EFFECTS OF RAMS, INCUBATION TIME AND DILUTION RATE ON THE MOTILITY AND LONGEVITY CHARACTERISTICS OF MERINO RAMS SPERMATOZOA

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ABSTRACT

The objective of the study was to determine the effects of rams, incubation time and dilution rate of Hepes synthetic oviduct fluid (HSOF) medium on the motility, and longevity of rams spermatozoa. Four fertile rams (R1, R3, R5, R6) were used in this study. Rams’ semen was collected by electroejaculation with 3 replications for analyzing the motility and longevity of spermatozoa. Fresh semen from rams was diluted by four dilutions (1:25, 1:20, 1:15, 1:10). To determine the effect of rams, dilution rate and incubation time on the motility and longevity of spermatozoa were analyzed by using variance one way classification. Results in this study showed that sperm motility (undiluted semen) at room temperature varied between rams. R1 and R5 were close in motility, as were R3 and R6. The longevity of spermatozoa from R1 and R5 was more significantly longer (P<0.05) than R3 and R6. The longevity average of undiluted semen at room temperature (23 °C) was 13.8 hours, whereas the longevity of diluted semen at 39 °C was 10.2 hours. The longevity of sperm diluted in HSOF medium was similar in all rams which is in contrast to the results attained for undiluted semen. There was no significant effect of the interaction between treatments. There was no significant effect of the dilution rate on the motility and longevity of spermatozoa, whereas there was significant effect of the incubation time on the motility and longevity of spermatozoa.

(Key words: Motility, Longevity, Incubation time, Dilution rate, Rams spermatozoa)

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(Kata kunci: Motilitas, Daya hidup, Masa inkubasi, Pengenceran, Spermatozoa)

Introduction
Motility and longevity of sperm are an important factor for sperm to reach the oviducts for fertilization. In a recent study, subjective motility of sperm was investigated in a number of species including goats (Gangadharan et al., 2001; Batista et al., 2002), and dogs (Risopatron et al., 2002). Progressive motility appears essential for the passage between the processes and folds of the utero-tubal junction before ovulation (Thibault, 1973).

Modification of Hapes-synthetic oviduct fluid (Tervit et al., 1972) medium has been used for sperm culture, working with oocytes in air and for in vitro fertilization (Parrish et al., 1988). Fertile life of sperm is prolonged within the female reproductive tract (Harper, 1994; Smith and Yanagimachi, 1991). In study in vivo may provide only limited information about the multiple events involved in sperm storage within the cervix or oviduct, different with in vitro coculture systems that have been developed during recent years by using Hapes-synthetic oviduct fluid (Gillan et al., 1997; Gomez et al., 1997). Bound sperm have been shown to be progressively released under in vitro coculture conditions, and this process should mimic what occurs in vivo in response to a still unknown physiological signal (Chian and Sirard, 1995). Motility and longevity of spermatozoa has been assessed by visual estimation using a microscope.

The aim of the present study was to determine the effects of rams, incubation time and dilution rate of Hepes synthetic oviduct fluid medium on the motility, and longevity of rams spermatozoa.
Material and Methods

Animal

Four rams (R1, R3, R5, R6) of proven fertility were used in this study. Semen was collected by electroejaculation using standard procedures (Evans and Maxwell, 1987). Rams semen was collected with 3 replications, for analyzing the motility, and longevity of spermatozoa.

Semen handling

Semen was collected into 15 ml sterile a plastic centrifuge tube (Rohre/tube, Särstedt, Germany) and a placed into polystyrene box warmed to 39 °C by bottles of warm water. The interval between semen collection and preparation for analysis of motility and longevity of spermatozoa was about 5 minutes. The study was conducted on semen from four rams and was repeated three times.

Sperm preparation

Fresh semen from rams was diluted at four dilutions (1:25, 1:20, 1:15, 1:10) in Hepes (15mM) buffered synthetic oviduct fluid (HSOF) (Tervit et al., 1972) and supplemented with 6.29 mg/ml Sodium chloride, 0.53 mg/ml Potassium chloride, 0.13 mg/ml Calcium chloride, 0.05 mg/ml Magnesium chloride, 2.11 mg/ml sodium hydrogen carbonate, 0.36 mg/ml L-Lactic acid, 0.04 mg/ml Sodium pyruvate, 3.2 mg/ml bovine serum albumin fraction V, 0.16 Potassium phosphate, 0.81 mg/ml D-glucose, 0.075 mg/ml penicillin G-potassium salt and 0.05 mg/ml streptomycin sulfate, 0.12 mg/ml kanamycin monosulfate, 0.06 mg/ml pyruvic acid. The diluted semen samples were held at 39 °C in microscope warm stage (LEC Instrument, Australia). This temperature was selected in order to approximate the intrafemale reproductive tract environment. At 0, 4, 8, 12, 24, 36 hours, a sample was collected and the motility and longevity determined.

Statistical analysis

Data were analyzed using SPSS software program (SPSS 11.0 Brief Guide, New Jersey). Data were analyzed by ANOVA univariate to determine the effect of rams, dilution rate and incubation time on the motility and longevity of spermatozoa.

Results and Discussion

In this study, characteristics of Merino semen showed individually variation, average of semen volume and sperm motility were 0.89 ml and 81.7% (Table 1), respectively with semen color of milky to thick creamy. The longevity average of undiluted semen at room temperature (23 °C) was 13.8 hours (range 2 to 30 hours) whereas the longevity of diluted semen at 39 °C was 10.2 hours (range 6 to 12 hours).

Results in this study also showed that sperm motility (undiluted semen) at room temperature varied between rams. Ram 1 and ram 5 were close in motility, as were ram 3 and ram 6 (Figure 1). In addition the longevity of spermatozoa from ram 1 and ram 5 more significantly longer than ram 3 and ram 6.

Sperm motility (diluted semen) at temperature of 39 °C was similar in all rams, and there was no significantly different between the rams (Figure 2). The longevity of sperm diluted in HSOF medium was similar in all rams which is in contrast to the results attained for undiluted semen. In addition there was no significant effect of the dilution rate on the motility and longevity of spermatozoa (Figure 3).

It has been recognized for sometime that mammalian spermatozoa have limited ability to survive in undiluted seminal plasma (Hamner, 1970; Ritar and Salamon, 1982; Ashworth et al., 1994; Paulenz et al., 2002). While there are factors in seminal plasma that have a detrimental effect on the viability of spermatozoa (Dott et al., 1979), exposure of semen to air increases the metabolic activity of spermatozoa in turn reducing the viability of spermatozoa because of lactic acid production.
and reduction of pH (Evans and Maxwell, 1987). The reduction of viability of ram spermatozoa in undiluted semen as measured by the motility of spermatozoa was confirmed in the studies.

An interesting observation was that there were marked differences between rams, suggesting that some rams have specific substances in seminal plasma that could be detrimental to the survival of spermatozoa incubated at 23 °C. This detrimental effect in some rams was apparently lost when the semen was diluted and incubated in HSOF medium, although spermatozoa in HSOF medium were not able to survive as long as spermatozoa in undiluted semen for two of the four rams that were studied.

<table>
<thead>
<tr>
<th>Ram (n = 4)</th>
<th>Semen volume (ml)</th>
<th>Sperm motility (%)</th>
<th>Semen color*</th>
<th>Sperm longevity**</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall</td>
<td>0.89 ± 0.38</td>
<td>81.7 ± 11.9</td>
<td>3.5 ± 0.78</td>
<td>13.8 ± 12.8</td>
</tr>
<tr>
<td>Range</td>
<td>(0.45 - 1.70)</td>
<td>(70 - 90)</td>
<td>(2 - 5)</td>
<td>(2 - 30)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(6 - 12)</td>
<td></td>
</tr>
</tbody>
</table>

* 0=clear, 1=cloudy, 2=milky, 3=thick milky, 4=creamy, 5=thick creamy
** at least the motility of sperm is 5%
Undiluted semen (at room temperature, 23 °C), Diluted semen (at 39 °C)

Figure 1. Effect of incubation time and rams (R1, R3, R5, R6) on the sperm motility of undiluted semen and diluted semen at room temperature (23 °C) and diluted semen (The results are a mean of three replications for each ram)
Figure 2. Effect of incubation time and rams (R1, R3, R5, R6) on the sperm motility of undiluted semen and diluted semen at 39°C in HSOF medium (The results are the mean of three replications for each ram).

Figure 3. Relationship between incubation time and dilution rate of semen (1: 25, 1: 20, 1: 15, 1: 10) in HSOF medium on sperm motility. The results are the mean of three replications for each ram.
Conclusion

Characteristics of Merino semen showed individually variation. Incubation time was affected the motility and longevity of spermatozoa, however the dilution rate was not affected the motility and longevity of spermatozoa.

References


Ritar, A.J. and S. Salamon. 1982. Effects of seminal plasma and of its removal and of egg yolk in the diluents on the

