

AN ABSTRACT OF THE THESIS OF

Kari Gamble for the degree of Master of Science in Animal Sciences presented
June 14, 2004.

Analysis of the Effect of the Potassium Ion on Fowl Sperm Motility

Abstract approved:

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Fowl sperm are believed to be immotile prior to ejaculation. The concentration of potassium found in deferent duct fluid is approximately 8 times higher than that found in blood plasma, which is 5 mM in *Gallus domesticus*. The effect of ionic potassium on fowl sperm motion was tested in a series of five experiments. Initially, the following argument was made: if 40 mM KCl inhibited sperm motility in vitro, then deferent duct K^+ might act to inhibit sperm motility prior to ejaculation. A preliminary experiment demonstrated that extracellular potassium had no effect on sperm mobility ($P \geq 0.05$). Greater replication was used in the second experiment, and, the same outcome was observed ($P \geq 0.05$). The third experiment confirmed that K^+ had no latent effect on sperm motility as measured by motile concentration with the Hobson Sperm Tracker over a 20-min interval ($P \geq 0.05$). The fourth experiment was a preliminary experiment in which the effect of 1% (vol/vol) dimethyl sulfoxide (DMSO) on motile concentration had no effect ($P \geq 0.05$). Experiment five demonstrated that glybenclamide, a mitochondrial K^+_{ATP} channel blocker, decreased

sperm motility in a dose-dependent manner ($P \leq 0.001$). This experimental outcome was consonant with the predicted effect of sulfonylureas on mitochondrial volume and respiration. In summary, the notion was rejected that a high concentration of K^+ within deferent duct fluid inhibits sperm motility. However, the collective data sets may afford an explanation for the origin of deferent duct fluid K^+ , which remains a mystery. Fowl deferent duct fluid contains millimolar amount of glutamate, and fowl sperm have N-methyl-D-aspartic acid (NMDA) channels. It is proposed that K^+_{ATP} and NMDA channels act in concert to enable a K^+ efflux from sperm during sperm maturation in the excurrent ducts of the testis.

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Analysis of the Effect of the Potassium Ion on Fowl Sperm Motility

by

Kari Gamble

A THESIS

submitted to

Oregon State University

in partial fulfillment of

the requirements for the

degree of

Master of Science

Presented June 14, 2004

Commencement June 2005

Master of Science thesis of Kari Gamble presented on June 14, 2004

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I understand that my thesis will become part of the permanent collection of Oregon State University. My signature below authorizes release of my thesis to any reader upon request.

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ACKNOWLEDGEMENTS

I would like to express my sincere appreciation towards Drs. Fred Menino, Annie Qu, and Jerry Heidel for serving on my graduate committee. Dr. David Froman, as my major professor, inspired me to think outside of the box and guided me to become a better scientist and presenter. Thank you for the privilege of working with you. I would like to thank Allen Feltmann for all his help. His skilled assistance, attitude, and willingness to listen helped me to complete my project. I would like to recognize the Chester M. Wilcox Memorial Scholarship for financial support. I would also like to thank my fellow graduate students and friends including Cecily, Jessica, Camie, Annette, Michelle, and Amy for allowing me to bounce ideas off of them and for their support.

I want to thank my parents for all their love and support. Last, but definitely not the least, my husband, Wesley for his support, patience, love and understanding. I couldn't have done this without you.

CONTRIBUTION OF AUTHORS

Dr. David Froman, as my major advisor, provided funding for the project and participated in the experimental design and data collection.

Mr. Allen Feltmann assisted in making the reagents and everywhere else as needed.

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INTRODUCTION

The anatomy of the reproductive tract of birds is quite different from that of mammals (Lake, 1966). The fowl does not possess accessory reproductive organs commonly found in domestic mammals, e.g. seminal vesicles, prostate gland, Cowper's gland. Domestic fowl have a sperm concentration of 5 to 7 billion sperm per mL, and a high concentration of glutamate and potassium (Freeman, 1984). The fowl possesses a cloaca into which the colon, ureter, and a vas deferens empty (Nickel et al., 1977). Interest in the physiology of avian semen has centered mainly on the common domestic fowl (*Gallus domesticus*) and the turkey (*Meleagris gallopavo*) (Lake 1966).

Reviere (1968) stated that the duration of spermatogenesis in the fowl is short. From the division of the primary spermatocytes to the end of meiotic prophase it takes an estimated 5-6 days. After division of the secondary spermatocytes, the spermatids elongate on the 10th and 12th day, ending spermiogenesis. The sperm are then suspended in seminiferous tubule fluid prior to their passage through the excurrent ducts. The sperm cell is made up of a head, neck, mid-piece, and tail. According to Thurston and Hess (1987), fowl sperm are vermiform cells that contain a conical acrosome and a helix of 25 to 30 mitochondria. At the nucleus, fowl sperm are about 0.5 μm in diameter and have an overall length of 90 μm (Thurston and Hess, 1987).

Esponda and Bedford (1985) stated that the suspensory fluid's volume and composition are altered prior to sperm entry into the epididymal duct and finally the deferent duct. Deferent duct fluid, also referred to as seminal plasma, is the product of

this process. Prior to ejaculation, fowl sperm are essentially immotile (Ashizawa and Sano, 1990).

The chemical composition of fowl deferent duct fluid has been known for approximately 40 to 50 years (Lake, 1966). The primary cations found in deferent duct fluid include Na^+ , K^+ , and Ca^{2+} (Lake, 1984). Calcium has been shown to be a stimulating factor for sperm motility (Ashizawa and Wishart, 1987). Calcium uptake within the mitochondria matrix is mediated by a uniporter, whereas Ca^{+2} efflux is mediated by a sodium/calcium exchanger (Mojet, et al., 2001). Froman (2003) proposed that calcium acts as a motility agonist by activating phospholipase A_2 (PLA_2). Endogenous fatty acids are released from PLA_2 for β -oxidation within the mitochondrial matrix. ATP is then synthesized enabling sperm to ascend the vagina and enter sperm storage tubules (Froman, 2003).

Calcium and sodium levels within deferent duct fluid are similar to that found in blood plasma. In contrast, the potassium concentration is about 8 times higher in the deferent duct fluid compared to blood plasma (Lake, 1984). Elsewhere in the body, e.g. extracellular fluid (ECF), bloodstream, and cerebral spinal fluid (CSF), concentrations of potassium are kept at a stable level to prevent hyperkalemia, a lethal condition. Hyperkalemia is prevented by aldosterone which is secreted from zona glomerulosa cells of the adrenal cortex (Guyton and Hall, 1996). These cells are sensitive to circulating angiotensin, a decrease in ECF sodium concentration, and an increase in ECF potassium concentration. Potassium is secreted into the distal convoluted tubules and cortical collecting ducts and excreted in urine at an approximate concentration of 60 mM (Guyton and Hall, 1996). Thus, deferent duct

potassium could be high due to secretion. However, potassium could also be coming out of sperm cells as implied by the observations of Ashizawa et al. (1988).

Garlid (1996) suggested that the physiological role for the mitochondrial potassium cycle is volume regulation. However, the fowl spermatozoon may be a cell that cannot accommodate changes in mitochondrial volume without loss of function due to its limited cytoplasm and closely adherent plasma membrane. The mitochondrial K^+ cycle includes concerted interplay between K^+ uptake via one or more channels and K^+ efflux via the K^+/H^+ exchanger (Bernardi, 1999).

The biological significance for the high levels of potassium in fowl deferent duct fluid remains unclear. Several fish species including chum salmon, rainbow trout, masu salmon, and char also have a high concentration of K^+ in their seminal plasma (Morisawa and Morisawa, 1990). Morisawa and Morisawa stated that within these fish species, potassium inhibited sperm motility, even at concentrations lower than that found in their seminal plasma. They went on to find that sperm became motile when the spermatozoa of these species were diluted in a K^+ -free medium and concluded that the high levels of K^+ found in these species' seminal plasma inhibited sperm motility. Therefore, the primary goal of the thesis research was to determine if high extracellular K^+ inhibited fowl sperm motility.

The origin of the high potassium found in fowl deferent duct fluid is also a mystery. There are two possible explanations. Either the potassium arises as an osmolyte from the excurrent duct epithelium or it originates from sperm cells, in particular from the mitochondria. Ashizawa et al. (1988) reported that the Na^+ -to- K^+ ratio associated with the mid-piece of fowl sperm increased as a function of sperm

passage through the deferent duct. If this is the case, then K^+ is leaving the mid-piece as Na^+ increases within the mid-piece. This supports the hypothesis that the potassium originates from fowl sperm mitochondria.

The $mitoK^+_{ATP}$ channel is selective for K^+ over Na^+ . According to Garlid and Paucek (2001), the $mitoK^+_{ATP}$ channel consists of a mitochondrial inward rectifying K^+ channel (mitoKIR) and sulfonylurea receptor (mitoSUR) facing the cytosol. In the presence of Mg^{2+} , the channel is inhibited by ATP, ADP, and long-chain acyl CoA esters but can be reactivated by GDP or GTP, the latter being the most potent (Bernardi 1999).

Inoue et al. (1991) performed a patch-clamp study of rat liver mitoplasts where a K^+ -selective channel was described to be inhibited by the plasma membrane K_{ATP} channel blocker glybenclamide and according to Inagaki et al. (1995), glybenclamide acted through a specific receptor that modulates the K_{ATP} channel.

The mitochondrial K_{ATP} channel has had insightful new implications for the physiological role of the mitochondrial K^+ cycle (Garlid, 1996). When open, the mitochondrial K_{ATP} channels shifted the balance from the K^+ uniport to the K^+/H^+ exchanger (KHE) catching up with the higher rate of K^+ influx (Brierley, 1978).

The primary role of the KHE was to compensate for unregulated K^+ leakage into the matrix making the KHE responsible for volume homeostasis (Garlid and Paucer, 2001). They also stated that KHE was essential for maintaining vesicular integrity in the face of high ionic traffic for the alkali cations including Na^+ , K^+ , Li^+ , Rb^+ , and Cs^+ across the inner membrane. The KHE was stimulated by an alkaline pH of up to pH 8.3 (Nakashima and Garlid, 1982) and allosterically regulated by matrix protons

(Beavis and Garlid, 1990). Garlid and Paucek (2001) stated that KHE is inhibited by a wide variety of amphiphilic amine, including phenothiazines, antidepressants, antihistamines, antiarrhythmics, and local anesthetics.

At the onset of experimentation, the role of the potassium cycle within fowl spermatozoa was unknown. Therefore, this thesis outlines a series of experiments in which the effect of 40 mM KCl on sperm motility was determined. Additionally, the research determined whether fowl sperm contain mitochondrial K_{ATP} channels.

Analysis of the Effect of the Potassium Ion on Fowl Sperm Motility

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ABSTRACT

Fowl sperm are believed to be immotile prior to ejaculation. The concentration of potassium found in deferent duct fluid is approximately 8 times higher than that found in blood plasma, which is 5 mM in *Gallus domesticus*. The effect of ionic potassium on fowl sperm motion was tested in a series of five experiments. Initially, the following argument was made: if 40 mM KCl inhibited sperm motility in vitro, then deferent duct K^+ might act to inhibit sperm motility prior to ejaculation. A preliminary experiment demonstrated that extracellular potassium had no effect on sperm mobility ($P \geq 0.05$). Greater replication was used in the second experiment, and, the same outcome was observed ($P \geq 0.05$). The third experiment confirmed that K^+ had no latent effect on sperm motility as measured by motile concentration with the Hobson Sperm Tracker over a 20-min interval ($P \geq 0.05$). The fourth experiment was a preliminary experiment in which the effect of 1% (vol/vol) dimethyl sulfoxide (DMSO) on motile concentration had no effect ($P \geq 0.05$). Experiment five demonstrated that glybenclamide, a mitochondrial K^+_{ATP} channel blocker, decreased sperm motility in a dose-dependent manner ($P \leq 0.001$). This experimental outcome was consonant with the predicted effect of sulfonylureas on mitochondrial volume and respiration. In summary, the notion was rejected that a high concentration of K^+ within deferent duct fluid inhibits sperm motility. However, the collective data sets may afford an explanation for the origin of deferent duct fluid K^+ , which remains a mystery. Fowl deferent duct fluid contains millimolar amount of glutamate, and fowl sperm have N-methyl-D-aspartic acid (NMDA) channels. It is proposed that K^+_{ATP}

and NMDA channels act in concert to enable a K^+ efflux from sperm during sperm maturation in the excurrent ducts of the testis.

(Key Word: potassium, fowl, sperm motility, mitochondria)

INTRODUCTION

Fowl sperm are considered to be immotile in deferent duct fluid throughout the vas deferens (Ashizawa and Sano, 1990). Fowl deferent duct fluid's primary electrolytes include sodium, calcium, and potassium (Lake, 1984). The concentration of K^+ in fowl deferent duct fluid is approximately 3 to 8 times higher than the level of potassium found in blood plasma, which is 5 mM in *gallus domesticus*. (Lake, 1966). Thus, the concentration of K^+ in deferent duct fluid would be considered hyperkalemic.

Morisawa and Morisawa (1990) explained that K^+ , even at a lower concentration found in seminal plasma of chum salmon, rainbow trout, masu salmon, and char, inhibited sperm motility. Spermatozoa from these species then became motile when diluted in a K^+ free medium. Therefore, spermatozoa taken from the male reproductive organ of these species appear to be immotile due to the high concentrations of potassium. We hypothesized that if extracellular potassium at a concentration similar to that found in fowl deferent duct fluid could inhibit the motility of ejaculated sperm, then potassium may be an agent that inhibits sperm motility prior to ejaculation.

MATERIALS AND METHODS

New Hampshire roosters ($n = 40$) were used as semen donors. All roosters were housed in individual cages measuring 48 x 30 x 60 cm (L x W x H) with 14 hr light: 10 hr dark. Feed and water were provided *ad libitum* and they were 30 to 36 weeks of age. All reagents were purchased from Sigma Chemical Co., St Louis, MO unless noted.

The first experiment entailed a paired comparison using an ejaculate from each of 40 males. Sperm concentration and mobility were determined spectrophotometrically according to Froman and Feltmann (1998). Sperm were diluted to a concentration of 5×10^8 sperm per mL with either 50 mM N-Tris (hydroxymethyl)methyl-2-aminoethanesulfonic acid (TES), pH 7.4, containing 128 mM NaCl and 2 mM CaCl_2 or 50 mM TES, pH 7.4, containing 40 mM KCl, 88 mM NaCl, and 2 mM CaCl_2 . Data were analyzed using a paired t-test with 39 df (Ramsey et al., 2002). The experiment was repeated in which a nested design was used, i.e. duplicate observations were made per treatment per ejaculate. In this case, data were analyzed using nested ANOVA (Ramsey et al., 2002).

The third experiment used an ejaculate from each of 10 males diluted to 5.0×10^8 sperm per mL with 50 mM TES pH 7.4, containing 40 mM KCl, 88 mM NaCl, and 2 mM CaCl_2 . This medium was also used to prepare sperm for computer-assisted sperm motion analysis as outlined by Froman and Feltmann (2000). Sperm suspensions were incubated at 41°C and motile concentration was measured after 5, 10, 15, and 20 min incubation. Data were analyzed by two-way ANOVA (Ramsey et al., 2002).

The fourth experiment entailed a paired comparison using ejaculates from 15 males. Sperm suspensions were prepared for computer-assisted sperm motion analysis according to Froman and Feltmann (2000). A 300- μL subsample was pipetted into each of two 12 x 75 mm culture tubes. A 3- μL volume was removed from each tube. Thereafter, either a 3- μL volume of 50 mM TES, pH 7.4, containing 128 mM NaCl and 2 mM CaCl_2 was returned (control) or a 3- μL volume of dimethyl sulfoxide (DMSO). The motile concentration within each suspension was measured after preincubating the test tube for 2 min at 41°C. The effect of 1% (vol/vol) DMSO on motile concentration was determined by ANOVA using a randomized complete block design (Ramsey et al., 2002)

The fifth experiment used ejaculates from each of 10 males. Sperm suspensions were prepared for computer-assisted sperm motion analysis according to Froman and Feltmann (2000). A 300- μL subsample was pipetted into each of four 12 x 75 mm culture tubes. A 3- μL volume was removed from each tube and replaced with either DMSO or glybenclamide in DMSO to create final concentrations of 0, 75, 150, or 300 μM (wt/vol) glybenclamide in 1% (vol/vol) DMSO. The motile concentration within each suspension was measured after preincubating the test tube for 2 min at 41°C. Data were analyzed by ANOVA using a randomized complete block design (Ramsey et al., 2002)

RESULTS

The first experiment demonstrated no significance difference ($P \geq 0.05$) in sperm mobility in response to 40 mM K^+ (Table 2.1). The population mean and standard deviation of the control group were estimated to be 0.54 ± 0.203 absorbance units,

respectively. The population mean and standard deviation of the sperm treated with K^+ were estimated to be 0.56 and 0.194 absorbance units, respectively.

TABLE 2.1: Effect of 40mM K^+ on sperm mobility

¹Measured by sperm penetration of 6% (wt/vol) Accudenz at 41°C from an overlay of extended semen; sperm penetration induced a change in absorbance at 550 nm.

Treatment	Roosters (n)	Sperm mobility ² (absorbance)
Control	40	0.54 ± 0.203
40 mM KCl	40	0.56 ± 0.194

²Each value is a mean ± SEM.

Treatment of sperm with 40 mM K^+ had no effect ($P \geq 0.05$) as evidenced by nested ANOVA (Table 2.2).

TABLE 2.2: Summary of nested ANOVA testing effect of 40 mM K^+ on sperm mobility¹

Source of variation	Degrees of freedom	Sum of squares	Mean square	F-value
Rooster	39	2.89	0.07	7.61****
Treatment	40	0.39	0.01	0.74

¹Duplicate observations of sperm mobility were made per treatment (control versus 40 mM K^+) per ejaculate from each of 40 males.

**** $P \leq 0.0001$.

Table 2.3: Summary of nested ANOVA testing effect of 40 mM K⁺ on motile concentration over a 20-min incubation at body temperature¹

Source of variation	Degrees of freedom	Sum of squares	Mean square	F-value
Rooster	9	0.803	0.089	3.93*
Time	3	0.013	0.004	0.20

¹ The effect of time was tested by making measurements after 5, 10, 15, and 20-min of incubation at 41° C.

* $P \leq 0.01$.

The population mean and standard deviation of the sperm treated with K⁺ were estimated to be 0.56 and 0.157 absorbance units, respectively. Experiment three (Table 2.3) demonstrated that K⁺ did not have a latent effect on sperm motility ($P \geq 0.05$).

Experiment four showed no significant difference in motile concentration between the control and 1% DMSO ($P \geq 0.05$). The population mean and standard deviation of the control group were estimated to be $1.19 \pm 0.189 \times 10^6$ sperm per mL, respectively. The population mean and standard deviation of the sperm treated with 1% DMSO were estimated to be 1.18 ± 0.230 sperm per mL, respectively (Table 2.4). In experiment five (Table 2.5), the four concentrations of glybenclamide demonstrated a dose response on motile concentration ($P \leq 0.0001$).

TABLE 2.4: Effects of 1% DMSO on sperm motile concentration^{1,2}

Treatment	Roosters (n)	Motile Concentration (x10 ⁶ /mL)
Control	15	1.19 ± 0.19
1% DMSO	15	1.12 ± 0.23

¹Each value is a mean ± SEM.

²Computer-assisted sperm motion analysis used to fine MOTC.

Table 2.5: Effect of glybenclamide on motile concentration¹

Glybenclamide (μ M)	Motile Concentration (x 10 ⁶ /mL) ²	Predicted motile sperm (%) ³
0	1.11 ± 0.05 ^A	92.5
75	0.80 ± 0.04 ^B	66.7
150	0.56 ± 0.05 ^C	46.7
300	0.19 ± 0.03 ^D	15.8

¹ Measured with a Hobson Spermtracker.

²Each value is a mean ± SEM.

^{A,B,C,D} Means within a column differed at $P \leq 0.0001$.

³ Based on concentration 1.2×10^6 sperm/mL in sperm suspension.

DISCUSSION

Sperm mobility is the net movement of a sperm cell population against resistance at body temperature. Froman and Feltmann (2000) used computer assisted sperm motion analysis to demonstrate that motile concentration was correlated with sperm mobility. Motile concentration reflects the behavior of individual sperm. Both sperm

mobility and motile concentration were used as endpoints in the present research.

The high mobility males were determined prior to these experiments using the mobility assay as outlined by Froman et al. (1999).

The primary objective was to determine if the concentration of potassium found in deferent duct fluid inhibited sperm mobility. A large number of replicate males were used in the first experiment. A paired comparison was conducted using all the high mobility males on hand. No difference was found ($P \geq 0.05$). To validate this result the experiment was repeated with greater replication. The sperm mobility within each individual ejaculate was measured in duplicate per treatment. The third experiment confirmed the conclusion that 40 mM potassium does not inhibit sperm motion. Using the related variable, motile concentration, it was learned that there was no latent effect as opposed to an immediate effect. The results of these three experiments provide strong evidence that the high potassium is not what inhibits sperm motility in the deferent ducts.

Having demonstrated that extracellular K^+ had no effect on sperm motion, the effect of intracellular K^+ remained a question. The concentration of intracellular K^+ is 180 mM (Garlid, 1996). We hypothesize that the high concentration of K^+ found in fowl deferent duct fluid originates from the sperm cells, in particular the mitochondria. In general, potassium cycles between the cytoplasm and mitochondrial matrix in living cells (Garlid, 1996). Therefore, we believe that potassium leaves the sperm mitochondria and due to the limited cytoplasm the potassium does not readily return and eventually enters the deferent duct fluid. The potassium concentration in the mitochondrial matrix is estimated to be 180 mM (Garlid, 1996). Therefore, prior to

ejaculation, there could be a net loss of potassium from the sperm cells to deferent duct fluid.

According to Halestrap (1994), sulphonylureas, e.g. glybenclamide, decrease K^+ entry into the mitochondria leading to an inhibition of respiration and fatty acid oxidation, resulting in a decrease in sperm motile concentration. Experiment five demonstrated that glybenclamide does decrease fowl sperm motile concentration in a dose dependent manner, demonstrating that fowl sperm mitochondria have K^+_{ATP} channels ($P \leq 0.0001$). Garlid (1996) stated that mitochondrial K^+_{ATP} (mito K^+_{ATP}) channels consist of an inward-rectifying K^+ channel (mitoKIR) and a sulphonylurea receptor (mitoSUR).

Garlid et al. (1996) found that the mitoSUR faces the cytosol and has regulatory sites for Mg^{2+} , GTP (an agonist), and long-chain acyl CoA esters or ATP (antagonist). We hypothesize that the mito K^+_{ATP} channels are closed as the immotile sperm travel through the vas deferens due to the high levels of ATP present. The mito K^+_{ATP} channel is inhibited by physiological concentrations of ATP (Edwards and Weston, 1993). The KHE on the inner mitochondrial membrane uses H^+ to drive K^+ out of the mitochondrial matrix and eventually into the deferent duct fluid. The efflux of K^+ via the KHE passes through the outer mitochondrial membrane through non-specific porin by facilitated diffusion (Bernardi, 1999). Glutamate activates the NMDA-receptor channels, which are permeable to Ca^{2+} , Na^+ , and K^+ (Aidley and Stanfield, 1996), on the plasma membrane of spermatozoa. Therefore, the millimolar amounts of glutamate found in fowl deferent duct fluid (Freeman, 1984) can activate the NMDA

channels allowing a K^+ efflux resulting in the high K^+ concentration in the deferent duct fluid.

According to Ashzawa and Wishart (1987), calcium is the main stimulating factor for sperm motility. Using a calcium chelator, Froman (2003) determined that sperm incubated in a calcium-depleted medium were eventually rendered immotile. He concluded that it was the efflux of Ca^{+2} that enabled a progressively smaller population of sperm to be motile at any give time point. Froman (2003) also demonstrated that long-chain fatty acids, produced by Ca^{+2} driven phospholipase A_2 (PLA_2), are essential for fowl sperm motility. When long-chain fatty acids are not produced, β -oxidation within the mitochondrial matrix cannot occur, therefore in accordance with Halestrap (1994), ATP is not synthesized and sperm are immotile.

In conclusion, our data demonstrated that the high K^+ content in deferent duct fluid is not what inhibits sperm motility in vivo. However, the potassium flux across the inner mitochondrial membrane affects sperm motility.

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CONCLUSION

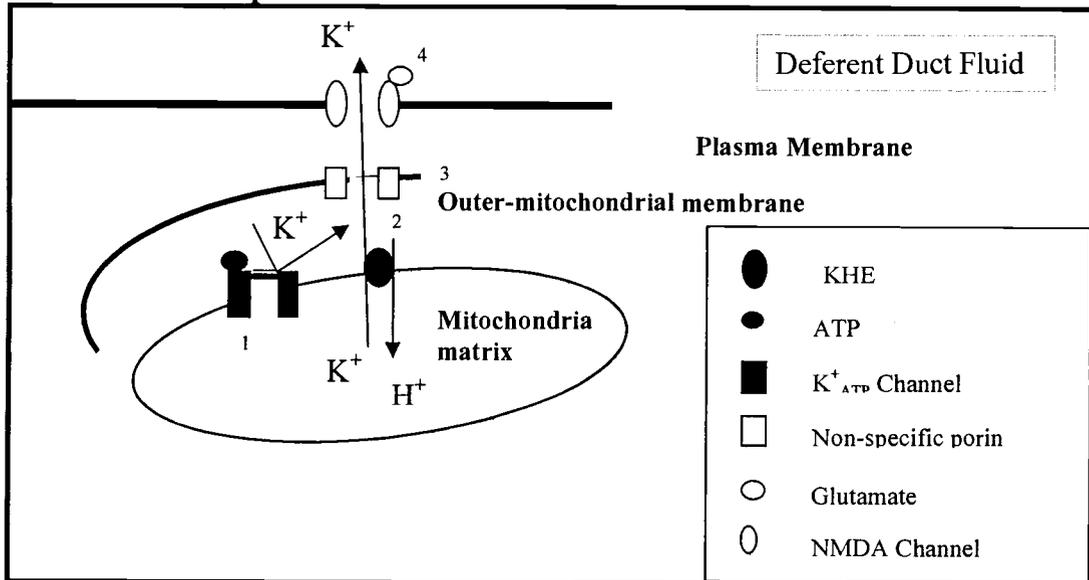
The initial hypothesis in this thesis was that sperm motility might be inhibited by 40 mM KCl, a level comparable to that in deferent duct fluid. However, that proved not to be the case as evidenced by a series of experiments. Therefore, the reason for the high concentration of potassium found in fowl deferent duct fluid is still not understood.

However, I have proposed a model from the collected data explaining the possible origin of the high potassium in the deferent duct fluid. Earlier two possible explanations were proposed: either the potassium arises as an osmolyte from excurrent duct epithelium or it originates from the sperm's mitochondria. My model (Fig 3.1) proposes the latter. According to Ashizawa (1988), sodium and potassium increase

and decrease respectively within the mid-piece of fowl spermatozoa as they pass through the deferent duct.

My final experiment demonstrated that fowl sperm mitochondria have K^+_{ATP} channels. My model proposes that as immotile sperm travel through the deferent duct, the $mitoK^+_{ATP}$ channel is closed, blocking K^+ influx. Inhibition of potassium entry into the mitochondria allows the KHE to dominate using H^+ to drive K^+ out of the mitochondrial matrix (Halestrap, 1994). The efflux of K^+ passes through the outer mitochondrial membrane then the plasma membrane and out into the deferent duct fluid via facilitated diffusion and *N*-methyl-D-aspartic acid (NMDA) channels respectively (Figure 3.1).

FIGURE 3.1: Proposed model for K^+ efflux



¹ $MitoK^+_{ATP}$ channel closed via ATP keeping K^+ outside the mitochondrial matrix.

² The KHE contributes to the K^+ efflux from mitochondrial matrix.

³ K^+ passes through the outer-mitochondrial membrane via facilitated diffusion.

⁴ Glutamate binds to and activates the NMDA channel. K^+ enters deferent duct fluid.

Glutamate activates the NMDA channels on the plasma membrane of the spermatozoa, which are permeable to Ca^{2+} , Na^+ , and K^+ (Aidley and Stanfield, 1996).

A millimolar amount of glutamate is found in fowl deferent duct fluid (Freeman, 1984). Therefore, glutamate might activate the NMDA channels and thereby enable a K^+ efflux resulting in the high K^+ concentration in the deferent duct fluid. The model, in part, also uses the potassium cycle to explain why fowl sperm are immotile prior to ejaculation. The KHE uses H^+ to drive K^+ efflux rather than driving ATP synthase. Therefore, ATP is not synthesized and sperm are immotile. This model is consistent with the relationship between mitochondrial volume and respiration rate (Halestrap, 1994).

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