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Effects of pH, Lactate, and Viscoelastic Drag on Sperm Motility: A Species Comparison[1]

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ABSTRACT

Little or no motility is observed when sperm from 5 mammalian species are incubated in vitro in their cauda epididymal fluid (CEF). We examined the effects of pH, lactate, and viscoelastic drag on sperm motility to determine whether these factors are responsible for this inhibition of motility. The pHs of CEF from bull, dog, rat, guinea pig, and hamster were 5.8, 6.2, 6.9, 6.9, and 7.2, respectively. The lactate concentration of epididymal semen collected from anesthetized animals ranged from 0.6 to 0.9, but increased almost 10-fold in samples from rats or dogs when measured 2 h postmortem. Increasing the pH of CEF to 7.0 resulted in the initiation of full motility for bull and dog sperm. Suspensions of sperm in buffer at various pHs (from 4.0 to 7.6) produced a sigmoidal motility curve for all species. All species, including bull and dog, showed almost full motility in buffer at a pH equal to the pH of their own CEF. Motility of bull and dog sperm showed greater inhibition with decreasing pH when suspended in CEF instead of buffer. The addition of 15 mM lactate, which has been shown to lower sperm intracellular pH, shifted the motility versus pH curves of all species toward higher pH. In bull and dog the addition of lactate produced a motility profile that was indistinguishable from that in their own CEF. The viscoelastic drag of the CEF of only two species, rat and hamster, was sufficiently high to inhibit sperm motility. We conclude that the low pH of the CEF from bulls and dogs plus the presence of lactate is sufficient to cause inhibition of motility. Rat and hamster sperm motility inhibition can be explained by the high viscoelastic drag of their CEF. Guinea pig CEF, which has a high pH and a relatively low drag, may inhibit by some other mechanism.

INTRODUCTION

Several mechanisms have been proposed to account for the inhibition of sperm motility in caudal epididymal fluid (CEF) from a variety of different species (for a review of this literature, see: Usselman and Cone, 1983; Acott and Carr, 1984; Carr and Acott, 1984). Recently, bovine cauda epididymal (CE) sperm were shown to be inhibited, in vitro, in a pH-dependent manner by a quiescence factor present in CEF; the pH of neat semen collected any time from 5 min to 6 h postmortem from bovine epididymides is 5.8 (Acott and Carr, 1984). At this acidic pH, an epididymal quiescence factor(s) immobilizes bovine CE sperm. The addition of lactate or other permeable weak acids, e.g., pyruvate, D-lactate, propionate, or β-hydroxybutyrate (BHB), to an osmotically balanced buffer mimics this inhibitory effect. Nonpermeable acids such as glutamate and succinate have no effect. Babcock et al. (1983) have shown that permeant weak acids such as lactate lower the intracellular pH (pH_i) of bovine CE sperm. We have presented evidence that low sperm pH_i is responsible for the in vitro inhibition of motility by bull CEF (Acott and Carr, 1984).

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However, rat CE sperm quiescence is maintained by a large, mucin-like glycoprotein, immobilin, that appears to mechanically immobilize the sperm due to the high viscoelasticity that it imparts to the CEF of the rat (Usselman and Cone, 1983).

In this paper, we examine the pH, lactate concentration, and relative viscoelastic drag of the CEF from bull, rat, dog, hamster, and guinea pig. We also examine the effect that lactate has upon the pH profiles of the different species to determine whether lactate produces an inhibitory effect similar to its effect on bull sperm motility.

MATERIALS AND METHODS

Collection of Semen and Fluid

The epididymides from bull and dog were obtained, respectively, from a local abattoir and a local veterinary clinic and semen was extracted within 3 h after excision. The dogs were anesthetized prior to removal of the tissue. Cauda epididymal semen from these animals was collected by retrograde flushing of the vas deferens. The pH of the semen was measured immediately after removal using a pH meter or pH paper calibrated with standardized buffers (Acott and Carr, 1984). Cauda epididymal sperm and fluid were separated by centrifugation at 700 × *g* for 20 min at room temperature followed by 1000 × *g* for 10 min.

For collection of semen from rat and dog to be used for estimates of in vivo lactate levels, the animals were anesthetized with pentobarbital and one epididymis was exposed but not severed. The tubules were then punctured with a scalpel blade and the semen was collected in a tube containing 0.5 ml of 3 M perchloric acid (PCA). For postmortem samples, the other epididymis, still attached to the testis, was excised and stored at room temperature for 2 h prior to semen collection.

Rats, hamsters, and guinea pigs were killed in a CO_2 gas chamber. The epididymides were removed and the cauda squeezed with forceps to increase the pressure. The tubules were then punctured with a scalpel blade and the semen was collected in a small plastic tube.

Ejaculated human semen was obtained from the OHSU Infertility Service Lab, allowed to liquify, and centrifuged at 700 × *g* for 5 min. The supernatant was removed and the sperm were resuspended in assay buffer.

Motility Assay

Semen was diluted 1:100 in buffer containing 20 mM 2-(*N*-morpholino)ethanesulfonic acid (MES), 45 mM KCl, 105 mM NaCl, and 5 mg/ml bovine serum albumin (BSA) at various pHs; 15 mM lactate (Sigma Chemical Co., St. Louis, MO) was added where indicated and the pHs were readjusted. Bull and dog samples were then incubated for 15 min at 37°C to allow full motility to be achieved. Aliquots (15 μl) were placed on a prewarmed slide and assessed for motility. Rat, hamster, and guinea pig sperm motility declined with time so these sperm were assayed for motility immediately after dilution. Bull and dog sperm were also diluted 1:100 in their own CE fluid and incubated for 15 min at 37°C prior to motility assays. The pH of the sample was then adjusted by the addition of 1 N NaOH or HCl and motility was measured again. The addition of equal amounts of 1 N NaCl to controls produced no change in motility.

The motility assay was the same as that described previously (Carr and Acott, 1984) except that video recordings were made to allow for multiple scoring of the same sample or objective scoring (Katz and Overstreet, 1981). Briefly, motility was assessed visually by experienced observers who were not aware of the experimental details. Two parameters were recorded: the percentage of motile sperm and a vigor score (based on the intensity of flagellar activity) from 0 to 10. The vigor score was multiplied by 10 and these two parameters were averaged and are reported as "Motility Units." All experiments were done at least twice and each sample was analyzed in duplicate. The results are presented as means ± SEM.

Lactate Assay

The concentration of lactate was determined using a fluorimetric method (Passonneau, 1974). Neat epididymal semen was added to 0.5 ml 3 M PCA and then frozen until assayed. For in vivo samples, the semen from the left epididymis of 5 rats or 2 dogs were pooled in a tube containing 3 M PCA. The other epididymis was removed and the semen extracted 2 h postmortem (see "Collection of Semen and Fluid"). The lactate assays on bull, dog, and rat were done twice and the results are presented as means ± SEM. The lactate concentration for hamsters and guinea pig were taken from Jones (1978) and converted to millimolar concentrations.

Viscoelastic Drag

Viscoeleastic drag measurements have been described in detail (Usselman and Cone, 1983). Briefly, we measured the time required for a steel ball (approx. 0.67 mm in diameter) to fall 3 cm through a glass tube (approx. 1.12 mm inside diameter) filled with sample. For comparison, glycerol produced a drag of 134 ± 8 s, whereas the drag of water was below the level of resolution of the instrument (less than 0.2 s).

RESULTS

Comparison of Motility, pH, Drag, and Lactate Concentration

Neat semen, collected from the cauda epididymidis of 5 different species, was analyzed for sperm motility, pH, relative viscoelastic drag, and lactate concentration (Table 1). The sperm from rat, guinea pig, and hamster are non-motile. Bull sperm are only slightly motile, and dog sperm showed some motility in two out of seven experiments. The pHs of bull and dog CEF are low (pH 5.8 and 6.2, respectively)

TABLE 1. Comparison of motility, pH, viscoelastic drag, and lactate concentration from epididymal semen from 5 different species.

	Bull	Dog	Rat	Guinea pig	Hamster
Motility in neat CEF	20 ± 5	5 ± 4	0	0	0
CEF pH	5.8 ± 0.11	6.2 ± 0.17	6.9 ± 0.10	6.9 ± 0.14	7.2 ± 0.10
Drag (s/3 cm)	<2	<2	133 ± 48	13 ± 9	30 ± 11
Lactate (mM)					
In vivo	–	0.6 ± 0.1	0.9 ± 0.2	0.7*	0.9*
Postmortem	8.0 ± 1.8	5.6 ± 2.1	8.1 ± 2.5	–	–

*Data taken from Jones (1978) and converted to mM.

when compared to the pHs of guinea pig, hamster, and rat CEF (all near neutrality). The viscoelastic drag of rat CEF is much higher than that of the other species, although the drag of the CEF of the hamster and guinea pig are also appreciable; the drag of CEF from the dog and the bull are very low. The lactate concentrations of semen collected from anesthetized animals before the epididymis had been removed (in vivo) ranged from 0.6 mM to 0.9 mM. If the epididymides of the dog or rat are excised and the semen collected 2 h later (postmortem) the lactate concentration has increased approximately 9-fold. The pH of the semen collected in vivo from the rat and dog is not significantly different from the postmortem values (data not shown).

Species Exhibiting Lactate Effect at Physiologic pH

Bull and dog CE sperm were diluted into CEF or into osmotically balanced buffer with or without the addition of 15 mM lactate, and the pH was adjusted to the indicated values (between 4.0 and 7.6). These samples were then incubated at 37°C for 15 min and their motility was assayed (Fig. 1). The motility of dog sperm exhibited more inhibition at higher pH than that of bull sperm for all the conditions used. The addition of 15 mM lactate to the buffer shifted the motility versus pH curve toward the right for both species, although the change in the pH corresponding to the change in half-maximal motility for bull sperm was approximately twice that observed for dog sperm. The curve for the motility of both types of sperm in buffer with added lactate is indistinguishable from the motility versus pH curve obtained in their own CEF. In both species, at the pH of their own neat CEF (5.8 for bull and 6.2 for dog; see dashed vertical line in Fig. 1), low motility is observed in either CEF or buffer plus lactate, while nearly maximal motility is observed in buffer without lactate.

Species Exhibiting Lactate Effect but Not at Physiologic pH

Rat and hamster CE sperm are similar to those from the bull in that their motility in buffer is relatively insensitive to pH (Fig. 2). The sperm from both rat and hamster do show a significant decrease in motility upon the addition of lactate to the buffer. However, at the pH of their own CEF (6.9 for rat and 7.2 for hamster; see the dashed vertical line in Fig. 2) there is no significant difference between the motility of the sperm in the presence or absence of lactate.

Species Exhibiting Only Small Effects of Lactate at Any pH

Inhibition of the motility of guinea pig CE sperm in response to decreasing the pH of the buffer occurs at much higher pH than it does for the other species tested (Fig. 3). The addition of 15 mM lactate to the buffer produced only a slight effect upon guinea pig CE sperm motility and, at the pH of its CEF, the effect of lactate is small.

The effect of lactate upon human ejaculated sperm in buffer is relatively small even at the lower end of the motility versus pH curve. This is probably not within the physiologic range, although we could find no literature values

for the pH of human CEF. Human ejaculated sperm are approximately 60% motile when measured in seminal plasma (data not shown). The pH of human seminal plasma is around 7.5 and the lactate concentration is 4 mM (Zaneveld and Chatterton, 1982).

DISCUSSION

We have previously proposed two different mechanisms to explain the quiescence of sperm in semen collected from the cauda epididymidis (Usselman and Cone, 1983; Acott and Carr, 1984; Carr and Acott, 1984). One is based

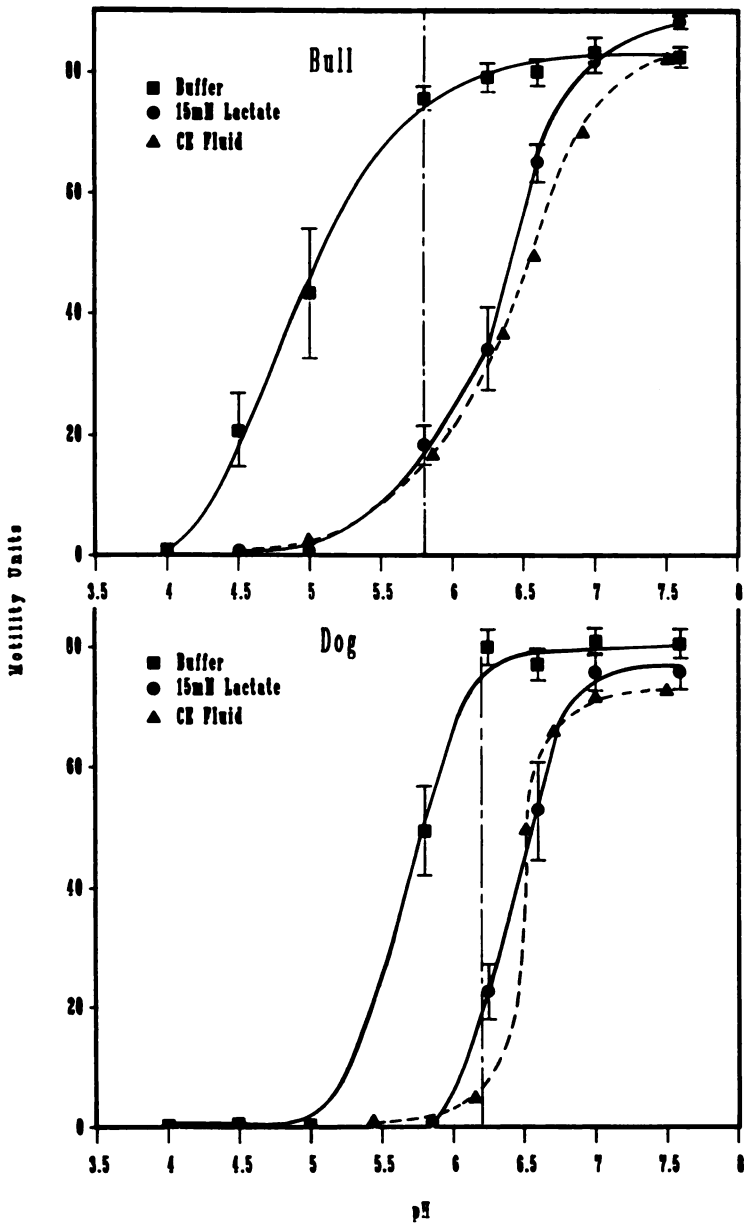

FIG. 1. Dependence of caudal sperm motility from bull (*upper panel*) and dog (*lower panel*) upon pH in buffer (*squares*), buffer plus 15 mM lactate (*circles*), and CEF (*triangles, dashed line*). The *vertical dashed lines* represent the measured pH of the CEF and error bars represent the standard error of the mean.

upon the lowering of the intracellular pH of the sperm by permeant weak acids (e.g., lactate) or other factor(s), at acidic extracellular pH. The other is based upon the mechanical immobilization of the sperm by the high viscoelasticity of CEF. Epididymal sperm from 5 species and human ejaculated sperm were analyzed to determine whether their motility was susceptible to regulation by either of these mechanisms.

Dog and bull epididymal sperm are similar in several regards. The pH of the epididymal semen from both species is relatively acidic, the viscoelastic drag is very low, and the postmortem lactate concentration is relatively high. Most importantly, at the pH of their CEF, both species show little or no motility in CEF or in

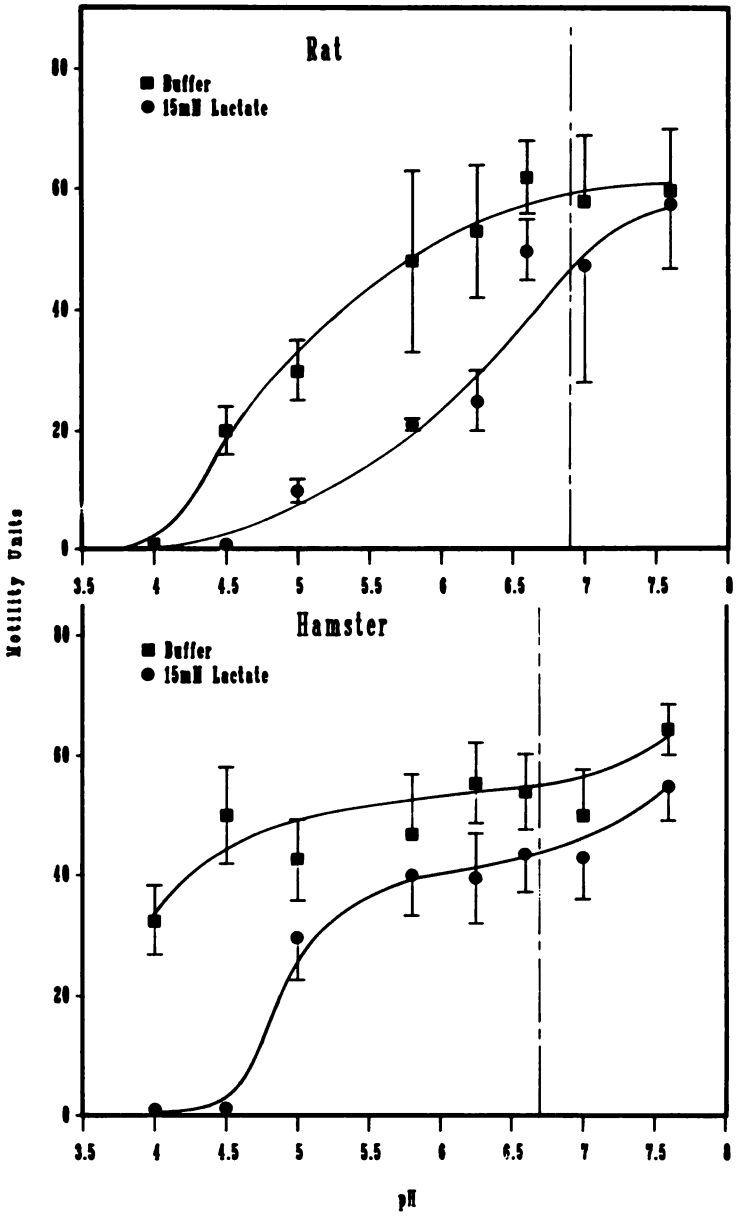

FIG. 2. Dependence of caudal sperm motility from rat (*upper panel*) and hamster (*lower panel*) on pH in buffer (*squares*) and buffer plus 15 mM lactate (*circles*). The *vertical dashed lines* represent the measured pH of the CEF and error bars represent the standard error of the mean.

buffer plus lactate and show almost full motility in buffer alone. Lowering the external pH effectively raises the concentration of lactic acid because only the associated form of the molecule is membrane permeable and therefore capable of lowering the intracellular pH. The characteristics of the sperm from these species are compatible with the hypothesis that their motility in CEF may be regulated by the intracellular pH mechanism.

Although rat and hamster sperm do respond to the addition of lactate, it has little or no ef-

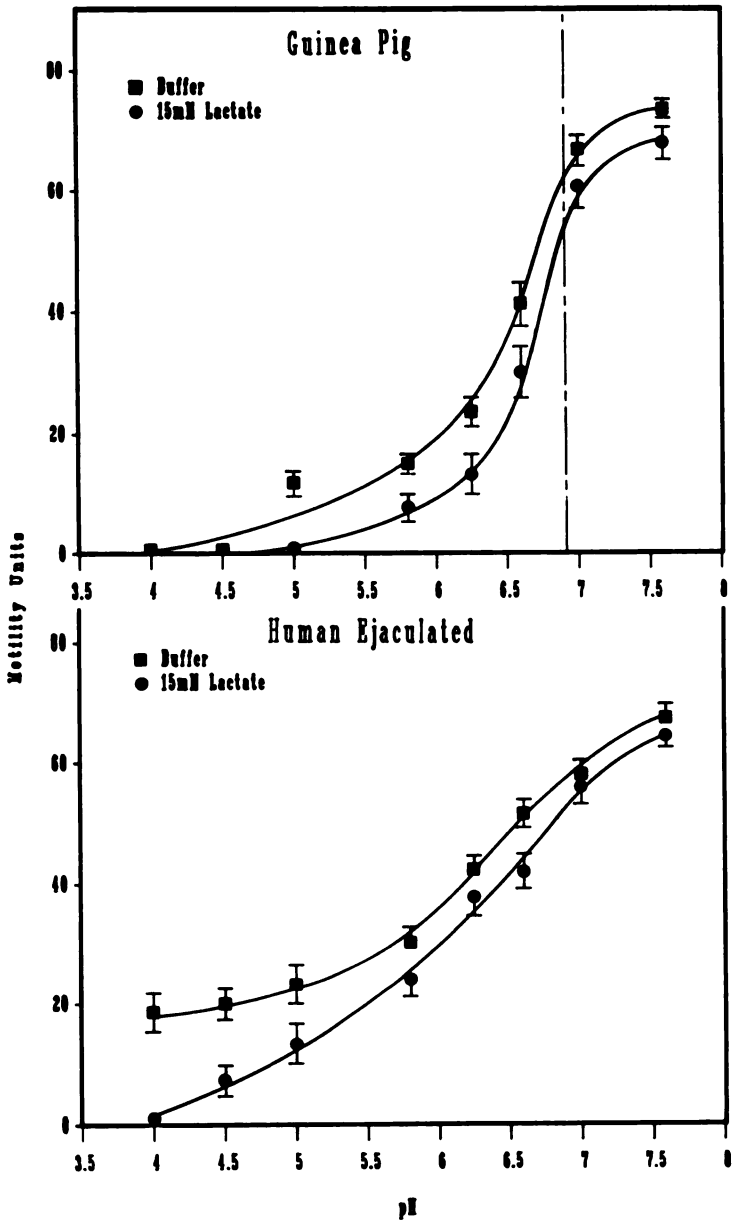

FIG. 3. Dependence of sperm motility from the caudal epididymis of the guinea pig (*upper panel*) and from human ejaculate (*lower panel*) on pH in buffer (*squares*) and buffer plus 15 mM lactate (*circles*). The *vertical dashed line* represents the measured pH of guinea pig CEF; no value was available for human CEF. The error bars represent the standard error of the mean.

fect at the pH of their CEF. Rat and hamster are similar in that they both have a viscoelastic drag sufficiently high to mechanically immobilize the sperm. The pH of both fluids is around 7, so the concentration of the associated form of lactate is comparatively low. The sperm from these species appear to be regulated by the viscoelastic CEF mechanism, but seem unlikely to be regulated by the intracellular pH mechanism.

Guinea pig sperm show very little response to lactate. Their CEF has a pH near neutral and a relatively low drag compared to rat. Neither of the above mechanisms seem to explain the quiescence of sperm in their CEF. It is interesting to note that guinea pig sperm are more sensitive to lowering of the extracellular pH than are the sperm from the other species. One might speculate that the membranes of the guinea pig sperm are more permeable to H^+ ions (or that they are less able to maintain pH_i homeostasis) than are the membranes of the bull sperm, and therefore the intracellular pH of the guinea pig sperm can be lowered without a permeant weak acid to carry the protons across. The possibility of other, completely unique mechanisms for the regulation of guinea pig epididymal sperm also exist.

Human ejaculated sperm do not appear to be particularly sensitive to lactate at pH values near those of the ejaculate (pH 7.5) or even lower values, although the pH of their CEF is unknown to us. We have been unable to obtain human epididymal sperm for these studies, but they may behave quite differently from ejaculated sperm. Recently, Turner and Reich (1984) reported that human epididymal sperm are non-motile in neat CEF. They also found that the viscoelasticity of human CEF is very low (personal communication). It will be interesting to examine these sperm to determine whether they are similar to either bull or rat sperm.

Caution must be used in extrapolation of these studies to the "in vivo" or "in situ" situation. The measurement of only postmortem motility, lactate, and pH values could be misleading. It is very difficult to determine what is actually occurring in vivo. Even though reviews of the male tract generally assume that epididymal sperm are immotile (Mann and Lutwak-Mann, 1981; Zaneveld and Chatterton, 1982), a large increase in lactate could explain the in vitro motility inhibition in species with low fluid pH. White et al. (1959) went to considerable effort to extract ram epididymal sperm from living animals without exposing them to external factors and found them to be motile. On the other hand, although we have hypothesized that lactate may be responsible for quiescence in species with low CEF pH, other proton carriers or unknown factor(s) in epididymal fluid may also reduce internal sperm pH in vivo and thereby cause quiescence. The pH_i control mechanism is also interesting in that the sperm's trek through the female tract involves many changes in external pH.

In summary, dog and bull CE sperm quiescence in vitro can be modulated by the intracellular pH mechanism. Rat and hamster CE sperm are more likely to be immobilized by the CE fluid viscoelasticity mechanism. Guinea pig sperm motility is probably regulated by another mechanism. Further studies will be required to draw realistic conclusions about the mechanism that regulates the motility of human epididymal sperm.

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