INHIBITION OF MOTILITY OF BOVINE, CANINE AND EQUINE SPERMATOZOA BY ARTIFICIAL VAGINA LUBRICANTS

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ABSTRACT

The effects of four vaginal lubricants on progressive spermatozoal motility were evaluated. Neat semen was exposed to 0, 5, or 10% (w/v) of H-R, sterile K-Y, nonsterile K-Y or Maxilube lubricating jellies for 10 min at 37°C and then extended to 10 x 10^6 spermatozoa/ml. Spermatozoal motility was evaluated after 0, 1, 2, 4 and 6 or 8 h of incubation at 37°C. For bovine spermatozoa, sterile K-Y jelly at 10% suppressed motility ($P<0.05$), but nonsterile K-Y, H-R and Maxilube jellies had no effect. Maxilube was toxic ($P<0.01$) to canine spermatozoa and is not recommended for use during collection or insemination of canine semen. Exposure of equine semen to 10% H-R jelly had no effect on spermatozoal motility, whereas 10% sterile K-Y, nonsterile K-Y or Maxilube jellies suppressed ($P<0.05$) motility. For all three species, the new, nonsterile K-Y jelly was no more deleterious to spermatozoal motility than the old, sterile K-Y jelly, and H-R jelly also was satisfactory. Fertility tests are required to determine the effect of these products on fertility.

INTRODUCTION

It is essential to lubricate the artificial vagina (AV) when collecting semen from a bull, dog, ram or stallion. Although vaseline or white petroleum jelly can be used, these products are difficult to remove from the liner of the AV. For about four decades, sterile K-Y jelly (Johnson & Johnson Co., New Brunswick, NJ) has been used for AV lubrication. Even though sterile K-Y is accepted as nontoxic

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to spermatozoa, a literature review and discussions with pioneers in artificial insemination (J. O. Almquist, F. I. Elliot and G. W. Salisbury, personal communications) failed to identify experiments establishing this as fact. In the U.S.A., over 200 million dairy cows have been inseminated with semen collected in AVs lubricated with sterile K-Y jelly. This product was recommended (1) for collecting stallion semen although toxicity studies were not undertaken (B. W. Pickett, personal communication). Sterile K-Y jelly also has been used to lubricate AVs when collecting canine semen.

In the spring of 1982, Johnson & Johnson Co. changed the formulation of K-Y jelly. The new product is marketed under the same order number as the old product but is designated as nonsterile. The new, nonsterile K-Y jelly contains chlorhexidine gluconate. This compound is both bacteriostatic and spermicidal when present in appropriate concentrations. Discussions with a representative of Johnson & Johnson Co. (S. Brittian, personal communication) revealed that Johnson & Johnson Co. routinely cultures each lot of the new product and never has found microorganisms. Because of the change in formulation, including inclusion of chlorhexidine gluconate, it was deemed essential to determine if nonsterile K-Y jelly was deleterious to bovine, canine or equine spermatozoa. Ideally, an investigation of spermatozoal toxicity would include fertility testing. Since we did not have the resources to conduct such tests, we evaluated the effects of sterile K-Y jelly, nonsterile K-Y jelly, and two competitive products on the progressive motility of spermatozoa.

MATERIALS AND METHODS

The effects of four water-soluble lubricants on the motility of bovine, canine or equine spermatozoa were evaluated in separate experiments. Tubes representing one lot of Maxilube personal lubricant (Mission Pharmacal Co., San Antonio, TX), H-R sterile lubricating jelly (Holland-Rantos Co., Inc. Piscataway, NJ), sterile K-Y lubricating jelly and nonsterile K-Y lubricant (Johnson & Johnson Co., New Brunswick, NJ) were used.

For each species, a 4x3 factorial experiment was used to evaluate each jelly, at concentrations of 0, 5 and 10% (w/v). At least nine ejaculates per species were collected using an AV lightly lubricated with sterile K-Y jelly. Aliquots of neat semen were pipetted into culture tubes containing premeasured amounts of jellies at 37°C. Tubes were vortexed gently to mix semen and jelly, and the spermatozoa were exposed to lubricant for 10 min at 37°C. Aliquots of the mixtures then were pipetted into prewarmed culture tubes containing seminal extender. Semen was extended to 10 x 10^6 sperm/ml. Extenders that maximize retention of spermatozoal motility were used: egg-yolk citrate (2) for bovine, egg-yolk tris (3) for canine, and powdered milk (1) for equine semen. Culture tubes were randomized and incubated at 37°C.

The percentage of progressively motile spermatozoa was determined at 37°C by phase-contrast microscopy (400x) at 0, 1, 2, 4 and 8 h for bovine and canine spermatozoa and at 0, 1, 2, 4 and 6 h of incubation for equine spermatozoa.

For each treatment, the response was expressed as the change in progressive spermatozoal motility over time. The model was $Y_i = \alpha + \beta x_i + e_i$ and the intercept (α) and slope (β) were estimated by the method of least squares. It was hypothesized that exposure of spermatozoa to a deleterious jelly would yield either a lower intercept, a steeper slope or both, relative to control values ($P<0.05$).

RESULTS

Progressive motility of bovine and equine spermatozoa exposed to 5% of any lubricant was comparable ($P>0.05$) to the motility of control spermatozoa (data not presented). This also was true of canine spermatozoa, except for cells exposed to 5% Maxilube. At the 10% level, certain lubricants reduced spermatozoal motility and these data are presented. For each species, the 95% confidence interval of the regression line for the control treatment was less than plus or minus three percentage units.

Bovine Spermatozoa

The decline in progressive motility of spermatozoa exposed to 10% of each jelly was not different from the control (Figure 1). However, the intercept of 53% for the curve for spermatozoa exposed to 10% sterile K-Y was lower ($P<0.05$) than that of 56% for the control. Intercepts of curves for spermatozoa exposed to 10% nonsterile K-Y, H-R or Maxilube were not significantly different from that for the control curve. Thus, nonsterile K-Y, H-R and Maxilube were not toxic to bovine spermatozoa during a 10-min exposure.

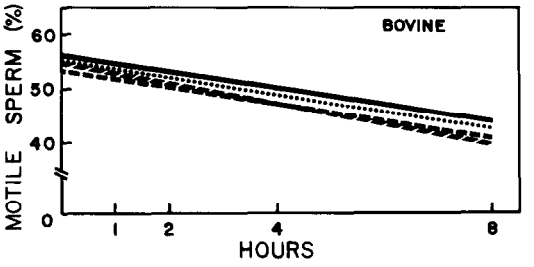

Figure 1. Motility of bovine spermatozoa exposed to 10% H-R or Maxilube (·····), sterile K-Y (------), nonsterile K-Y (–·–·–·) jelly or to no lubricant (——— - control) for 10 min before extension and incubation at 37°C.

Canine Spermatozoa

For spermatozoa exposed to 10% H-R, nonsterile K-Y or sterile K-Y jelly, both the intercepts and slopes of the response curves were similar (P>0.05) to the control intercept and slope (Figure 2). Since exposure of spermatozoa to either 5 or 10% Maxilube was extremely deleterious (P<0.01) to spermatozoal motility, Maxilube must contain one or more compounds toxic to canine spermatozoa. H-R, sterile K-Y and nonsterile K-Y jellies were not toxic to canine spermatozoa.

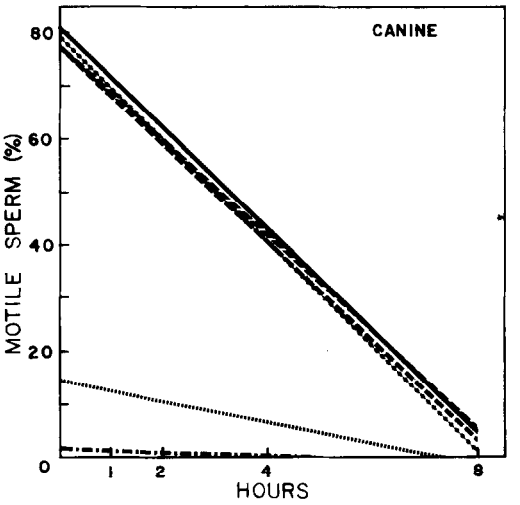

Figure 2. Motility of canine spermatozoa exposed to 10% H-R (·····), Maxilube (-·-·-·-), sterile K-Y (------), nonsterile K-Y (– – – –) jelly or to no lubricant (——— control) for 10 min before extension and incubation at 37°C. Data for spermatozoa exposed to 5% maxilube (·····) also are shown.

Equine Spermatozoa

There were significant differences (P<0.01) among response curves for progressive motility of spermatozoa exposed to 10% of each jelly or the control treatment (Figure 3). The responses fell in two groups. The intercept and slope for samples exposed to 10% H-R jelly did not differ (P>0.05) from those of the control treatment. Intercepts and slopes for samples exposed to 10% Maxilube, sterile K-Y and nonsterile K-Y treatments were comparable to but lower than control values. Exposure of spermatozoa to 10% Maxilube yielded the lowest intercept (Figure 3). Since the intercepts for sterile K-Y, nonsterile K-Y, and Maxilube were depressed, these lubricants may be slightly toxic. H-R jelly was not toxic to equine spermatozoa.

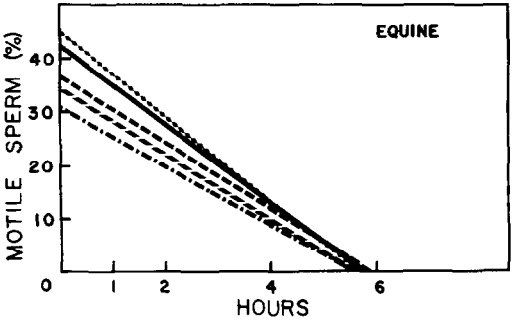

Figure 3. Motility of equine spermatozoa exposed to 10% H-R (·····), Maxilube (-·-·-·-), sterile K-Y (------), nonsterile K-Y (~~~~~) jelly or to no lubricant (——— - control) for 10 min before extension and incubation at 37°C.

DISCUSSION

Either H-R jelly or nonsterile K-Y jelly are acceptable substitutes for sterile K-Y jelly when collecting bovine, canine or equine semen. It seems unlikely that spermatozoa would be exposed to >10% jelly for over 10 min prior to extension for use in artificial insemination. Despite the absence of any deleterious effect of H-R jelly or nonsterile K-Y jelly on spermatozoal motility, a lubricant might alter some other spermatozoal property and, thus, decrease fertilizing capacity or ability to withstand freezing and thawing. Maxilube was very toxic to canine sperm and may be slightly toxic to stallion sperm. Maxilube should not be used to collect dog semen.

Fertility tests, which were not undertaken, are needed to conclusively determine if any product is or is not deleterious to fertility.

REFERENCES

1. Pickett, B.W. and Back, D.G. Procedures for preparation, collection, evaluation and insemination of stallion semen, Colorado State Univ. General Series 935 (1973).

2. Howard, T.H., Vasquez, L.A. and Amann, R.P. Antibiotic control of Campylobacter fetus by three extenders of bovine semen, J. Dairy Sci. 65:1596-1600 (1982).

3. Olar, T.T. Cryopreservation of spermatozoa and daily spermatozoal production of dogs, Ph.D. Thesis, Colorado State Univ. (1983).