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# Osmotic Damage as a Predictor of Motility Loss During Convective Desiccation of Bovine Sperm

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Current state-of-the art technologies are lagging in the application of desiccation storage to mammalian cells using nonreducing sugars. For bovine sperm, motility is irreversibly lost before reaching a sufficiently low moisture content necessary for preservation. It is hypothesized that much of the damage during drying is related to the osmotic stress encountered due to increased osmolarity of the extracellular environment. To test this hypothesis, we subjected sperm to liquid hyperosmotic environments for varying time-periods and measured their motility. We then extracted parameters for two models for motility loss based on these experiments: a first-order rate injury model (Fast or Slow) and a multi-modal (MM) injury model. The MM injury model incorporated an additional function accounting for damage induced by a time-independent osmotic change. Based on these models, we predicted sperm motility loss measured from natural and forced convective desiccation experiments. The MM injury model was able to closely bracket motility loss for desiccation as an osmotic change event with time-independent and time-dependent components. While the mechanistic basis of osmotic damage requires further exploration, the model can serve as a bracketing tool for predicting motility loss during desiccation based on excipients designed to minimize osmotic damage.



## Introduction

Motility retention is widely recognized as the standard for successful preservation and storage of sperm in the animal husbandry industry. Desiccation preservation using novel excipients could potentially allow for near ambient temperature storage of sperm. Successful desiccation preservation of sperm has been limited to retention of the DNA structure, which works using intra-cytoplasmic injection.[1,2] However, the more practical outcome in the animal husbandry industry is the retention of motility after convective desiccation.[1] Sperm motility is irreversibly lost prior to reaching moisture contents < 0.05 gm water/gm of dry weight necessary for stable ambient temperature storage.[3–5]

Loss of motility during desiccation is attributed at least in part to the osmotic stress due to dehydration (hyperosmotic) and corresponding rehydration (hypo-osmotic).[6–9] Therefore, quantifying the amount of osmotic-induced damage during desiccation is important. Although various mechanisms of osmotic damage have been postulated, including the critical volume hypothesis and the critical rate of volume change hypothesis, the complexity of the damage indicates other factors such as increased ionic inequilibrium due to osmotic efflux of intracellular water, mitochondrial depolarization,[10] protein denaturation from dehydration or phase change of membrane lipids,[11] and apoptotic cell death due to biochemical disequilibrium may play a role.[12] This has prevented the development of osmotic damage mechanisms and corresponding models. Water transport models abound in the literature quantifying membrane permeability parameters.[13,14] Rational excipient loading–unloading protocols are designed for cryopreservation applications by coupling the water transport model to critical volume hypotheses.[11,13,15,16] However, the effect of the volume change and the long-term exposure of the cells in a hyperosmotic environment have not been combined in a biological damage model to predict cellular damage. More specifically, this model does not exist for sperm motility loss.

First-order rate models are commonly used as a standard starting approach for quantifying biological damage as a function of transport events.[10,17] Although simplistic and far from mechanistic, it allows for the rapid characterization of a system as a function of stress and time. For desiccation preservation, Elliott et al.[18] and Liang et al.[19] developed cumulative osmotic damage models for mammalian cells and plant seeds, respectively, from their experimental data. However, they have not tested their models predictive ability for other systems. Our approach is to perform experiments for measuring the motility loss of bovine sperm in a liquid hypertonic environment, and to develop variations of first-order models based on these results. The model would then be used to predict sperm motility loss independently in a

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convective desiccation system. We hypothesize that a large part of the motility loss observed during convective desiccation is due to a series of escalating, hypertonic step changes during desiccation.[3–5] The model would serve two purposes: (1) To confirm the extent of convective desiccation osmotic damage captured by the liquid exposure experiments; and (2) to allow design and testing of excipients (in a liquid environment) that can minimize the osmotic damage. The focus of this article is the former.

## Materials and Methods

### Semen shipment

Fresh bovine semen samples from Holstein bulls were shipped every week from ABS Global (WI). Samples were received in a standard egg yolk Tris extender without glycerol in a ratio of 1:2 and stored at 4°C. Samples were packed in a foam brick refrigerant during shipment, which maintained the temperature at 4°C. Progressive motility of sperm was checked immediately on arrival and was typically over 80%. To ensure that there was no loss in cell function over time, all the experiments were performed within 3 days of sperm delivery; during this time, the motility did not fall below 70%.

### Hypertonic buffer exposure

In order to obtain the parameters for cell damage as a function of osmotic stress, sperm cells were exposed to known hypertonic conditions using Tyrode-based buffers. The composition of the isotonic buffer in 1 L of distilled water consisted of 6.82 g sodium chloride, 0.28 g potassium chloride, 0.35 g calcium chloride dehydrate, 0.09 g magnesium chloride hexahydrate, 2.51 g sodium bicarbonate, 0.048 g sodium phosphate, 1.08 g glucose, and 2.76 g HEPES, resulting in an isotonic buffer of 300 mOsm.[8] The reagents were purchased from Sigma Aldrich, St. Louis, MO. Additional buffers of 250 and 1000 mOsm were prepared by decreasing and increasing the amount of solutes by 0.83 and 3.33, respectively.

Sperm were initially suspended in 50 μL of an isotonic buffer (300 mOsm) in vials with an initial concentration of 7–10 million cells per mL, and were then subjected to hypertonic exposure by adding an appropriate volume of a 1000 mOsm Tyrode buffer. The final osmolarities of the hypertonic suspensions were 400, 450, 500, 600, and 800 mOsm. Samples were exposed to the constant hypertonic environment in these vials for times ranging between 5 and 90 minutes. All experiments were performed at room temperature.

### Evaluation of motility

Studies have shown that there is a significant difference in measuring motility during hypertonic conditions and in isotonic media after hypertonic exposure.[16,20] In the current study, we chose to evaluate the motility by resuspension in isotonic media in order to model the process of convective evaporation and subsequent rehydration more accurately. Cells were brought back to isotonic conditions by adding a proper volume of the 250 mOsm Tyrode buffer.

After the cells were brought back to isotonic conditions following hypertonic exposure, motility was evaluated visually under a bright-field microscope (Nikon Eclipse TS 100) using a hemocytometer, counting a volume of 1.8 μL. Because of the dilution, the sperm concentration on these motility counts varied between 50,000 and 300,000 cells/mL. Motility is expressed as the percentage of progressively motile sperm as observed by the user as a fraction of the total number of sperm (motile + immotile). The method of manual count of motile sperm has been used by our research group in previous studies.[4,5] The tests for hypertonic exposure were also performed by an outside collaborator (at ABS Global Inc, DeForest, WI) using Computer Assisted Semen Analysis (CASA) to verify our findings. All motility values are normalized with respect to the motility of a respective control sample suspended in isotonic buffer.

### Models for motility loss

Motility loss as a function of osmotic stress depends on the osmolarity to which the sperm are subjected and on the time of exposure to that specific osmolarity.[20] The simplest form is the first-order rate injury model, which is largely recognized as the standard for bio-damage models,[18,19] represented as:

$$\varphi_R(t) = \varphi_0 - \int_0^t f(M)dt \qquad \text{(Eq. 1)}$$

where $\varphi$ is the natural log of the motility percentage, $\varphi_0$ is the initial value of $Ln\ (100\%) = 4.605$, and $M$ is the deviation from isotonic conditions (300 mOsm). The subscript $R$ represents the version of the model, in this case, the first-order rate injury model. The integral represents the cumulative damage, with $f(M)$ effectively being the rate of motility loss as a function of the osmolarity. The form of this function was determined based on the hypertonic exposure experiments. Two different rates were used for these studies: fast and slow, indicated with subscripts $f_F$ and $f_S$ based on the experimentally observed data.

In these experiments, transport across the cell membrane is reduced to water excursions from the cell, since the solutes in the media are nonpermeant. The high Lp values for sperm[16] result in this volume shrinkage occurring within the first second of exposure, which will in turn result in motility loss. In our second model, we introduced an additional time-independent mode for injury to Eq. (1) to account for damage due to this initial transport event:

$$\varphi_{MM}(t) = \varphi_0 - \left( \int_0^M g(M) \cdot dM + \int_0^t f_s(M) \cdot dt \right) \qquad \text{(Eq. 2)}$$

where

$$G(M) = \int_0^M g(M)dM \qquad \text{(Eq. 3)}$$

Eq. (2) is defined as the multi-modal injury model, identified with the subscript $MM$. The term $G(M)$ accounts for immediate, irreversible, time-independent drop in motility after osmotic exposure. The second term accounts for the rate of damage over prolonged exposure, as seen in Eq. (1), in which we used $f_S$. To determine the necessary parameters for

the model, Eq. (1) and (2) were solved for the case of constant osmolarity used in the hypertonic buffer experiments. Eq. (2) can then be re-written as:

$$\varphi_{MM}(t) = \varphi_0 - \int_0^t \left( g(M)\frac{dM}{dt} + f_s(M) \right) \cdot dt \qquad \text{(Eq. 4)}$$

### Testing of model for variable osmolarity

The developed models were used to predict the loss in motility after exposure to step increases in osmolarity using hypertonic buffers. Starting with 50 μL of isotonic cell suspension, the osmolarity was increased every 20 min by adding the proper amounts of the 1000 mOsm buffer for the necessary increase in tonicity to 400, 500, and 600 mOsm, respectively. Motility measurements were taken at the end and at the beginning of each step (within 10 sec) by properly diluting the sample back to isotonic conditions using the 250 mOsm buffer. Figure 1 (dashed line) shows the osmotic history for this experiment. Piece-wise integration of Eqs. (1) and (4) was used to predict motility loss during these conditions.

### Prediction of motility during desiccation

Previous studies by our group focused on convective desiccation of sessile drops containing a suspension of cells, and the preservation of motility under these conditions.[4,5] The osmolarity increase from desiccation was determined based on gravimetric experiments.[19] Figure 1 displays the desiccation kinetics as deviation from isotonic conditions of a 10 μL sessile drop on a glass substrate under natural convective desiccation at 39% relative humidity (RH, black circles) using potassium carbonate, and under forced convection using a stream of nitrogen gas (white circles). These drying curves were used as input conditions for Eqs. (1) and (4) to determine the predicted values of motility. Table 1 lists the coefficients used for the trend lines of the desiccation kinetics.

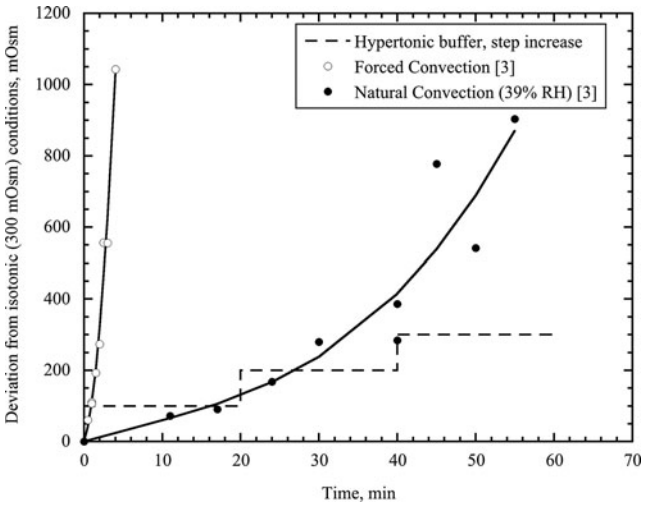


**FIG. 1.** Osmotic histories. Desiccation kinetics for the cases of natural and forced convection using potassium carbonate (39% RH) and a stream of nitrogen gas, respectively.[3] The hypertonic buffer experiment used liquid buffers instead of evaporation. Table 1 lists the polynomial coefficients for the trend lines.

## Results and Discussion

### Hypertonic buffer exposure

Figure 2 shows the experimental results for $\varphi$ as a function of exposure time to hypertonic Tyrode buffers of varying strength; error bars represent standard deviation from the experimental data ($n = 6$ samples for every data point, each pertaining to an independent experimental run). Results for motility loss using CASA were within the standard deviation of our manual counts, but were not included in the present work. The trend lines are a linear least-squares fit to the experimental data and indicate that motility drops exponentially with time for any given osmotic concentration, as $\varphi$ is the natural log of the percentage of motility. As expected, motility was affected both by hypertonicity and exposure time, with the motility decreasing at a faster rate when exposed to higher osmolarities. The same results were observed for a trehalose-based medium, which is the one more commonly used for desiccation studies, but they are not presented in this work. Since the sample needs to be mixed after initial exposure to hypertonic conditions, the earliest repeatable timeframe of 10 sec was chosen to allow for a homogeneous mixture.

We observe two distinct trends in Figure 2. As seen in the inset, the motility measured after 10 sec of exposure (the fastest measurement) indicates a drop in motility that occurs at a much faster rate, as opposed to the rate observed for $t > 10$ sec. The initial drop is also a function of the osmolarity, with a larger decrease in motility resulting from higher tonicity. We can infer from these results that there are actually two modes that cause loss of motility of the cells: one taking place the instant the cells are exposed to hypertonic conditions, and the other one causing a gradual decrease in motility from prolonged exposure to these conditions. Based on the known properties of hydraulic conductivity of bovine sperm cells, we know that the water excursions take place within the first second of exposure, so it is inferred that this initial loss in motility is caused by the initial transport event that results in volume shrinkage.

Prior studies have reported the loss of motility from immediate exposure to anisotonic conditions, without studying the effect of prolonged exposure.[16,20] In this study, we show that there are two distinct response regimes in bovine sperm behavior due to osmotic stress. The first is an immediate sharp drop in motility attributed to volume shrinkage from water excursions after exposure to hypertonicity. Another response occurs as the motility of the cell population decreases gradually from prolonged exposure. Liang and coworkers[19] offered some possible explanation of this damage in their study of the effect of rate of dehydration and cumulative desiccation stress in plant embryonic tissues by using a rate-based model for cumulative osmotic damage. They have analogized the embryonic tissues to a viscoelastic system, in which the rate of dehydration was attributed for a proportional damage through direct mechanical or physical stresses, and the effect of drying time was related to the effect of physiochemical or metabolic stresses. On the other hand, motility loss during prolonged hypertonic exposure, often

Table 1. Summary of Polynomial Coefficients for Best-Fit Trend Lines

| | *a0* | *a1* | *a2* | $R^2$ |
|---|---|---|---|---|
| Osmolarity increase kinetics from evaporation, mOsm(t). Time is in minutes | | | | |
| Natural convection[3] | – | $2.127\times10^{-1}$ | $2.799\times10^{-1}$ | 0.8803 |
| Forced convection[3] | – | 49.69 | 59.07 | 0.9415 |
| Model parameters | | | | |
| $f_S(M)$ (slow-rate) | – | $-6.428\times10^{-5}$ | $-2.296\times10^{-7}$ | 0.9954 |
| $f_F(M)$ (fast-rate) | – | $-7.597\times10^{-3}$ | $-2.189\times10^{-5}$ | 0.9899 |
| $G(M)$ | – | $1.804\times10^{-3}$ | $3.015\times10^{-6}$ | 0.9887 |
| $g(M)$ | $1.804\times10^{-3}$ | $6.03\times10^{-6}$ | – | – |

Based on $y=a0+a1*x+a2*x^2$. For evaporation kinetics, the function represents the increase from isotonic conditions in mOsm, and the independent variable is the time in minutes. For the model parameters, the independent variable is the increase from isotonic conditions, *M*, in mOsm. $R^2$ is the covariance coefficient for the respective least-squares fit.



referred to as solute exposure, could be attributed to (i) further imbalance of intracellular ions during cell volume regulation following hypertonic perturbations of the sperm, (ii) the role of the oxidative stress factors,[3] and/or (iii) initiation of cell apoptosis due to the inability of cells to compensate for osmotic stress.[21] The combination of the exposed osmolarity and the time clearly affect the eventual kinetics of motility loss that needs to be captured through a model. Eventually, we would like to ascertain what aspects of the behavior are found in convective desiccation kinetics.

### *Model for motility loss: Evaluation of parameters*

*A) First-order rate injury model.* Comparing Eq. (1) to the results from Figure 2, we can see that the function *f(M)* is essentially the rate of motility loss at a particular osmolarity. Based on the observed results from Figure 2, two different damage rates were calculated for this model, one based on the almost instantaneous motility drop for $t<10$ sec and one based on the gradual motility drop from prolonged exposure for $t>10$ sec. The second rate, deemed "slow" and denoted by $f_S(M)$, was calculated based on the slope of the trend lines from Figure 2, while the first rate called "fast" rate damage was determined from:

$$f_F(M)=\frac{\varphi_{10}-\varphi_0}{10\ \text{sec}} \qquad \text{(Eq. 5)}$$

where $\varphi_{10}$ is the natural log of motility after 10 sec of exposure, as measured experimentally. The rate model approach was used in an attempt to quantify osmotic damage using a well-established tool for biodamage. The "fast" rate determined for the first 10 sec of exposure was used to capture the initial damage due to volume excursions, even though the initial response may be significantly faster than that due to the high permeability to water of the cell membrane.

Figure 3 shows the values obtained for *f(M)* plotted against the respective osmolarity increase, with the fast and

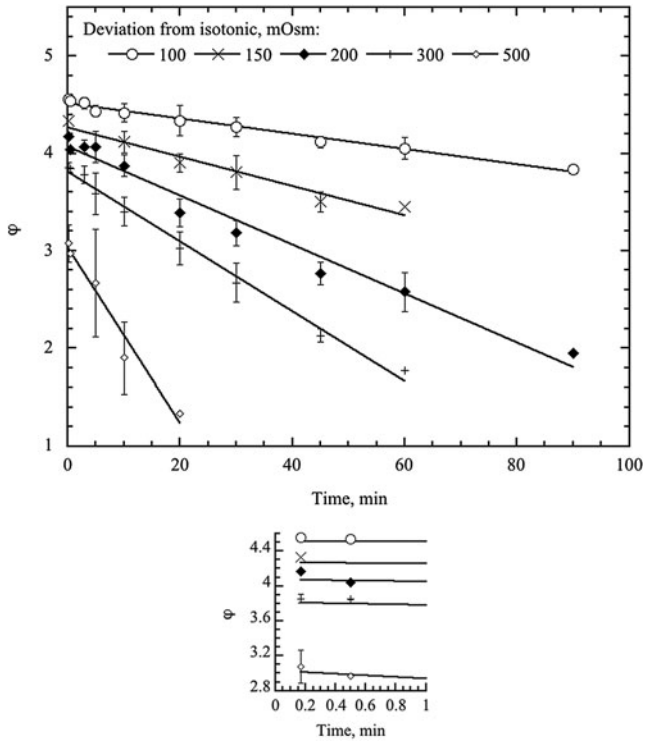


**FIG. 2.** Loss of motility from hypertonic buffer exposure. $\varphi$ represents the natural log of the percentage of motile cells, as measured at specific times during hypertonic exposure. The buffers used are indicated as a function of their deviation from isotonic conditions (300 mOsm). The *inset* shows a detailed view of motility drop for the first minute of exposure.

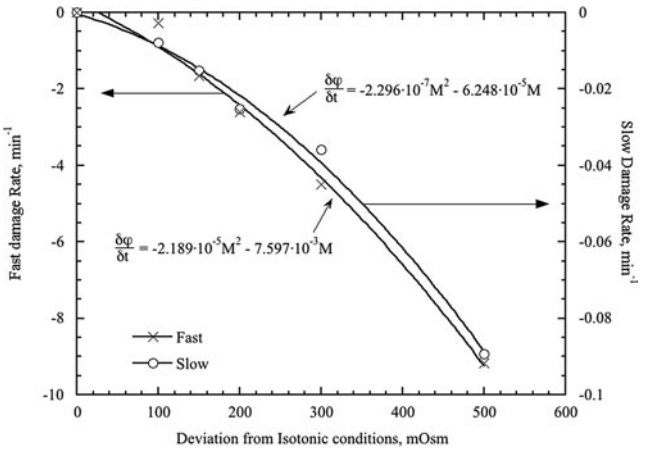


**FIG. 3.** Damage rate during hypertonic exposure. Damage rate is defined as the decrease rate of the natural log of motility, $\varphi$. Two rates are presented on two different axes. The slow rate is determined from the gradual motility drop that occurs at $t>10$ sec, as seen on Figure 2; the fast rate is determined as the initial drop in motility from 100% down to the value at $t=10$ sec. *Trend lines* represent a polynomial least-squares fit.

slow rates in different scales for comparison. The fast rate, $f_F(M)$, is shown to be about two orders of magnitude higher than the slow rate, $f_S(M)$. The polynomial trend lines from Figure 3 were used as the function, $f(M)$ on Eq. (1) for two damage rates on the first-order rate injury model. Table 1 summarizes the coefficients for the obtained trend lines.

*B) Multi-modal (MM) injury model.* An alternative approach would be to use the two observed damage time scales in one combined model. However, the exact timescale of the fast rate mode is rather arbitrary, and likely to occur within the first second of exposure due to the high hydraulic conductivity of the cell membrane.[8] Since it was clear that the time scale for the 'fast' injury was possibly an order lower than that of the 'slow' injury, we developed a time-independent and time-dependent MM injury model. This model includes a non-time dependent parameter, $g(M)$, to account for the seemingly instant drop in motility after hypertonic exposure, as detailed in Eqs. (2)–(4). By comparing Eq. (2) and (3) to the experimental results from Figure 2, we find that,

$$G(M) = \varphi_0 - \varphi_0' \qquad \text{(Eq. 6)}$$

where $\varphi'_0$ is defined as the intercept at t = 0 for the respective trend lines, effectively representing an immediate drop in motility after hypertonic exposure. Figure 4 shows the values of $G(M)$ determined by Eq. (6) as a function of the osmolarity, clearly showing how a larger drop in motility occurs at higher osmolarities. The coefficients for the trend line are shown in Table 1, and $g(M)$ is then readily evaluated as,

$$g(M) = \frac{dG(M)}{dM} \qquad \text{(Eq. 7)}$$

The model from Eq. (4), deemed the MM injury model, uses a time-independent factor in the function $g(M)$, added to our first-order rate injury model for the slow version of the rate equation, $f_S(M)$. This results in an instantaneous drop in motility after an increase in tonicity, followed by a motility decrease rate dictated by $f_S(M)$, as seen on Figure 2. Since convective desiccation processes consist on exposure to anisotonic environments for extended times, a proper prediction model has to account for the observed damage after immediate exposure, plus the gradual damage from prolonged exposure to hypertonic media. To the best of our knowledge, this is the first time that a model combines these two damage mechanisms.

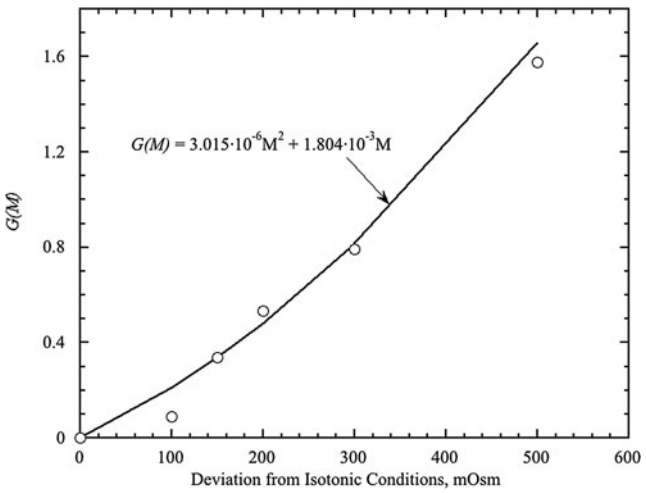


**FIG. 4.** Initial damage as a function of the osmolarity. The function $G(M)$ from Eq. (3) represents the initial damage and is determined as the drop in φ for t = 0 from 100% motility, as extrapolated from the results in Figure 2. *Trend lines* represent a polynomial least-squares fit.

## *Verification of model for a stepwise increase in osmolarity*

The models developed in the previous sections were used to predict motility loss during exposure to a stepwise increase in liquid hypertonic environment, as previously detailed in Figure 1 (dashed line). Figure 5 shows the results for motility retention during this experiment. The measured motility is shown to experience a drop just after every increase in osmolarity, followed by a period of gradual decrease during the following 20 min. The labels on the data points indicate that the measurement was performed either 10 sec before the increase of osmolarity or 10 sec after ($n = 4$ samples per every data point, each pertaining to one independent experimental run). This pattern was first observed in the results of Figure 2 for constant values of osmolarity, and it is clear from these results that the drop in motility does not only occur after leaving isotonic conditions, but every time the osmolarity is increased. This result gives a glimpse into possible damage kinetics during the process of convective desiccation, since it involves a continuous increase of osmolarity (Fig. 1, solid lines). In summary, it is evident that motility loss follows a bi-modal trend due to osmotic damage.

Figure 5 also shows the model predictions for the same osmotic history, by using the parameters specified in Table 1. For our first-order rate injury models, the slow rate model predicts a higher value of motility than the experimental results, while the fast rate model overpredicts the damage.

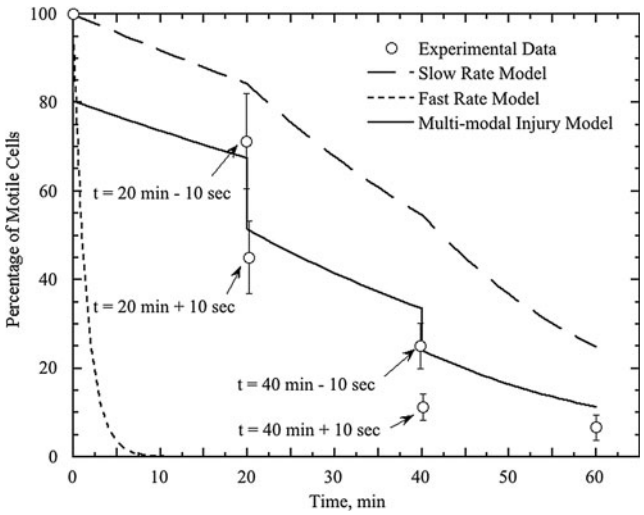


**FIG. 5.** Loss of motility during step changes in tonicity. Cells were exposed to the hypertonic buffer history specified on Figure 1, and motility was measured at various intervals, as shown through the data points. The model prediction is calculated from piecewise integration of Eqs. (1) and (4) for the first-order rate and multi-modal injury models, respectively.

Table 2. Error Analysis

| Injury model | Stepwise tonicity increase, % | Natural convection desiccation, % | Forced convection desiccation, % |
|---|---|---|---|
| First-order rate, fast | 26.6 | 27.2 | 15.2 |
| First-order rate, slow | 23.9 | 38.7 | 63.4 |
| Multi-modal (MM) | 9.3 | 12.8 | 22.6 |

Average absolute errors as a percentage of full scale as determined by $|(Mot_{exp}\text{-}Mot_{model})|$, where $Mot_{exp}$ is the experimental value of motility, and $Mot_{model}$ is the predicted value. The osmotic histories for each case can be seen on Figure 1.

Based on these differences, and the observations from experimental results, we see how the damage mechanisms that cause motility drop are not purely rate-based. While there is unequivocally a rate component to the osmotic damage, it appears as though a significant loss of motility occurs independent of the exposure time, as evidenced by the steep decreases measured.

The MM injury model is able to predict the motility loss under these conditions more accurately. Consistent with the experimental results, a sharp drop is observed at 20 and 40 min, followed by a gradual motility decrease. The model prediction is not as accurate near the end of the experiment for reasons unknown to us, and perhaps related to other damage mechanisms after prolonged exposure to increasing hypertonic conditions. Table 2 summarizes the errors obtained for each model. In summary, the MM injury model accurately reproduces sperm motility loss due to multi-step hypertonic exposure.

### Prediction of motility during desiccation

*A) Natural convection desiccation.* Motility retention from natural convection desiccation was measured in a previous study.[4,5] Figure 6 shows the experimental motility results for these experiments, along with the model predictions using the parameters from Table 1. The motility drops to about 35% of its initial value when the osmolarity is increased by 200 mOsm. The slow and fast versions of the first-order rate injury model show motility drops to about 80% and 0%, respectively, at this same point. In contrast, the MM injury model is able to predict the experimental results more accurately by displaying a comparable motility of 45%. The developed models are based on osmolarity exposure, so the close fit of the MM injury model indicates a strong role of osmotic damage during convective desiccation. However, while the MM injury model is the best fit, it slightly overpredicts motility retention by about 10%; this can be attributed to the other damage mechanisms that may play a significant role during convective desiccation, such as increases in viscosity that may impart mechanical stress to the cells.

*B) Forced convection desiccation.* For forced convection desiccation, the cells are exposed to high osmolarities for much shorter times. Figure 7 shows the experimental results for motility retention, as well as the model predictions for motility loss under forced convection desiccation. The experimental results are nearly identical to those seen under natural convection,[3] and the motility drops to about 30% at 200 mOsm in tonicity increase. The scarcity of the experimental data is due to the difficulty in achieving partial desiccation using forced convection.

As our first-order rate injury models are directly dependent on the exposure time, these predictions were expected to be quite different than those for natural convection desiccation, even if the experimental results are essentially the same. At the 200 mOsm point, the slow and fast rate models show a motility of about 98% and 30%, respectively. It is interesting to note that the fast-rate model is able to predict this motility drop with high accuracy. Since this model is based on the initial rate of motility loss, it is likely that this damage mode is the predominant effect during forced convection, hence the more accurate prediction. The slow-rate model predicts higher motility retention due to the short exposure times. The MM injury model is seen to slightly overpredict motility retention during forced convection when the

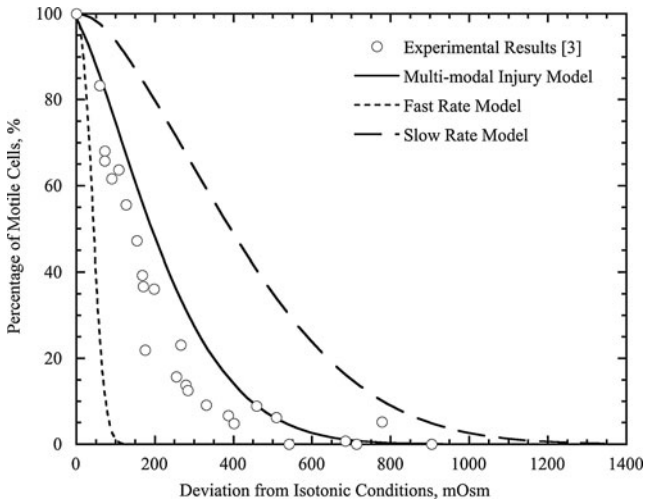


**FIG. 6.** Loss of motility during natural convection desiccation. Using motility results,[3] the models are used to predict loss of motility for natural convection desiccation. The model prediction is calculated from Eq. (1) and (4) for the first-order rate and multi-modal injury models, respectively.

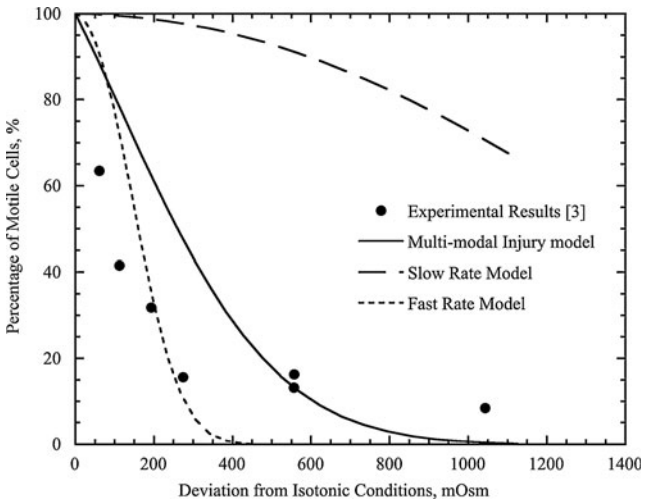


**FIG. 7.** Loss of motility during forced convective desiccation. Using motility results,[3] the models are used to predict loss of motility for forced convection desiccation. The model prediction is calculated from Eq. (1) and (4) for the first-order rate and multi-modal injury models, respectively.

osmolarity is below 800 mOsm, and it underpredicts motility when it is above.

### *Rationale for selection of models for convective desiccation*

The ability to quantify osmotic damage in sperm during desiccation is crucial in designing excipients and protocols to minimize the damage during the desiccation process. Since sperm motility is a much sought after end point and is a strong function of osmotic damage, our study focuses on sperm motility as a measure of osmotic damage. Our previous studies have also focused on motility loss during convective desiccation.[4,5]

Our first model is a first-order rate equation. These models tend to be a first step in evaluating biological injury due to biochemical, thermal or biophysical stresses. Several forms of Eq. 1 are widely used in studies of thermal damage in cells[22] or damage from cryoprotectant permeation;[17] hence our initial approach of using a rate-based model to predict motility loss as a function of osmotic damage and time of exposure. In our case, based on our liquid exposure experiments (Fig. 2), it was clear that sperm motility loss followed a two-step response. We therefore developed two sub-models, a fast and a slow rate model. The first mode (fast rate) is most likely related to volume shrinkage in the cell that occurs at a very rapid timescale due to the high *Lp* values of sperm.[14] The second mode (slow rate) is based on a more complex and not fully understood mode of damage. How these two probably different mechanisms play a role in convective desiccation is important to understand.

The MM injury model is based on combining these two responses. As can be seen from Figure 6, the MM injury model captures the motility response well for natural convection, while the first-order rate model (fast and slow) overpredict and underpredict the response, respectively. For forced convection (Fig. 7), it appears that the osmotic damage mechanism captured through the fast rate model tends to predominate. In summary, based on the two first-order rate models (fast and slow) and the MM injury model, we are able to capture possible motility loss behavior as a function of osmolarity during various protocols for convective desiccation. This allows us to somewhat bracket the osmolarity-driven motility loss during desiccation.

For both desiccation methods, the MM injury model overpredicts the motility damage. This potentially indicates that other biophysical and biochemical damage mechanisms may set in at high osmolarities. While this underscores the accuracy of osmotic damage models being the sole predictor of desiccation damage in a bovine sperm system, it also allows us to quantitatively understand more clearly the role of osmotic driven mechanisms (that have not been entirely elucidated in sperm systems) in desiccation preservation, and thereby study desiccation methods with emphasis on reducing osmotic stress.

### *Assumptions in the models*

Both approaches to the model for motility loss were based on the fact that the change in osmolarity of the extracellular environment is always positive; that is to say, our models would not work for cases in which there is partial rehydration in the middle of the experiment. It has been shown that gradual rehydration may improve the survival of cells,[7] and the modeling of this effect is well beyond the scope of the current study.

## Conclusion

Our motivation was to determine the percentage of injury to bovine sperm during desiccation that was due to osmotic exposure. We measured the motility loss of sperm by exposing them to liquid hypertonic buffers. The cell motility loss was found to be bimodal—a rapid drop initially during the first 10 seconds of the experiment, followed by a slower gradual decrease.

Two different versions of a model for motility loss were developed based on the results from the hypertonic exposure experiments: A first-order rate model (for fast and slow damage rate), and a multi-modal model, which adds a time-independent parameter in order to account for damage that occurs due to the initial water excursions immediately after exposure.

The developed models were used to predict motility loss first on sperm exposed to stepwise increases in osmolarity in a liquid environment, and then extended to the convective desiccation studies. For the liquid exposure, the MM injury model clearly presented the best results. For the convective desiccation experiments, the MM injury model provided better fits for the natural convection, while the fast model gave a better fit for forced convection. Overall, the multi-modal model seemed to capture the slope of the motility curve better than the first order model, indicating it to be a reliable predictor of osmotic damage during desiccation. This will aid in future studies of the damage kinetics that lead to loss of motility.

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## Author Disclosure Statement

No competing financial interests exist.

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