Comparison of Two Diluents for Freezing Semen of Local and F₁ Goats

ABSTRACT

Semen from 3 katjang and 10 F₁ (katjang female × German Fawn male) bucks were frozen using tris-citric-acid-yolk and skim milk diluent. Neat, post thaw and post thaw stress percent motility of sperms in the semen with skim milk diluent was found to be slightly better than the tris-citric-acid-yolk diluent although this difference was not statistically significant (P < 0.05).

INTRODUCTION

Corteel et al. (1982) has reviewed the work of different researchers on the use of different diluents for processing and freezing of goat semen, and recommended pre-washing before dilution of semen. The present report compares the efficiency of two methods used for diluting semen prior to freezing with regard to sperm motility in local and F₁ (local ♀ × German Fawn ♂) bucks.

MATERIALS AND METHODS

Ten F₁ bucks, with ages ranging from 324 to 702 days at the initiation of the trial, and three local males with maturated sets of teeth but of unknown age, were selected from the nucleus herd of the University of Malaya. These males were subsequently trained to serve an artificial vagina by putting an oestral doe in a specially constructed holding crate next to a fence. Two ejaculates were collected from each buck for each of the two treatments of washing and dilution. The first treatment consisted of both washing and dilution of semen by using tris-citric acid-egg yolk buffer. Tris-citrate buffer solution for washing and skim milk extender for diluting the semen were used in the second treatment (Nelson and Lin, 1983).

A. Procedure for preparing tris-citrate buffer

The basic tris-citrate buffer solution may be prepared once a week, and stored at 5°C. Constituents of buffer are:

- Tris 3.3190g
- Citric acid 1.8210g
- Dextrose 1.0000g
- Streptomycin 0.5000g
- Penicillin 50,000 I.U.

Double distilled water is added to produce 100ml of tris-citrate acid buffer. This solution should have a pH of 7.20 and an osmolality of 323m Osmol/l. The pH and osmolality should be checked and adjusted before the complete diluent is to be used.

B. Tris-citric acid egg yolk extender

- Tris citric acid buffer 76%
- Yolk 20%
- Glycerol 4%
The egg yolk should be obtained from fresh eggs (less than 24 hours old). The intact yolk may be rolled on a clean paper towel, then punctured to release the yolk. Care should be taken not to contaminate the egg yolk with egg white or yolk membrane.

**C. Skim milk diluter**

Milk powder with 1% fat (highest quality) 10gms
2x distilled water containing 0.01M glucose anhydride 100ml
Penicillin G 50,000 I.U.
Streptomycin 50mg

**Technique of Freezing**

Semen was processed soon after collection by first placing the semen sample in the water bath at 30–35°C. Semen was then washed 1:9 by volume with tris-citric buffer. The mixture sample was centrifuged for 10 minutes at 600 G's. The supernatant was removed with a pipette.

1. **Dilution:** On the basis of concentration, volume and percent progressive motility the total number of motile spermatozoa was estimated. The sample was then diluted with unglycerolated skim - milk diluter at 30–35°C to a final concentration of 800 million spermatozoa per ml of diluted semen.

2. **Cooling and equilibration:** Samples were placed in a 250ml beaker of water at one hour, and a maximum of two hours in the cold room (10°C). After cooling, the semen was glycerolated with 14% glycerol diluter (86% of skim milk diluter + 14% glycerol) in the cold room in the three steps at 10 minutes interval giving a final concentration of 400 million motile spermatozoa per ml of extended semen.

3. **Freezing:** After equilibration for 10 minutes, semen was drawn into .5ml fresh straws and placed horizontally on a freezing tray. The tray was lowered into nitrogen vapour to −110°C gradually from the mouth of nitrogen tank, and held at that temperature for two minutes. The straws were then lowered directly into the liquid nitrogen. These straws should be stored at −196°C until they are used.

Evaluation of semen was on the basis of the changes in percent motility of sperms from initial collection through processing, post thaw after one day of storage and post thaw stress of 3 hours in a 35°C water-bath. Motility estimates were based on average of estimates made by the two authors and checked by one or more technicians.

**RESULTS AND DISCUSSION**

Table 1 shows an average decrease of about 58% in the motility estimates from neat semen to post thaw using tris-citric acid-yolk and only 55% when skim milk is used. The % motility loss from neat to post thaw was 65% and 67% respectively. Post thaw stress motility values when compared to post thaw were 81 and 72% respectively for the above two diluters.

Although the skim milk diluter was more effective in protecting goat spermatozoa through freezing by 45.3% as compared to 21.2% for the tris-citric acid-yolk (TCY) diluter, yet TCY appeared to maintain the spermatozoa through the stress period slightly more effectively.

Three bucks from whom semen was collected appear to have semen values below those considered to be useful for breeding (Table 1). Buck No. 540 had fair semen before freezing but most of the semen was killed during freezing. Buck No. 553 had extremely low sperm concentration in all ejaculates collected and Mad produced very small quantity of semen. This suggests that approximately one-third of the bucks tested should be culled because they either have low semen volume/sperm concentration or their semen does not freeze well.

The proper comparison of breed and age effect was not possible because of the smaller number of animals in the local group, of which one did produce a very low volume of semen.

Percent motility of post thaw semen after 3 and 6 months of storage has been estimated for some animals. These estimates are approximately the same as the estimate given in Table 1 for post thaw motility. Thus transfer of germ plasm to village farmers' herds from the University nucleus herd should be possible with artificial insemination.
TABLE 1
Average percent motility of spermatozoa in 13 bucks

<table>
<thead>
<tr>
<th>Type</th>
<th>Number</th>
<th>Tris-citric acid-yolk diluter</th>
<th>Skim milk diluter</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Vol.</td>
<td>Con. ($\times 10^9$)</td>
<td>% Motility</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Neat</td>
<td>Post thaw</td>
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<td>F₁</td>
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<td>4.0</td>
</tr>
<tr>
<td>F₁</td>
<td>549</td>
<td>0.7</td>
<td>5.3</td>
</tr>
<tr>
<td>F₁</td>
<td>583*</td>
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<td>0.6</td>
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<tr>
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<tr>
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<td>Average</td>
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<td>4.43</td>
</tr>
</tbody>
</table>

*Semen of these bucks does not appear to freeze well.

**The total spermatozoa in this buck's semen is very low.
REFERENCES


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