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CASE REPORT / OLGU SUNUMU

DNA damage of cumulus cells in a cow with brucellosis

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SUMMARY

The purpose of this study was to determine the effect of *Brucellosis* on DNA integrity in cow cumulus cells by comet assay. The DNA damage was determined using by comet assay method in cumulus cells separated from Grade A and B oocytes aspirated from the ovaries of a Hollstein cow referred to local abattoir for conditional slaughtering. The DNA damage in cumulus cells seperated from Grade A and B oocytes rates were detected as 59% and 47%, respectively. In conclusion that the presence of *B.abortus infection in a cow* was concerned to be common DNA damage in cumulus cell.

Key Words: Comet assay, cow, cumulus cell, DNA damage

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Brusella tanısı konmuş bir inekte kumulus hücrelerinde DNA hasarı olgusu

ÖZET

Sunulan çalışmada Brusella tanısı konmuş ve şartlı kesim için mezbahaya getirilmiş olan Holstein ırkı bir inekten alınan ovaryumlardan aspirasyon metodu ile toplanan A ve B kalite oositlere ait kumulus hücrelerinde Comet assay yöntemi ile DNA düzeyindeki hasarın belirlenmesi amaçlandı. Sonuç olarak A ve B kalite cumulus hücrelerinde sırasıyla %59 ve %47 oranlarında DNA hasarı tespit edildi. Sonuç olarak Brusellozis'li bir inekte kumulus hücrelerinde DNA hasarının yaygın olduğu kanaatine varıldı.

Anahtar kelimeler: Comet assay, inek, kumulus hücresi, DNA hasarı

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INTRODUCTION

Brucellosis is an economically important disease of cow caused by the gram-negative bacterium *Brucella abortus* and an important zoonosis in worldwide. Infection occurs in cow of all ages but is most common in sexually mature animals, especially dairy cows. *B. abortus* has a tropism for the pregnant uterus, udder, testicle and accessory male sex glands, lymph nodes, joint capsules, and bursae.[1] Late gestation abortion is the predominant clinical sign of *B. abortus* infection in cows resulting in reproductive failure and a decrease in milk production.[1,2]

Cumulus cells play a pivotal role in oocyte maturation and maintenance. In primary follicles which contain growing oocytes, the surrounding granulosa cells start to proliferate and can be distinguished as two specific populations during antrum formation: (a) cumulus granulosa cells, which enclose the oocyte with the corona cells as the innermost layers; and (b) mural granulosa cells which line the follicular wall.[3,4] In developing follicles, these cells are coupled to the growing oocyte through gap junctions as a functional syncytium that facilitates the transfer of signals as well as nutrients into and out of the oocyte and between follicle cells.[5,6] Cumulus cells secrete and assemble extracellular matrix molecules essential for ovulation and subsequent fertilization.[7]

Reactive oxygen species (ROS) may have a regulatory role in oocyte maturation, folliculogenesis, ovarian steroidogenesis and luteolysis. There is a delicate balance between ROS and antioxidant enzymes in the ovarian tissues. The antioxidant enzymes neutralize ROS production and protect the oocyte and embryo.[8]

The single cell gel electrophoresis or Comet assay is a sensitive, reliable and rapid method for DNA double- and single-strand breaks, alkali-labile sites and delayed repair site detection in eukaryotic individual cells.[9] There are several studies[10,11] show that the effects of infections and heat stress using Comet assay method, but there is no report the effect of Brucellosis on the cumulus cells. The aim of this study was to determine the effect of *B. abortus* infection on DNA integrity in cow cumulus cells by comet assay.

CASE HISTORY

The material of this case report was collected from the ovaries of a cow infected with B. abortus slaughtered at a local abattoir. The diagnosis of B. abortus was made by the complement fixation test (CFT), rose Bengal plate test (RBPT) and standard tube agglutination test (STAT). The STAT test gave positive result at a titre of 1/1280. The oocytes were aspirated from ovaries using 20 G needle connected to a 10 ml sterile syringe. Follicular contents were transferred in to 60 mm glassed petri dish. Oocytes were collected and evaluated under stereomicroscope. The oocytes were classified on the basis of their morphological appearance: Grade A (oocyte with more than 4 layers of cumulus cells and homogenous cytoplasm); Grade B (oocyte with completely or partially 2-4 layers of cumulus cells and homogenous cytoplasm) and Grade C (denuded oocyte with non-homogenous cytoplasm).[12] The cumulus cells were separated from the immature oocytes of Grade A and Grade B by mechanical method and pipetted into the phosphate buffer saline (PBS). All the chemicals were purchased from Sigma Chemical Co. (St. Louis, MO, USA) unless indicated otherwise.

A Comet assay reagent kit for single cell gel electrophoresis (Trevigen, Inc., Gaithersburg, MD) was used. The alkaline comet assay protocol as described by Trevigen was employed. The protocol is a modification of the method originally described by Singh et al.[13] After the last washing step of cumulus cells were carefully suspended 70 µl 1% low melting point agarose in PBS kept at 37°C, and were rapidly pipetted on to comet slide and allowed to solidify under a 24x60 mm coverslip at 4°C until subsequent use.

The slides were removed from the lysing solution and placed in a horizontal gel electrophoresis tank containing fresh 4 °C alkaline solution (300 mM NaOH, 1 mM Na_2-EDTA, pH10). The DNA was left to unwind for 20 min. before electrophoresis. Electrophoresis was conducted for 20 min. using 25 V and 300 mA current in an ice cold tank. After electrophoresis the slides were drained and neutralized with 3-5 min. washing steps with neutralization buffer (0.4 M Tris–HCl, pH7.5) and stained with ethidium-bromide in PBS. The

slides were covered with a coverslip placed in a humidified airtight container to prevent drying of the gel and were visualized in fluorescence microscope.

The images of 100 randomly chosen nuclei were analyzed visually. Observations were made at magnification 400× using a fluorescent microscope (Olympus, Japan). Each image was classified according to intensity of the fluorescence in the comet tail and given a value of 0 (all undamaged), 1, 2, 3 or 4 (all damaged), so that the total score of the slide could be between 0 and 400 arbitrary units (AU) (Figure 1A, B).[14]

Total comet score of cumulus cells separated from Grade A and Grade B oocytes was 236 AU (59%) and 188 AU (47%), respectively.

DISCUSSION

The purpose of this study was to determine the effect of acute phase *B. abortus* infection on DNA integrity of cumulus cells by comet assay. *Brucella abortus* was typical of the original pathogens selected for the study in embryo pathogen research why it was subject to national control and eradication programs in cow and because the organism was found in the reproductive tract.[15] Sparling and Springfellow[16] were reported that the probability of Brucella persisting in the uterus of normal cycling cow was further tested by culturing cervical mucus twice weekly from 16 artificially infected heifers that were bacteremic during the period of sampling. Brucella was only isolated from the heifer in bacteremic phase of infections. Accumulated evidence indicates that exposure of preimplantation embryos to *B. abortus* in the uteri of superovulated, infected cows are unlikely. Addittionally, it has been shown that embryo-washing procedures insure freedom from *B. abortus* even without antibiotics.[17,18] However, there were no reports about the effect of B. abortus infection on bovine cumulus cells. In the present case report, it was detected that acut phase *B. abortus* infection affected negatively the DNA integrity of cumulus cells.

Success in Assisted Reproductive Techniques (ART) are influenced by gamete and embryo quality but the assessment of these parameters has been thwarted by the lack of reliable biomarkers. Follicular fluid and cumulus oophorus cells may provide biomarkers due to their close relationship to the oocyte. These cells produce antioxidants and thus protect the oocyte from oxidative damage exerted by ROS. Which are important molecules resulting from normal cell metabolism that may be either harmful or beneficial to living systems. At low or moderate concentrations they are important for many physiological processes, but in conditions of oxidative stress. ROS can damage cellular lipids, proteins or DNA, affecting their normal function and implicating them in various human pathologies as well as in the normal processes associated with ageing.[19] In healthy aerobes, various intracellular antioxidant compounds oppose the damaging actions of ROS. In the female reproductive system, ROS and antioxidants perform physiological roles during folliculogenesis, oocyte maturation, luteal regression and fertilization.[8] Enhancing of ROS production in granulosa cells and an increase in the marker of DNA oxidative damage, 8-hydroxy-20-deoxyguanosine in granulosa and COCs, were associated with lower oocyte fertilization ability, lower embryo quality and reduced implantation success.[20,21] Furthermore, oxidative stress also appears to be associated with the pathology of reproduction and development, as is the case for endometriosis,[22-25] and Beytut and Kamiloğlu[26] reported that B. abortus infection induced oxidative stress was reduced the vitamin E, A and β-carotene levels and GSH-Pxactivity in the erythrocytes of cow. Serefhanoğlu et al.[27] reported that oxidants were increased and antioxidants were decreased in patients with brucellosis. In the present study, our result was showed that the *B. abortus* affected the DNA integrity of cumulus cells dramatically.

In conclusion that the presence of *B.abortus* infection in cow was concerned to be common DNA damage in cumulus cell. However, the results between cumulus cells separated from Grade A and Grade B were similar.

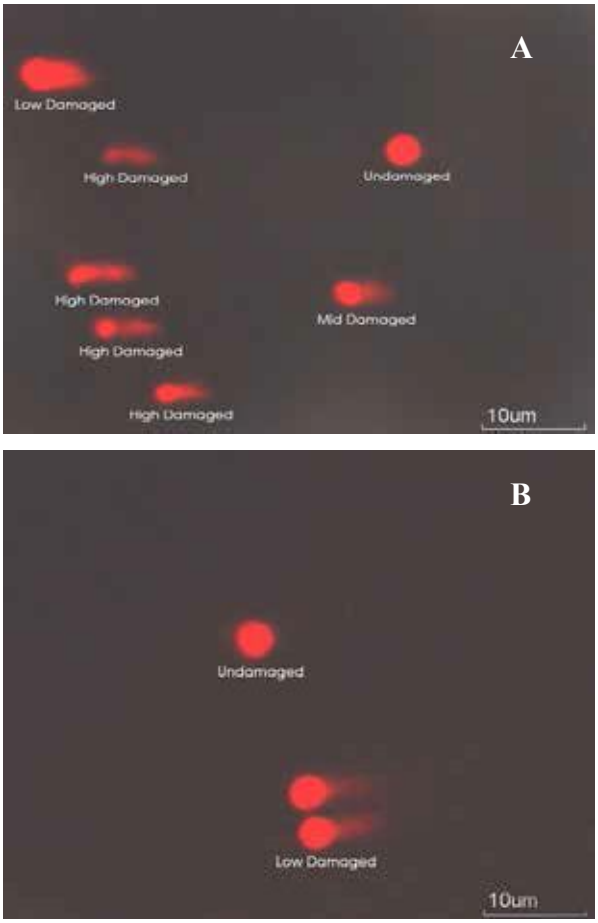

Figure: In A and B, different types of DNA damage in cumulus cells

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