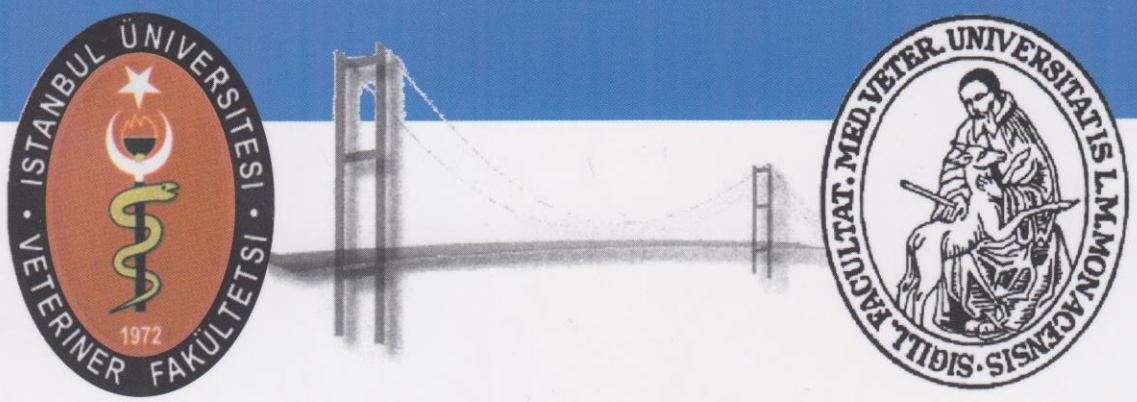

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The effects of high temperatures thawing techniques and cold shock on spermatological traits in bull semen

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Objectives: Storage of bull semen in a container is damaged due to different environmental temperatures during post-thawing processes. It was confirmed that bull semen are damaged from these processes when exposed to 12°C or low environmental temperatures post-thawing in field conditions. However, there isn't an agreement on how much the stated cold shock is affected after different post-thawing temperatures. In this study, it is aimed to develop a thawing technique that is more resistant to cold shocks and practicable in field conditions.

Materials and Methods: In this study, four Holstein bulls were used to frozen semen in 0.25 ml. straws. Semen of each bulls were thawed in 45 seconds at 37°C (group A=Control group), in 15 seconds at 50°C (group B) and 5 seconds at 70°C (group C) and post-thawing cold shock (300 seconds at 5°C) was applied to the control group. After spermatozoa motility and morphological examinations are performed sperm samples were incubated at 35°C for 120 minutes and spermatological traits were repeated. Semen samples from the control and treatment groups were placed in an incubator taking into consideration the medium conditions (Modified buffered Hepes medium) and the time needed for spermatozoa to reach the fertilization site. Motility and morphological defects were determined comparatively with phase contrast microscopy and sperm fertility analyzer (SFA-500). The Hancock solution was used for morphologic examination of spermatozoa (acrosome, other and total). In Computer aided sperm fertility analyzer, motility and movement were performed in according to the technique.

Results: No significant difference was observed between group A (control) and C, with respect to both motility and morphological abnormalities, cold shock and after the incubation. With respect to acrosomal and total morphological defects rate in group B, a significant increase was observed in comparison with the group A and C.

Conclusion: Artificial insemination procedure should begin as soon as possible after completion of thawing process. The shorter thawing technique in 5 seconds 70°C can be used alternatively especially lower environmental temperatures.

Key words: Bull, semen freezing, post-thaw, temperature, sperm traits.

Improved enzyme-immunoassay for the detection of cephapirin in milk

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Cephapirin is bactericidal, broad-spectrum, first-generation cephalosporin antibiotic, which is administered to dairy cows as a veterinary drug for intrauterine treatment and at the beginning of dry period for metaphylaxis of udder inflammation. Cephapirin can cause allergic reactions in beta-lactam sensitized persons, may promote the development of pathogenic bacteria resistant to antibiotics and, more important for dairies, interfere with manufacturing of dairy products by inhibition of bacterial starter cultures. Thus, an efficient control system for cephapirin residues in milk is essential in aspects both of public health and of good dairy practice.

Within the EU the maximum residue limit (MRL) for cephapirin in milk has been set at 60 µg/kg, the corresponding US value 'tolerance level', specified by the FDA, is at 20µg/kg. For efficient control of milk for residues, enzyme immunoassays (EIA) are suitable routine techniques. Monoclonal antibodies (mAbs) for the detection of cephalosporin have recently been described (1) but the sensitivity of the cephapirin assay (IC_{50} 40µg/L) was not sufficient to reliably detect this antibiotic at least at the FDA level. A further approach resulted in the development of mAbs with higher affinity. These antibodies were used in this study for the establishment of an improved EIA.

To optimize the EIA for the analysis of cephapirin in milk samples, different dilution buffers were tested. Best results were obtained by using antibiotic-free UHT milk (< 0.3 % fat content) as diluent both for the preparation of the standard curves and for dilution of the defatted samples. Under optimized EIA conditions, the detection limit was 5.1 µg cephapirin/L thus enabling the specific and sensitive detection of cephapirin in milk at the FDA level. The mean recovery rate for cephapirin in artificially contaminated milk samples was 86.7% with an average coefficient of variation of 10.9%. Thus, the improved cephapirin EIA is able to detect the antibiotic in milk with high accuracy and precision.

(1) Bremus A., R. Dietrich, L. Dettmar, E. Usleber & E. Märtlbauer (2012): A broadly applicable approach to prepare monoclonal anti-cephalosporin antibodies for immunochemical residue determination in milk. Anal. Bioanal. Chem. 403:503–515.