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Detection the AI dead sperms via florescent microscope with the investigation of DNA fragmentation by electrophoresis

KEYWORDS

Anmar Jasim Mohammed	Saad Akram Hatif
Veterinary med college /Baghdad	Veterinary med college Baghdad, Genetic and fertility

ABSTRACT *This study included the evaluation of semen samples in the artificial insemination center of Iraqi. Ejaculated semen was collected from thirteen mature Holstein bulls by artificial vagina , semen samples subjected to examination in the deferent stages of preparation , like collection , dilution , cooling and freezing point . Sperms stained by florescent dye (ethidium bromide) subsequent investigation of the sperm viability via florescent microscope , in addition the investigation of mid piece defect . Molecular inspection inclusive the genomic DNA extraction by (phenol – chloroform method) , total DNA product running in the agarose gel by electrophoresis to detect the DNA fragmentation . The feature of DNA fragmentation reinforce with the result of sperm colored (orange- reddish) of sperm head and , another defect in the midpiece , like swelling , thin , irregular , absent, and short .In conclusion , the manipulation of the AI semen preparation lead to decline sperms quality , increase sperms dead , and DNA fragmentation.*

Introduction

Artificial insemination is a technique that can help treat certain kinds of infertility in both human and animals .(AI) is the most valuable breeding management tool available to dairy cattle breeders to improve the genetic

potential and performance of their herds 1. During the production of sex-sorted spermatozoa from bull semen, the cells are exposed to a number of potential hazards including: dilution, centrifugation, incubation, exposure to DNA stains,these factors may affect the survival capacity and fertilization potential of the sperm 2 . Assessment of male fertility potential usually initiates with observing semen analysis values 3 . Apoptotic DNA fragmentation is a natural fragmentation that cells perform in apoptosis (programmed cell death) , DNA fragmentation is a biochemical hallmark of apoptosis .Apoptosis is mediated by proteolytic enzymes called caspases, which trigger cell death by cleaving specific proteins in the cytoplasm and nucleus 4 . It is also known as Caspase Activated Nuclease, DNA Fragmentation 5 .

Therefore this study included:

1-Evaluation the semen collection ,the concentration ,PH , volume , mass activity , individual motility before cooling , individual motility after cooling , and after freezing .
2-Ethidium bromide staining of the AI sperms
3- Florescent microscope examination sperms.
4- Extraction genomic DNA of sperms ,
5-Detection the DNA fragmentation by electrophoresis.

Materials and methods

Semen samples collected from thirteen Holstein bulls Intended to be used for Artificial insemination in the center of AI of Iraq . This solution prepared by dissolving 0.25 gm ethidium bromide in 50ml DW to get final concentration 5 mg/ ml 6 . Ethidium Bromide stain used for detection the dead sperms by staining the DNA in the head and midpiece .Floresent microscop used for examination and investigation florescent stained of DNA of sperms Genomic DNA was isolated from sperms in the semen samples under aseptic condition according to (phenol – chloroform method utilized for nucleic acid by lyses buffer solution (100 nM NaCl , 1 nM ETDA , 10 nM Tris-HCLpH8.0) with 5% SDS 7.

The solution for electrophoresis from Tris –acidic buffer 1x , casting of agarose gel 1%, Ethidium bromide ,and DNA loading & electrophoresis .Agarose gel electrophoresis used for analysis of DNA fragmentation . Assissing cell death by detecting DNA fragments using agarose gel electrophoresis 8 .

Results and discussion

This study was conducted on the semen samples were collected from 13 Holstein bulls in the artificial insemination center . All semen samples were evaluation at the semen concentration ,PH, volume, mass activity , individual motility before semen cooling , individual motility after semen cooling ,and individual motility after freezing at the maximum and minimum level . discarded the samples were evaluated less than 40% in **table 1** . The most commonly used cutoff for normal sperm motility is greater than or equal to 50% In other study men with motility over 45% and otherwise normal sperm numbers tend to be fertile. The World Health Organization's 5th edition of "normal semen analysis" values are shown 40% . 9 The low quality of sperms lead to low fertility and low the live birth rate High sperm DNA fragmentation in couples undergoing assisted reproduction techniques is associated with lower LBR 10. Therefore this study concentrated about the DNA fragmentation of sperms . by extracted the DNA related with the florescent dyes of chromosomal DNA .

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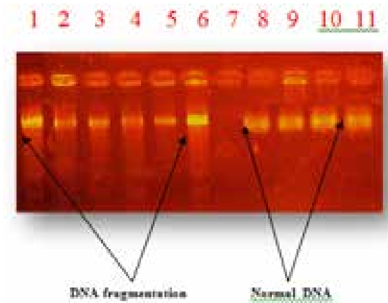
Table 1: Shows the semen evaluation concentration ,PH volume,mass activity, individual motility before cooling after cooling and after freezing .

Month	Type of test						
	Concentration m/ml	PH	volume	mass activity %	individual motility before cooling%	after cooling%	Individual motility (after freezing)%
First month	2117- 429,6	6.8 to 6.5	9- 5.5	60% - 20%	70% - 30%	60 -20	60- 20
Second month	1300 – 527.8	6.8 – 6.5	8.5 - 4	60-20	65-20	65-20	65-10
Third month	1501- 424.3	6.8-6.5	11-2.5	60-10	65-20	65-20	60-20
Fourth month	1500 - 429.6	6.8.6.6	11-3.5	55-40	70- 50	70-50	65-50

*The samples evaluated less than 40% discarded

Genomic DNA extracted running in the electrophoresis the samples were appeared the fragmentation extracted from the cooling and freezing semen sample **figure1** . while the normal bands looked in the semen samples were get from the semen collected and from the first steps of semen dilution . the results reflect the side effect of manipulation from the first steps of semen collection to the final step of storage . The reason dates back to the, tempreture , hard handiling , PH , humidity of environmental condition , it is the stress factor participate for liberate the radical oxygen species reason for damage of mitochondrial DNA with the enzyme of cells 11-12 and 13 in addition the second reason may be revealed to the effect of tissue culture media which is used in vitro fertilization 14. the defect led to apoptosis , the program of cell death corresponding with the loss plasma membrane permeability. Which is encourages the dye (ethidium bromide)introducing into cells and intercalating of chromosomal DNA with the staining if cell by using UV light Ethidium bromide will stain only cells that have lost membrane integrity 15. Sperms apoptosis revealed florescent staining by ethidium bromide , represented deferent cases collection , dilution ,cooling and freezing in **figure 2**

The result gives the variation in the sperms viability in the different stages of semen preparations , some defect take place in the mid piece , included mitochondrial DNA . It has been suggested that insufficient mtDNA repair, and the subsequent accumulation of mutations in the mitochondrial genome, can lead to mitochondrial dysfunction and cell death 16. The electrophoreses revealed the feature of DNA fragmentation reinforce with the result of sperm colored (orange- reddish) of sperm head by ethidium bromide .Nucleic acids that have been subjected to electrophoreses through agarose gels maybe detected by staining and visualized by illumination with UV light 17 .In conclusion , the manipulation of the AI semen preparation lead to decline sperms quality , decrease alive sperms ,increase the percent of dead sperm , and DNA fragmentation of dead sperm .



DNA fragmentation **Normal DNA**

Figure 1: Chromosomal DNA bands on 1% agarose gel after one hour electrophoresis at 50 volt shows the DNA fragmentation in lane (1 to 6) and normal DNA in lane (8 to 11) .

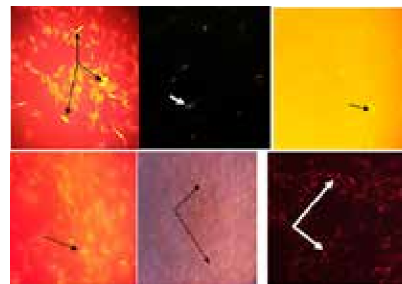


Figure 2: Shows sperms by fluorescence microscope, ethidium bromide (EB) staining is used to visualize nuclear changes are characteristic of apoptosis, deferent sperms apoptosis revealed deferent cases collection , dilution ,cooling and freezing .

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