

The Integrity of Frozen Spermatozoa

Proceedings of a Round-Table Conference

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This report has been reviewed by a group other than the authors according to procedures approved by a Report Review Committee consisting of members of the National Academy of Sciences, the National Academy of Engineering, and the Institute of Medicine.

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PREFACE

For the past few years the U.S. National Committee for the International Institute of Refrigeration has been conducting short conferences devoted to the presentation and discussion of research papers in the field of low-temperature phenomena as they relate to the life sciences and the application of the results. At these conferences the presentation of each paper is followed by a discussion period during which the conferees comment on the presented paper or offer additional information. As a service to the scientific community and the general public the National Academy of Sciences is publishing the papers and the pertinent discussions. It should be noted, however, that neither the National Academy of Sciences, the U.S. National Committee, nor the conferees approve or disapprove opinions or results presented in this publication.

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INTRODUCTION

Bernard LaSalle

Semen preservation includes selection of donors, collection, addition of cryoprotectant, stabilizer, diluent and preservatives, freezing, thawing, macroscopic and microscopic examinations, microbiological tests, immunobiological tests, genetic tests, insemination, and evaluation of fertility and offspring.

It is interesting to recall that artificial insemination was first practiced in horses by the Arabs, in the fourteenth century. In the last half of the eighteenth century, Jacobi, Spallanzani, and Rossi experimented on fish, frogs, silkworms, and dogs. In the nineteenth century, several papers on artificial insemination were published by gynecologists. It is only since the end of the nineteenth century, and the beginning of the twentieth century, that the technique of artificial insemination was developed and extensively applied to livestock.

Russian scientists, led by Ivanov, contributed greatly to the improvement of the livestock industry in their country, through artificial insemination. They were the first, in 1907, to begin writing extensively on its application in horses, cattle, sheep, swine, fowl, furbearers, and wild animals. Scientists of Europe, America, and Asia studied and pursued the Russians' work.

Interesting reviews on the subject of this conference may be found in

- *The Veterinary Record*,
- *The Technique of Artificial Insemination*, Imperial Bureau of Animal Genetics, 1933
- *Revue Internationale d'Agriculture*, 1934
- *Recueil de Medecine Veterinaire*, 1935
- *Germ Plasm Resources*, American Association for the Advancement of Science, 1961

J. K. Sherman presented "State of the Art of Banking Frozen Human Semen" at our 1972 USNCIIR round-table conference, "A Review of Problems Associated with Biological Integrity at Low Temperatures." From the beginning of artificial insemination in the fourteenth century, through the first three decades of the twentieth century, semen was generally preserved at body temperature, and protected against cold shock, for use immediately or within one-half hour after collection. The need for improving semen preservation was obvious.

The Russian workers developed and used buffered sugar solutions as diluents, to increase the volume and prolong the life of semen. These diluents preserved semen for a few days at room temperature, but did not protect spermatozoa against cold shock. In 1932, 560,000 mares, 2,000,000 cows, 3,000,000 ewes, and 200,000 sows were artificially inseminated in Russia. The practice was then generally nonexistent or only emerging in most other countries.

To transport semen to our widely distant farms, the American workers developed egg-yolk-phosphate buffer-citrate diluents, which made it possible to slowly cool diluted semen from body temperature to 7°C in 90 min. and to stop motility and reduce the loss of fertility to 5 percent daily for 3 or 4 days.

Ivanov reported, in 1923, "Experiments on the Disinfection of Sperm in Mammals, Especially in Relation to Dourine in Horses." He mixed infected semen with salvarsan (arsphenamine) and neosalvarsan (neoarsphenamine), 1:10,000, to kill *Trypanosoma equiperdum*, without affecting fertility.

The American scientists pioneered the use of antibacterial agents, such as sulfanilamide, penicillin, and streptomycin. They also pioneered, in their own way, the use of diluents containing glycerol, glycine, sugars, milk, whole egg, skim milk, buttermilk, dried milk, CO₂, dimethyl sulfoxide, ethylene glycol, and others.

Although attempts to preserve semen by freezing were made toward the end of the nineteenth century, they were not successful until the late thirties and early forties of this century, when only human semen was found to survive freezing at the low temperatures of liquid gases. Semen freezing became routine after the breakthrough of the French and British scientists who discovered the protective effects of glycerol. Their initial work with frog and fowl semen was soon extended to semen of other animal species including man.

Pretreatment of bovine semen with glycerol, slow cooling, freezing, and storage in liquid nitrogen at -196°C has since been used extensively and routinely, with great success. Several breakthroughs have been reported for the preservation of the fertility of boar semen through freezing and thawing. All this has made possible great progress in livestock improvement by increasing several thousandfold the potential progeny of better-selected sires or donors and by allowing ample time for extensive evaluation and testing for genetic qualities, freedom of genetic defects and agents of infectious diseases, for international transport, and for long-range breeding programs.

The history of semen preservation, the current literature, and this conference prompt me to share with you the expectation that greater emphasis will be placed on:

- Selection and care of donors
- Long-range breeding and livestock breeding programs
- Testing donors for all diseases common to the species
- Aseptic collection and handling of semen
- Testing semen for all known pathogens and undesirable contaminants
- Improved cryoprotective diluents and cryogenic preservation
- Addition of antibacterial, antifungal, antimycoplasmal, and other preservatives
- Cleaning, disinfection, and sterilization of all facilities, equipment, instruments, containers, freezers, and liquid nitrogen tanks.
- Sterile filtration of liquid nitrogen
- Recordkeeping
- Regulations
- Other precautions to prevent the use of contaminated, infectious, dangerous, or worthless semen.