VÄLISAUTORID

THE USE OF SEMEN FOR TESTING THE TOXICITY OF MATERIALS

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All new batches of materials used for manufacture of straws, inseminating sheaths and instruments that are in contact with semen should be tested for the toxicity to spermatoza. The reagents used for evaluation of semen may affect physiological responses of semen and give erroneous results.

Introduction

The progressive method of semen packaging into reed and paraffin paper tubes was first suggested by V. Milovanov in 1936 (Milovanov, 1938). Later, E. Sorensen (Pakėnas, 1993) in Denmark and R. Cassou (Cassou, 1950) in France applied this method by changing natural straws for synthetic ones. Various kinds of plastic are used for manufacture of straws depending on the semen freezing technique. For example, E. Sorensen, R. Cassou and P. Pakėnas used, respectively, cellulose acetate (Milovanov, 1972), polyvinyl chloride (Cassou, 1950) and polystyrene (Ginkevičius, 1976; Pakėnas, Ginkevičius, 1980). Each kind of plastic was adapted to a specific semen freezing technique. It has been observed that the use of new batches of polypropylene of the same kind for the manufacture of 0.25 cm^3 straws often resulted in lower physiological responses of semen after its freezing. Thus, it became evident that each batch polypropylene should be tested for its toxicity as regards spermatozoa. Most of chemicals and antibiotics used for semen dilution are toxic to spermatozoa (Balašov, 1980). This is most important when the main semen quality indicators – postthaw motility and survival time of spermatozoa are determined (Milovanov, 1938; Pakėnas, 1993). It is considered that there is a positive correlation between postthaw motility and viability of spermatozoa and fertility of semen (Kurbatov, 1988). The exactness of the physiological parameters of semen may be highly dependent on the material used for the manufacture of straws and the reagents used for semen freezing and evaluation (Instruktsija…, 1981), however, semen quality should always meet certain minimum requirements (Kurbatov, 1988).

The present study was designed to determine the effects of straws, inseminating sheaths and sodium citrate on physiological responses of semen.

Materials and Methods

The study was conducted at the Animal Reproduction Laboratory of the Lithuanian Institute of Animal Science in 1999. Spermoatozoa motility was determined in scale numbers (10 scale numbers for 100% of spermatozoa with progressive motility) visually using a hot stage microscope. The survival time (r) and absolute survival rate (S) of spermatozoa were determined at 38±0.5 and 4±0.5 °C temperatures. In order to determine spermatozoa motility, a thaved straw was wiped with a cheesecloth napkin, the ends of the straw were cut down with sharp scissors and the contents of the straw was emptied into a glass bottle containing 0.75 cm^3 of 2.9% sodium citrate solution warmed up to 38±0.5 °C. After the initial evaluation of the spermatozoa motility, the test-tubes with semen were corked up, placed into a water thermostat and evaluated repeatedly in 5 hours. It is possible to determine spermatozoa motility by incubation of the straw filled with semen at 38±0.5 °C for 5 hours, however, 2.9% sodium citrate solution should be used for evaluation and motility should be determined using the whole contents of the straw (Pileckas, 1997).

Both fresh and frozen bovine semen was used in the study.

The toxicity of straws to spermatozoa was determined by filling up the straws made from the new batch of polypropylene with thawed semen. Both ends of the filled up straws were sealed with glass balls. A certain amount of semen was incubated in glass tubes. 1 cm^3 of thawed semen was poured into the glass tubes and 150 mg of cut to pieces straws were added into each of the tubes. Then the semen was stored at 38±0.5 °C for 15 minutes, and afterwards the initial motility of spermatozoa determined.

The toxicity of inseminating sheaths to spermatozoa was determined by mixing the collected bovine ejaculates and diluting them with a lactose extender* at a rate of 1:10. 5 ml of diluted semen (control) was poured into each tube and 150 mg of cut to pieces sheaths were added into each tube. 5 cm^3 diluted semen was packaged into each inseminating sheath the ends of which were sealed.

Four batches of sodium citrate delivered by different suppliers were tested by preparing 2.9% sodium citrate solutions. Bovine semen diluted with this solution (0.75 cm^3 of natrium citrate and 0.25 cm^3 of semen) was incubated at 38±0.5 °C for 5 hours. The toxicity of sodium citrate to spermatozoa was tested by determining postthaw motility, survival time (r) and absolute survival rate (S) of spermatozoa.

Results and Discussion

The study indicated the necessity of testing plastics for toxicity each time a new batch of polypropylene used for straw manufacture is delivered (Fig. 1). Semen incubation at 38±0.5 °C for 15 minutes had no effect on the initial motility of spermatozoa when the semen was poured into the tested straws that were sealed with glass balls. Semen incubation together with cut to pieces polypropylene straws reduced spermatozoa motility by 10.2% in comparison with the control sample and semen incubated in the tested straws sealed with glass balls. Motility of spermatozoa was, respectively, by 73.7 ($P<0.001$) and 68.4% ($P<0.001$) lower both if the semen was incubated in the tested straws for 5 hours at 38±0.5 °C and if it was incubated in the tubes together with the cut to pieces straws in comparison with the control sample.

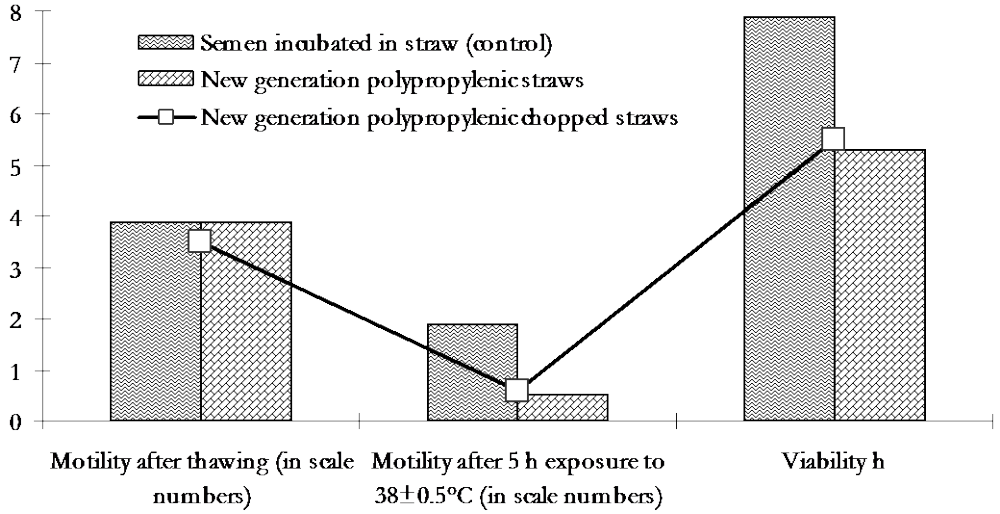

Fig. 1. Straw toxicity to spermatozoa using frozen semen

The difference in spermatozoa motility after 5 h incubation at 38±0.5 °C was insignificant for both tested samples and made up, respectively, 0.5±0.16 and 0.6±0.09 scale numbers.

The survival time of spermatozoa in the control sample was 7.9±0.25 h, in the tested straw 5.3±0.19 h and in the sample with cut to pieces straws 5.5±0 h, and these values were, respectively, by 32.9 ($P<0.001$) and 30.3% ($P<0.001$) lower in comparison with the control sample.

Fresh semen was used to determine the toxicity of sheaths. 5 cm^3 of diluted semen was packaged into inseminating sheaths and sealed.

Some more semen was incubated together with the cut to pieces sheath (150 mg of polypropylene and 5 cm^3 of semen diluted with lactose extender). The third part of semen was used as the control sample. The initial motility of spermatozoa after incubation at 38±0.5 °C for 15 minutes was not affected and amounted to 7±0 scale numbers for all samples. Spermatozoa motility in the sealed sheath after 5 h incubation at 38±0.5 °C was 4.5±0.54 scale numbers, i.e. it was by 15.1% lower in comparison with the control sample and semen incubated together with the cut to pieces polypropylene (Fig. 2). The absolute survival rate was by 10.8 and 13.2% lower in comparison with the control sample, however, the differences were statistically insignificant.

* 11.5 g lactose, 5 cm^3 glycerol, 20 cm^3 egg yolk, 100 cm^3 bidistilled water.

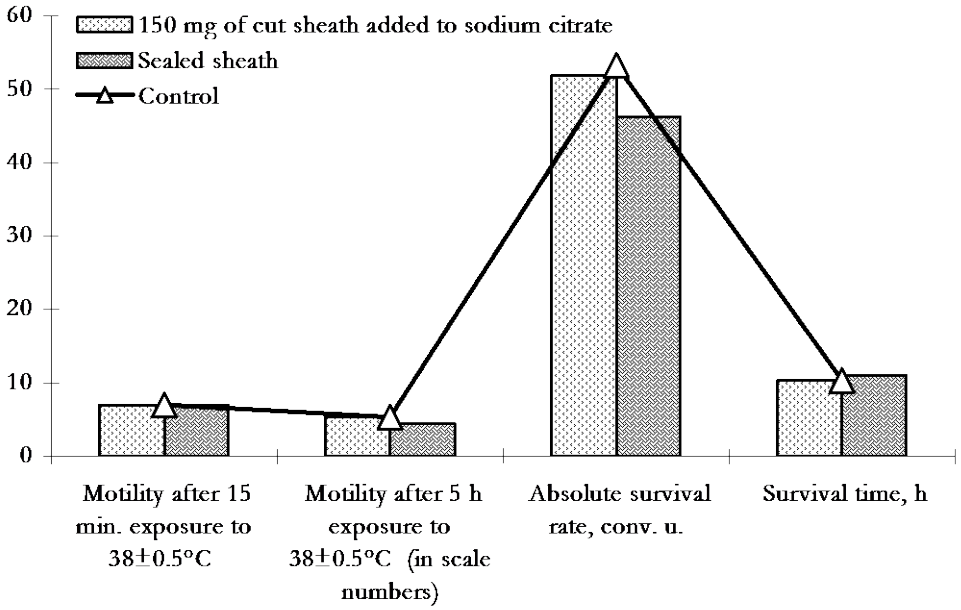

Fig. 2. Determination of material toxicity using fresh semen at 38±0.5 °C

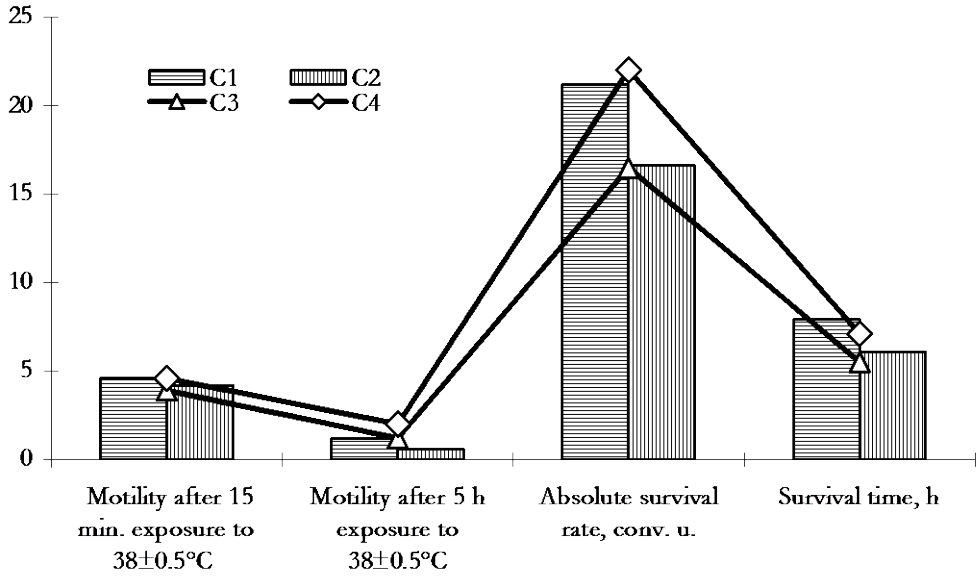

Fig. 3. Physiological indicators of frozen bovine semen on using different batches of sodium citrate for sample preparation

The toxicity of sodium citrate to spermatozoa was tested by using four batches of sodium citrate that were numbered C1, C2, C3 and C4 (Fig. 3). The testing results indicated that postthaw spermatozoa motility was similar for batches C1 and C2 if the semen was evaluated in 15 minutes after incubation. However, if the semen was evaluated after 5 hour incubation at 38±0.5 °C, spermatozoa motility using sodium citrate C4 was by 40% ($P<0.001$) and 70% ($P<0.001$) higher than that for batches C1 and C2, respectively, and was equal to that of batch C3. The absolute survival rate of spermatozoa amounted to 21.2±0.89 conv. units for batch C1, 16.6±0.39 conv. units for batch C2, 16.5±0.79 conv. units for batch C3 and 22.0±1.07 conv. units for batch C4. The survival time of spermatozoa amounted to 7.9±0.21, 6.1±0.16, 5.5±0.0 and 7.1±0.22 hours, respectively. Testing ejaculates of the same bull indicated that separate batches of sodium citrate had influenced the exactness of the results which might be decisive when physiological responses of semen are at issue.

Conclusions

The toxicity of straws was revealed when semen was incubated at 38±0.5 °C for 5 hours. Testing of the toxicity of the material to spermatozoa may be carried out both by incubation of the semen in the straw and by adding the cut to pieces straw to the semen.

New batches of the same polypropylene used for manufacture of straws and sheaths should be tested for their toxicity to spermatozoa. Sodium citrate should also be tested for toxicity, because otherwise semen evaluation may give erroneous results that reflect not the quality of semen but that of sodium citrate.

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