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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PENGARUH PEJANTAN, MASA INKUBASI DAN PENGENCERAN TERHADAP MOTILITAS DAN DAYA HIDUP SPERMATOZOA DOMBA MERINO

INTISARI

Penelitian ini bertujuan untuk mengetahui pengaruh pejantan, masa inkubasi dan pengenceran dengan menggunakan bahan pengencer *Hepes synthetics oviduct fluid* (HSOF) terhadap motilitas dan daya hidup spermatozoa domba. Empat domba jantan fertil (R1, R3, R5, R6) digunakan dalam penelitian ini. Sperma domba ditampung dengan menggunakan elektroejakulator dengan masing-masing tiga kali ulangan, untuk dianalisa motilitas dan daya hidup spermatozonya. Sperma segar diencerkan dengan empat pengencer (1:25; 1:20; 1:15; 1:10). Untuk mengetahui pengaruh pejantan, pengenceran dan masa inkubasi terhadap motilitas dan daya hidup spermatozoa digunakan analisis varian *univariate*. Hasil penelitian menunjukkan bahwa motilitas spermatozoa yang tidak diencerkan pada temperatur kamar bervariasi diantara pejantan. R1 dan R5 motilitasnya mendekati sama, demikian pula antara R3 dan R6. Daya hidup spermatozoa pada R1 dan R5 secara nyata ($P<0,05$) lebih lama dari pada R3 dan R6. Rerata daya hidup spermatozoa yang tidak diencerkan pada temperatur kamar (23 °C) adalah 13,8 jam, sedangkan daya hidup sperma yang diencerkan pada suhu 39°C adalah 10,2 jam. Daya hidup spermatozoa yang diencerkan didalam HSOF medium adalah relatif sama pada semua pejantan, yang mana ini kontras terhadap hasil pada sperma yang tidak diencerkan. Tidak menunjukkan interaksi yang nyata diantara perlakuan. Tidak ada perbedaan yang nyata pada angka pengenceran terhadap motilitas dan daya hidup spermatozoa. Walau demikian ada pengaruh masa inkubasi terhadap motilitas dan daya hidup spermatozoa.

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

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 Hepes-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 Hepes-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, Sarstedt, Germany) and a placed into polystrylene 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-potasium 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 intra-female 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 1 Data average (mean ± SEM) of semen volume, motility of spermatozoa, semen color and longevity of spermatozoa used in this study

Ram (n = 4)	Semen volume (ml)	Sperm motility (%)	Semen color*	Sperm longevity** Undiluted semen	Diluted semen
Overall	0.89 ± 0.38	81.7 ± 11.9	3.5 ± 0.78	13.8 ± 12.8	10.2 ± 2.4
Range	(0.45 - 1.70)	(70 – 90)	(2 – 5)	(2 – 30)	(6 – 12)

* 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)

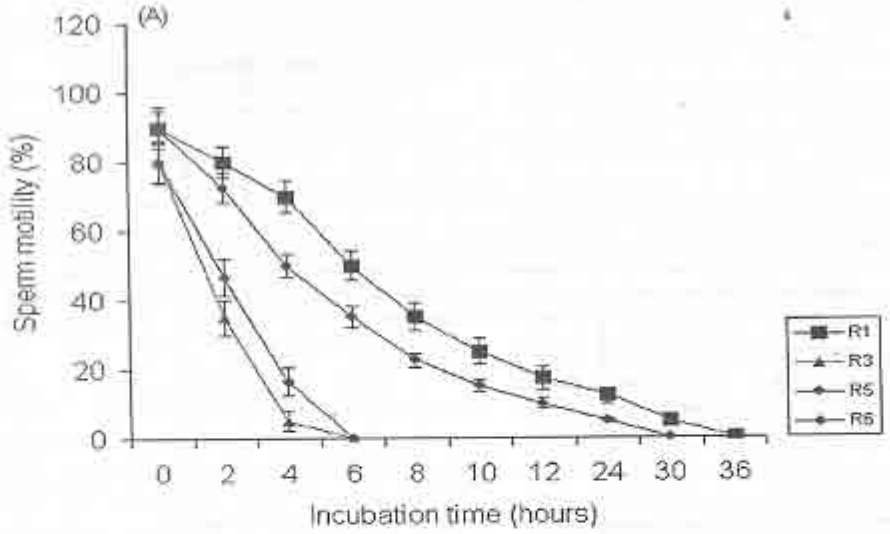

Figure 1. Effect of incubation time and rams (R1, R3, R5, R6) on the sperm motility of undiluted semen and diluted semen at room temperature (23°C) and diluted semen (The results are a mean of three replications for each ram)

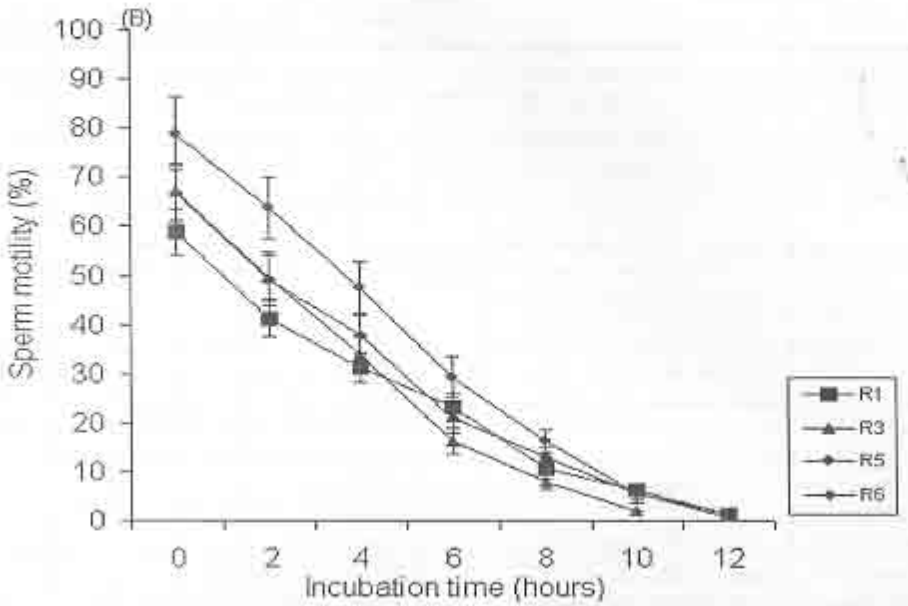

Figure 2. Effect of incubation time and rams (R1, R3, R5, R6) on the sperm motility of undiluted semen and diluted semen at 39°C in HSOF medium (The results are the mean of three replications for each ram)

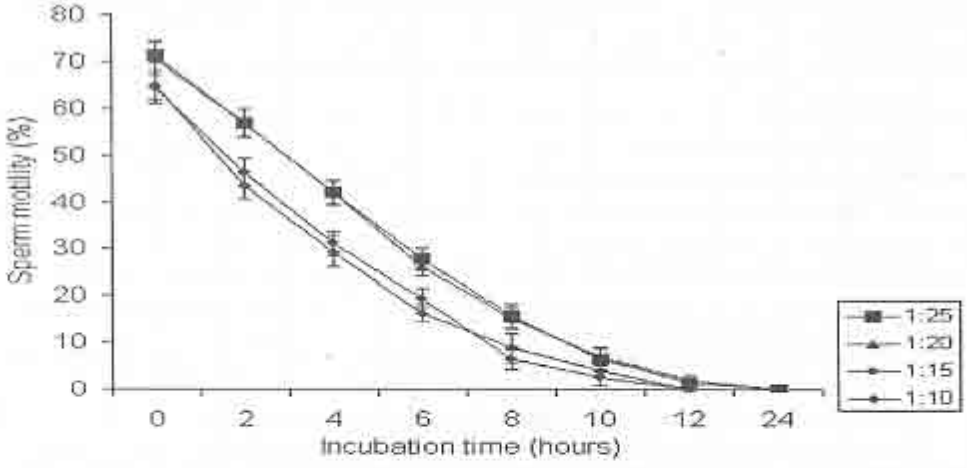

Figure 3. Relationship between incubation time and dilution rate of semen (1: 25, 1: 20, 1: 15, 1: 10) in HSOF medium on sperm motility. The results are the mean of three replications for each ram.

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.

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