE, Barbee RR, Magyar PL, Bunch DO, O'Brien DA (2006) Expression of the spermatogenic cell-specific glyceraldehyde 3-phosphate dehydrogenase (GAPDS) in rat testis. Mol Reprod Dev 73:1052–1060

Wu TF, Chu DS (2008) Sperm chromatin: fertile grounds for proteomic discovery of clinical tools. Mol Cell Proteomics 7:1876–1886