Molecular Characterization and Gene Expression of Heat Shock Protein 70 (hsp70) Gene in the Semen of Iraqi Holstein Bulls and Arabi Rams

By
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This study was conducted at the Animal Farm of College of Agriculture, University of Basrah, (longitude 47.7433690, latitude 30.5627250 north of Basrah), and the fields of Artificial Insemination Center in Abu Ghraib, the General Company for Livestock Services (longitude 44.1922070, latitude 33.3095550 northwest of Baghdad), during the winter season for the months November, December 2015 and January 2016, and the summer season for the months April, May and June, 2016. To achieve all the study requirements, the laboratories work at the Laboratory physiology, Molecular Genetics Laboratory, College of Agriculture, University of Basrah; the Central Research Unit, College of Veterinary Medicine, University of Basrah; the Criminal Laboratory Department of Investigation, Criminal Evidence Directorate, Basrah Governorate Police; as well as laboratories of the Abu Ghraib, Artificial Insemination Center; in addition to the First BASE Laboratories, Laboratory in Selangor Malaysia, were used. The study included two experiments:

**The first experiment :**

This experiment was designed to detect the genetic polymorphism of *hsp70* gene in the semen of Bulls, their relationship to certain physical and chemical semen characteristics, the level of gene expression in the season winter and summer, and the evaluation of the bulls by finding the breeding value of certain traits in the fresh and frozen semen of the Iraqi Holstein Bulls, back to the Artificial Insemination Center / Abu Ghraib / Baghdad, 30 bulls were used in this experiment.

**Second Experience :**

This experiment was designed to detect the genetic polymorphism of *hsp70* genes in the semen of Rams, their relationship with some physical and chemical semen characteristics, the level of gene expression in the season winter and summer, and the evaluation of the rams by finding the breeding value of some traits in the fresh and frozen semen of local Arabi rams, back to the Agricultural Research Station / College of Agriculture / University of Basrah, 20 Rams were used in this experiment.

**Results of the study :**

**I. The first experiment :**

1) After DNA extracted, the quantity and purity of the DNA were determined by the Nano drop device, the purity ratio was 1.8. A Polymerase Chain Reaction (PCR) carried out, the amplification product was approximately 1926bp. Nucleotide Sequence analysis was done to detect polymorphism and compared with the genotypes of the *hsp70* gene in the Gene Bank. There are three Genotypes of *hsp70* gene were obtained in bulls :

**A**: **The Genotype A**, this genotype is the closest to heat shock protein *hsp70* gene in the Holstein bulls in Gene Bank. This polymorphism was exposed to be genetic mutations at position No. 6, the Nucleotide (C < G), No. 174 (C < G), No. 282 (A < G) and position No. 1339 (T < C). All mutations are silent that did not encoding to any new amino acid, (16 Bulls).

**B: The Genotype B**, the nucleotides were substituted at different positions, position No.114 (G < A), No. 174 (C < G), No. 282 (A < G), No. 1339 (T < C), No.1451 (C < A), No.1590 (A < G), No. 1695 (C < T) and position No. 1719 (G <T), these genetic mutations are all silent except the mutation at the position No. 1451 is a missense mutation, encoding for new amino acid, (6 Bulls).

**C: The Genotype C** , the nucleotides were substituted at different positions, position No. 114 (G < A), No. 174 (C < G), No. 282 (A < G) No.1339 (T < C), No. 1590 (A <G), No. 1695 (C <T) and position No. 1719 (G <T), all mutations are silent, ( 8 bulls).

2) The results of the Multiple Sequence Alignment (MSA) of *hsp70* gene in the bulls showed that the convergence ratio of A, B and C were 99.73%, 99.51% and 99.56%, respectively, with the same gene in the Holstein bulls recorded in Gene Bank. The MSA results also showed that the B genotype gave the lowest ratio to the studied genotypes, followed by the C genotype and the A genotype, which showed the highest approximation with the genotypes studied in the Gene Bank for the Holstein bulls, sheep, goats, pigs and humans.

3) Genotype B, has shown significantly higher ($P < 0.05$) than the C genotype and A genotype in all physical semen characteristics studied, it gave the highest rate of mass and individual motility, the highest percentage of live sperm and the lowest rate of abnormalities, both in fresh and frozen semen in a period of one month and two months freezing and in both seasons of the study.

4) Genotype B showed Significantly higher ($P < 0.05$) towards the C and A genotypes in all chemical semen characteristics studied, It gave the highest percentage of the Acrosome integrities and gave the lowest level of concentration of enzymes ALT, AST and ALP in the frozen semen and freezing periods in a month and two months and in both seasons of the study.

5 ) After RNA extraction, synthesise of cDNA and carry out Quantitative Real-time PCR (qRT-PCR) analysis, genotype B showed Significantly higher ($P < 0.05$) than genotype C and A in the level of gene expression of heat shock protein *hsp70* gene in the summer season.

6) The bulls belong to genotype B were superior in the valuation on the basis of the breeding value of motility, live sperm and the abnormalities in the fresh and frozen semen for a month and two months followed by the C than A.

**II. Second Experience :**

1) After DNA extracted, the quantity and purity of the DNA were determined by the Nano drop device, the purity ratio was 1.8. A PCR carried out and the amplification product was in approximately 1926bp, bands were visualized by gel electrophoresis. Nucleotide sequence analysis was done to detect polymorphism compared to that of the *hsp70* gene in the Gene Bank. There are two genotypes of *hsp70* gene were obtained in rams :

**A: The A genotype**, showed no match with other nucleotides sequence of other group neither heat shock protein *hsp70* gene in Gene Bank, the nucleotide in position 1308, the nucleotide T was substituted by C (T < C), a silent genetic mutation that did not encoding a new amino acid, (10 rams).

**B: The B genotype**, in comparison with the A genotype , the Nucleotide C was substituted with G (C <G), a missense genetic mutation that resulted from change the amino acid encoding at the site 514, a genetic mutation not previously recorded in rams except in Chinese sheep. In addition, nucleotide G was substituted by nucleotide A (G < A) in the position 1524, a silent genetic mutation (10 rams).

2) The results of the (MSA) of *hsp70* gene in the rams showed that the ratio of A and B genotypes were 99.89% and 99.84% respectively with the same gene in rams in the Gene Bank. The MSA of the genotype B gave the lowest ratio to the same gene in sheep, Holstein bulls, goat, pig and human

3) The genotype B Significantly higher (P <0.05) than the genotype A in all Physical semen characteristics studied, it showed the highest rate of mass and individual motility and the highest percentage of live sperm and the lowest rate of abnormalities, in fresh and frozen semen in a period of a month and two months freezing and in both seasons of the study.

4) The genotype B Significantly higher (P <0.05) than the genotype A in all chemical semen characteristics, it showed the highest percentage of the Acrosome integrities, and showed the lowest level of concentration of enzymes ALT, AST and ALP in the frozen semen and freezing periods of month and two months and in both seasons of the study.

5) The result of Quantitative Real-time PCR (RT-PCR), showed the B genotype significantly higher ($P < 0.05$) than genotype A in the level of gene expression of heat shock protein *hsp70* in the summer season.

6) The genotype B of rams was assessment superior in the breeding value in both status of motility, live sperm and abnormalities in fresh and frozen semen for a month and two months followed by rams within the genotype A.

**III.** Phylogenetic tree analysis of *hsp70* genotypes in the Holstein Iraqi bulls, Arabian rams and genotypes of some animals and humans showed a strong and common convergence of the heat shock proteins of bulls and rams.