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شبكة المعلومات الجامعية

بسم اللّه الرحمن الرحيم

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التوثيق الالكتروني والميكروفيلم

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التوثيق الإلكتروني والميكروفيلم

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بالرسالة صفحات
لم ترد بالأصل

DILUTION AND FREEZING OF OVINE SEMEN

By

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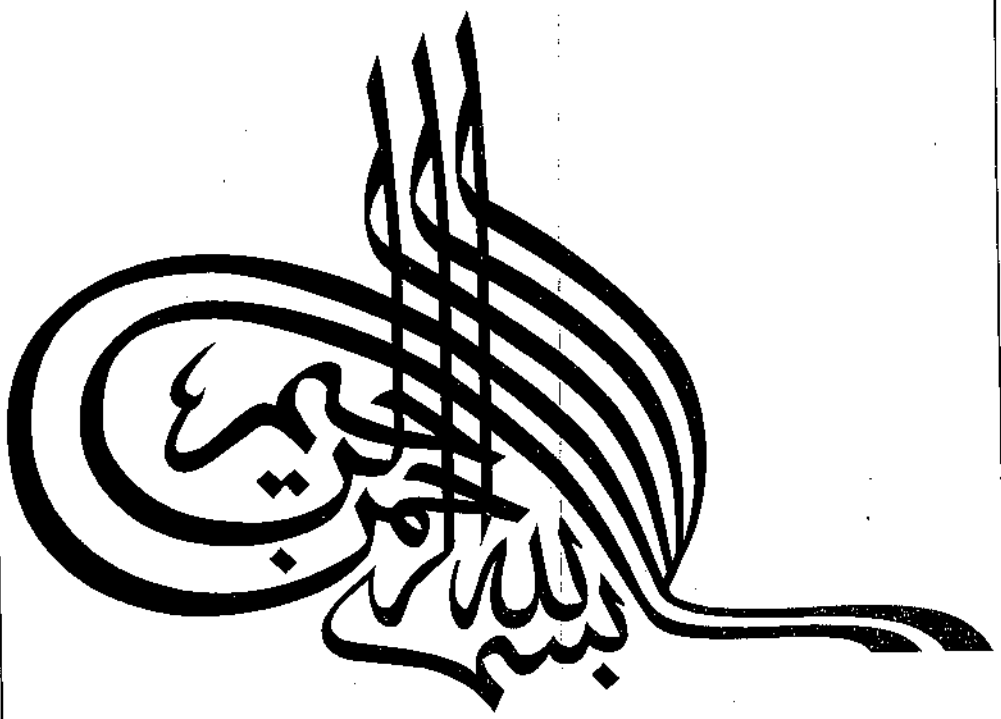
بسم الله الرحمن الرحيم

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INTRODUCTION

Genetic improvement in local sheep population represents the main and most important step for achieving satisfactory and economically efficient keeping of them. In the meantime, successful manipulation of artificial insemination (A.I) would accelerate the genetic improvement. In order to realize full potential of A.I in sheep, it depends largely on applying successful use of frozen semen, which is unfortunately still being beyond optimum. A prerequisite for appropriate technique for freezing of ram semen is finding an extender, which would maintain the motility of spermatozoa through out the various steps of semen processing (e.g., semen extension, cooling, equilibration, freezing and thawing).

Several factors must be taken into consideration in order to develop an appropriate procedure for freezing semen such as selecting the most convenient extender, as well as the proper cooling rate, egg yolk and glycerol concentrations, time and method of addition of the diluents, package, freezing and thawing conditions. Other general requirements are including ionic and non ionic substances to maintain the osmolarity and buffering capacity of the medium, existence of a source of lipoprotein or high molecular weight material to prevent cold shock such as egg yolk or milk. Furthermore, glycerol, propane-diol or DMSO would be also involved to offer cryoprotection, fructose or glucose as an energy source, and other additives such as enzymes and antibiotics (Vishwanath and Shannon, 2000).

The present work is aiming at studying the effect of five different extenders on freezability of ram spermatozoa and to develop

the protocol of processing technique involving method of diluent's addition, temperature at adding the diluents, glycerol level, cooling rate, equilibration period, freezing rate and thawing rate in order to increase the post-thawing recovery rate of ram spermatozoa.

REVIEW OF LITERATURE

The important factor influencing frozen storage of semen is the composition of the medium used for dilution of semen before freezing (Salamon and Maxwell, 2000). The seminal plasma alone provides only a very limited protection for spermatozoa against changes in temperature. Successful diluent is usually composed of different substances, which provide:

1. Proper *p*H and buffering capacity (buffer media).
2. Cryoprotectant agents against cryogenic injuries.
3. Nutrient and energy sources.
4. Antibiotics are commonly added to the diluents.

Data available in the literature indicate that several and serious trials have been already conducted during the 20th century in order to develop a successful preservation of mammalian spermatozoa under artificial conditions for extended period of time. Preservation of the spermatozoa frozen in the liquid nitrogen (at –196°C) aims mainly at reducing and arresting the metabolism of spermatozoa and thereby prolongs their fertile life. This technique becomes now the most applicable method, however, the post–thaw revival of the spermatozoa is still unsatisfactory, especially for ram spermatozoa. The following is a brief description of the most important efforts paid in this respect.

I – Main ram semen extenders:

Yashida (2000) stated that the sperm is a highly polarized and specialized cell with tripartite structure of head, mid-piece and tail. It

has lost the ability of biosynthesis, repair, growth and cell division during the last stage of spermatogenesis.

Extensive review concerning the improvement in reproductive technology, developments of semen dilution and conservation of mammalian spermatozoa is available in many publications (Hammerstedt *et al*, 1990; Foote and Parks, 1993; Maxwell and Salamon, 1993; Watson, 1995; Salamon and Maxwell, 1995, Royere *et al*, 1996; Holt, 1997; Vishwanath and Shannon, 2000; Woelders, 1997 and Foote, 1998). It clearly appears that the investigations with ram spermatozoa are comparatively less than that conducted for bull spermatozoa. In general, Salamon and Maxwell (1995) grouped the ram semen extenders on basis of chronological use or development into:

1. Citrate-sugar based extenders.
2. Milk extenders.
3. Di- and tri- saccharides–based extenders.
4. Tris–(organic) based extenders.
5. Other extenders.

1. **Citrate-sugar based extenders**

This means that the early tested ram extenders were based on utilization of citrate buffer combined with monosaccharide (glucose). In fact, this was due to the great success of such extenders for freezing of bull spermatozoa at that time. With adaptation of such extenders for freezing of ram spermatozoa the results had some limitations and most have been modified later (Salamon and Maxwell, 1995). First of all, opinions differed on the type of sugar to be used in the citrate media. Some investigators used 0.9–1.25% arabinose (Markovič, 1956 and