**BRIEF COMMUNICATION**

# Effect of lipids on ram spermatozoal motility

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## ABSTRACT

Lipids from egg yolk, milk and coconut were toxic to ram spermatozoal motility. The toxicity of egg yolk lipid was associated with the neutral lipid fraction. Motility was prolonged in the presence of coconut lipid.

**Keywords:** Semen; lipids; preservation; ram.

## INTRODUCTION

Approximately 90% of dairy cows and 1% of ewes are subjected to an artificial insemination (AI) procedure in New Zealand. This vast difference between the two species is attributed to the lower survival and fertility of ram spermatozoa upon storage. We hope to overcome this problem through increasing our knowledge on ram semen biochemistry and to use this knowledge in developing improved semen diluents. We undertook the development of a chemically-defined ram semen diluent (RSD-1), as biochemical interpretations are difficult when semen is stored in diluents containing complex biological fluids (milk, egg yolk etc). RSD-1 can support ram spermatozoal motility at 15°C for ~ 7 days and at 38°C for ~ 24 to 48 h (Upreti *et al.*, 1995), but with decreasing conception rate (Smith *et al.*, 1993). The availability of RSD-1 has however provided an opportunity to test components of complex biological fluids (lipids, proteins and carbohydrates) for their effects on spermatozoal motility and fertilising ability. We chose to study the effect of lipids, which are the major components of the membrane bilayer and influence both the membrane integrity and the activity of membrane bound enzymes (Jain, 1988).

## METHODS

Total lipids (TL) were extracted from milk-cream, egg yolk and coconut by chloroform : methanol (2:1, v/v) mixture (Folch, *et al.*, 1957). Total lipids from egg yolk were also fractionated into phospholipid (PL) and neutral lipid (NL) fractions by silicic acid chromatography (Upreti *et al.*, 1983). TL, PL and NL were added to RSD-1 (pre-saturated with $N_2$ gas) in amounts corresponding to 5% egg yolk. i.e. 0.365 g TL from egg yolk, milk and coconut respectively, 0.101 g PL-Egg yolk and 0.276 g NL-Egg yolk in 20 ml of RSD-1. The suspension was cooled to ice-cold conditions. Lipids were dispersed by sonication (3 bursts of 1 minute durations). A total of 6 treatment (T) diluents were used: T-1, RSD-1; T-2, TL-Egg yolk; T-3, TL-Milk; T-4, TL-Coconut; T-5, PL-Egg yolk and T-6, NL-Egg yolk. Semen from Romney x Poll Dorset rams (n=8) was collected using an artificial vagina, evaluated, diluted and cooled to 15°C as previously described (Upreti *et al.*, 1992). Aliquots of semen from each ram were diluted with each of the 6 diluents to a concentration of $100x10^6$ spermatozoa per ml, cooled to 15°C and stored for 0, 1, 2, 3 and 4 days under $N_2$. The stored samples were incubated at 38°C and motility parameters were measured using a Hobson Sperm Tracker (Smith *et al.*, 1995) at 0, 24, 48 and 72 h. The effect of various treatments on the sperm parameters motility immediately after transferring to 38°C (0 h) and the change in motility parameters over 24 h were statistically analysed using the ANOVA procedure in the Genestat statistical package. Separation of treatment means was tested using Fisher's LSD.

## RESULTS AND DISCUSSION

Motility was maintained for 24 h in most samples and for 48 h at 38°C in very few treatments (too few for detailed statistical analysis of motility data). It was not maintained for 72 h in any of the treatments. It was interesting to note that of the samples that did survive for 48 h, most of them were from TL-Coconut treatment (T-4). This is in accord with previous reports on beneficial effects of coconut milk on ram sperm motility (Chairussyuhur *et al.*, 1993). Differences in sperm motility between treatments were not statistically significant immediately after transferring to 38°C (0 h) for all storage periods up to 4 days.

Motility parameters dropped significantly between 0 and 24 h of incubation at 38°C. The data on change in % motility and velocity parameters are shown in Table 1. Similar trends were observed for the other parameters (data not shown).

These results showing a detrimental effect of NL-Egg yolk on spermatozoal motility, support previous preliminary findings (Upreti *et al.*, 1993). Such toxic effects are likely to be membrane mediated as incorporation of NL, particularly cholesterol, are likely to increase membrane

**TABLE 1:** Change[a] in percent motile and velocity parameters[b] of ram spermatozoa between 0 and 24 h at 38°C. (Pool means for cell storage times).

| | Parameters | | | |
|---|---|---|---|---|
| Treatments | % motile | VCL | VAP | VSL |
| T-1: RSD-1 | -16.7 | -67.8 | -55.4 | -48.0 |
| T-2: TL-Egg yolk | -30.4 | -75.9 | -65.2 | -56.9 |
| T-3: TL-Milk | -27.3 | -70.9 | -61.6 | -53.7 |
| T-4: TL-Coconut | -25.1 | -68.4 | -61.3 | -53.3 |
| T-5: PL-Egg yolk | -28.1 | -77.1 | -63.8 | -55.6 |
| T-6: NL-Egg yolk | -39.2 | -115.2 | -85.1 | -74.8 |
| Mean | -27.8 | -79.2 | -65.4 | -57.0 |
| LSD (P=0.05) | 16.2 | 35.8 | 20.2 | 13.2 |

[a] = The motility data was collected on 8 semen samples from different rams.

[b] = VCL, curvilinear velocity; VAP, angular path velocity and VSL, straight line velocity.

perturbations. These perturbations would be minimised with the inclusion of most phospholipids (except lysolecithin) in the diluent. Adverse effects of TL-coconut on motility were less than the TL- from other biological fluids and the PL and NL fractions of the egg yolk. Although, the change in velocity parameters over the first 24 h for TL-coconut was greater than the RSD-1, only the spermatozoa diluted in TL-coconut retained their motility for 48 h when incubated at 38°C, from samples stored for 2 days at 15°C (data not shown). Further studies are recommended to explore the apparent ability of TL-coconut to prolong spermatozoal motility. Such an effect is likely to be due to the major differences in the chemical composition of lipids from coconut and egg yolk. TL-Egg yolk contains cholesterol and unsaturated fatty acids, whereas TL-Coconut is enriched in saturated fatty acids. It is known that the unsaturated hydrocarbon chain perturbs membrane bilayer more than the saturated chain (Upreti *et al.*, 1980).

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