

Preservation of Sperm Motility and Acrosomal Integrity during Transport of Semen for Artificial Insemination in Asian Elephants (*Elephas maximus*)

Wendy K. Kiso¹

Dennis Schmitt^{2,3}

Janine Brown¹

Deborah Olson⁴

Elizabeth Crichton⁵

Budhan Pukazhenth¹

¹ Center for Species Survival, Smithsonian's National Zoological Park, Washington D.C., USA

² Missouri State University, Department of Agriculture, Springfield, MO, USA

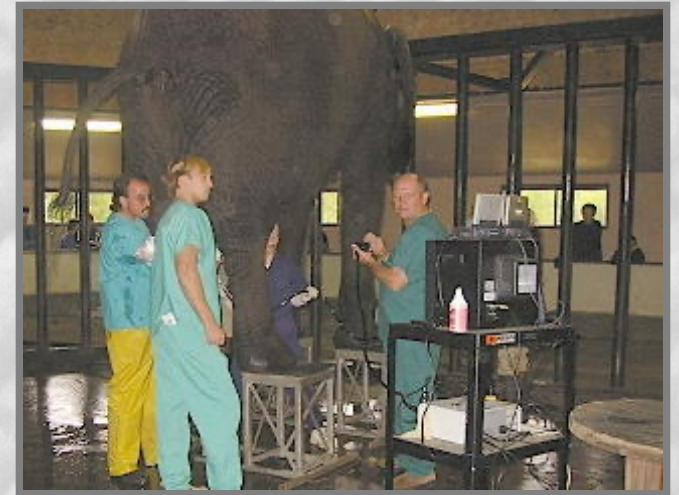
³ Ringling Bros. Center for Elephant Conservation, Polk City, FL, USA

⁴ Indianapolis Zoo, Indianapolis, IN, USA

⁵ XY Inc., Fort Collins, CO, USA

INTRODUCTION

- Artificial insemination (AI) has become an important technology for genetic management of captive elephants
- Semen storage and cryopreservation are important research priorities for elephant conservation
 - Genome Resource Bank (GRB)
 - Genetic diversity of *in situ* and *ex situ* populations



INTRODUCTION

- Semen storage remains a challenge
- Fresh-extended semen is routinely used for AI due to limited success with cryopreservation
- Fresh-extended semen often exhibit suboptimal sperm survival after short-term storage and transport (i.e. <12 h)
- Compromised sperm survival negatively impacts:
 - AI efficiency
 - Efforts to cryopreserve sperm
 - Progress in sperm sex sorting

OBJECTIVES

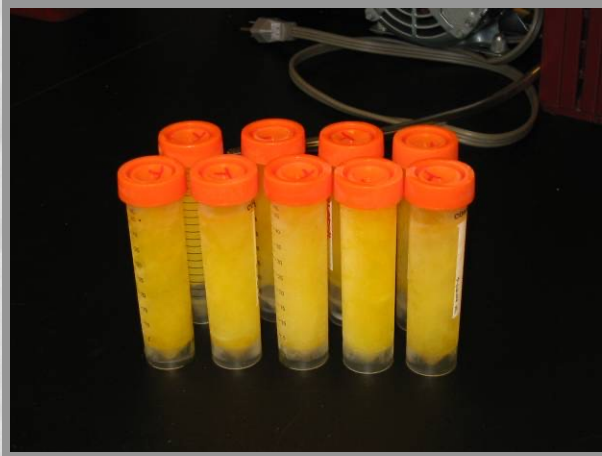
To identify:

- Consistent and reliable semen donors
- Optimal media and storage temperature to enhance sperm survival for:
 - Shipment for AI
 - Sperm cryopreservation
 - Sperm sex sorting



SEMEN EXTENDER

Diluent added to fresh semen to 'extend' and prolong sperm survival during storage (or transport)



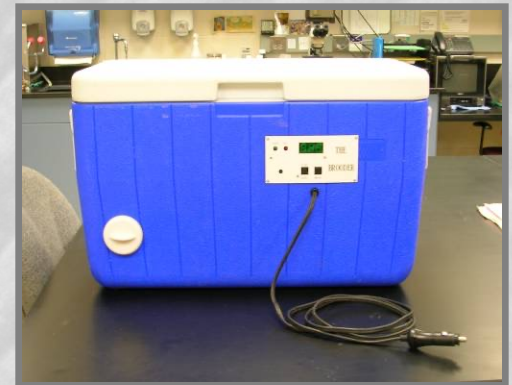
Contains various ingredients to help stabilize membranes and/or protect cells from external environment

(e.g. egg yolk/skim milk, buffers, sugars, antibiotics, detergents, etc.)

METHODS

Semen Collection

- Asian elephant bulls located at various facilities throughout North America
 - 12 Asian bulls; 136 ejaculates
 - 4 Asian bulls; 1 ejaculate/bull
- Semen was collected utilizing the rectal massage technique
- Ejaculates were collected into a modified palpation sleeve attached to an insulated 50 ml centrifuge tube
- The ejaculate was then immediately placed in an Brooder box maintained at body temperature (35° C)



METHODS

Ultrasound Assessments

Ultrasonography is used to assess the fullness of ampullae (site of sperm storage) before and after semen collection



FULL AMPULLAE



**PARTIALLY FULL
AMPULLAE**



EMPTY AMPULLAE

METHODS

Semen Evaluation

- Ejaculates are evaluated for:
 - overall quality (i.e. viscosity, color, +/- urine contamination)
 - volume (ml)
 - motility (% total and % progressive)
 - pH
 - osmolality (mOsm/L)
 - concentration ($\times 10^9$ spermatozoa/ml)
 - viability (Eosin-nigrosin)
 - **acrosome status** (SpermacTM stain)



SpermacTM stain, Asian elephant

ACROSOME STATUS EVALUATION

NORMAL

Intact



DAMAGED

Vesiculating



**Vesiculated &
Damaged
equatorial region**



Missing



METHODS: **Treatments**

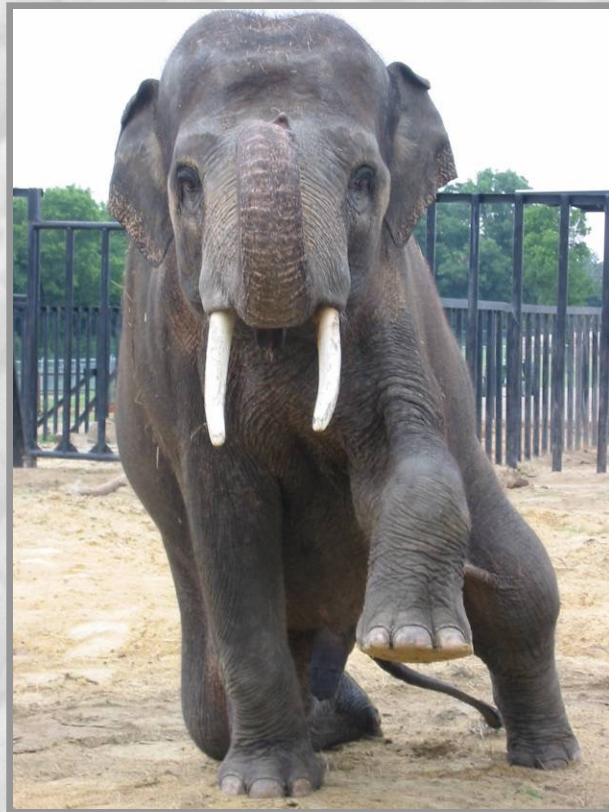
- Only ejaculates exhibiting $\geq 50\%$ total motility were included in the study
- Ejaculates were aliquoted into a 6 x 3 factorial design
 - 6 treatments:
 - Neat semen (Control)
 - TL-HEPES (BioWhittaker™)
 - TEST Yolk Buffer (Refrigeration Medium; Irvine Scientific)
 - Biladyl (Minitube)
 - Modena (SGI)
 - INRA 96 (IMV) + 4% egg-yolk
 - 3 storage temperatures:
 - Body temperature (35°C)
 - Ambient temperature (21-23°C)
 - Cooled (4-5°C)

METHODS

Assessments

- Spermatozoa were assessed for:
 - Motility (% total, % progressive)
 - Acrosome status
- Time intervals: 0, 1, 2, 4, 6, 12 and 24 h

RESULTS



SEMINAL CHARACTERISTICS

ASIAN ELEPHANTS (12 Bulls; N = 136 ejaculates)*

Volume (ml)	41.3 ± 6.1	(0-494)
Concentration (x 10⁹ sperm/ml)	1.1 ± 0.1	(1-2.7)
Ejaculate concentration (x 10⁹ sperm/ml)	43.5 ± 6.9	(25-302)
Total motility (%)	28.7 ± 2.1	(0-95)
Progressive motility (%)	24.5 ± 2.1	(0-95)
Normal spermatozoa (%)	54.8 ± 4.4	(0-95)
Acrosome intact sperm (%)	18.0 ± 3.3	(0-79)
Viability (%)	31.6 ± 3.8	(0-97)
Osmolality (mOsm)	258.4 ± 5.0	(81-561)
pH	7.2 ± 0.1	(4.8-9.5)

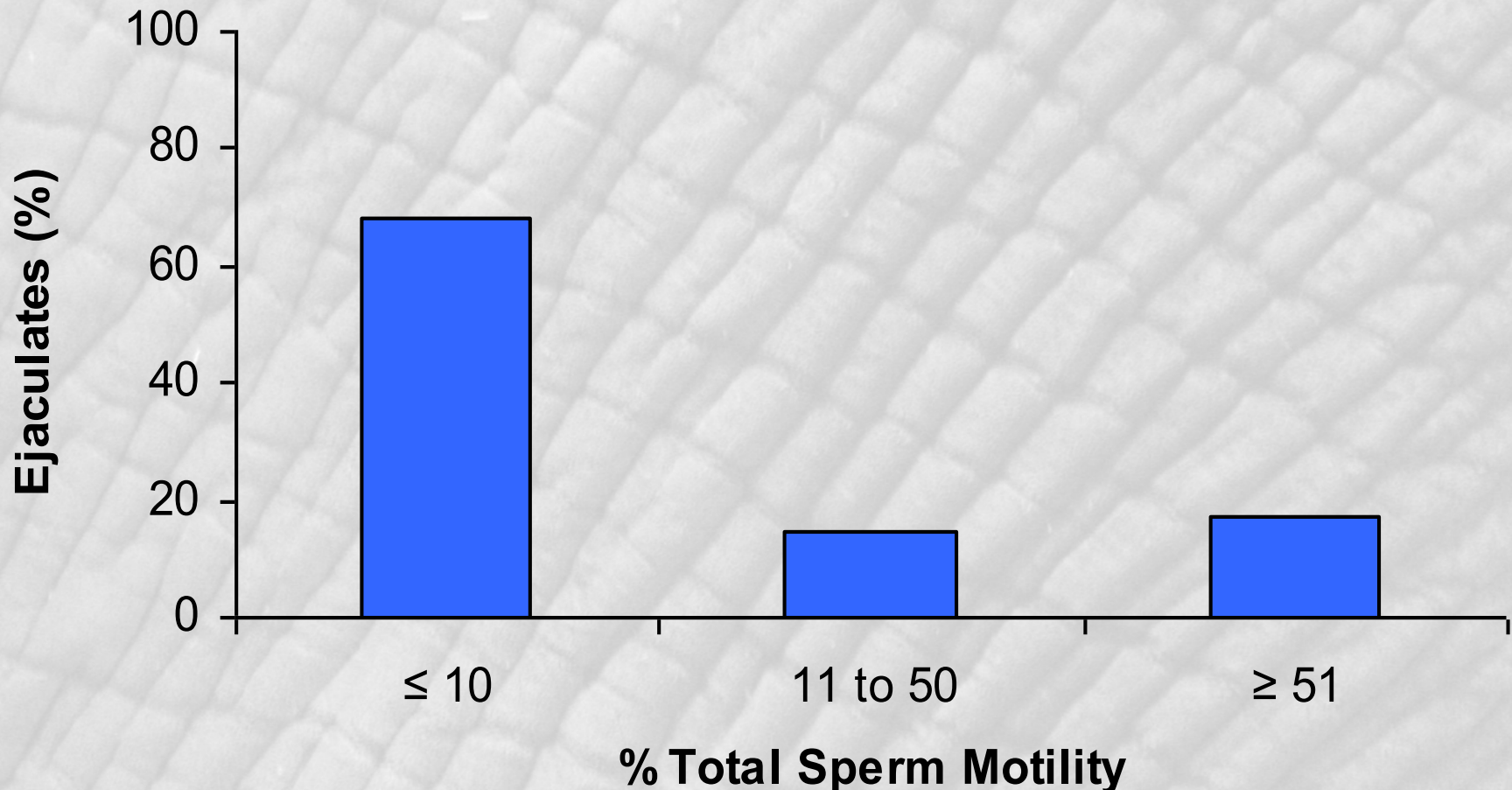
* **Samples collected from Jan 2006 – Present**

Values are Mean ± SE
Values in parenthesis = range



VARIATION IN EJACULATE QUALITY: **Sperm Motility**

ASIAN ELEPHANTS (12 Bulls; N = 136 ejaculates)*



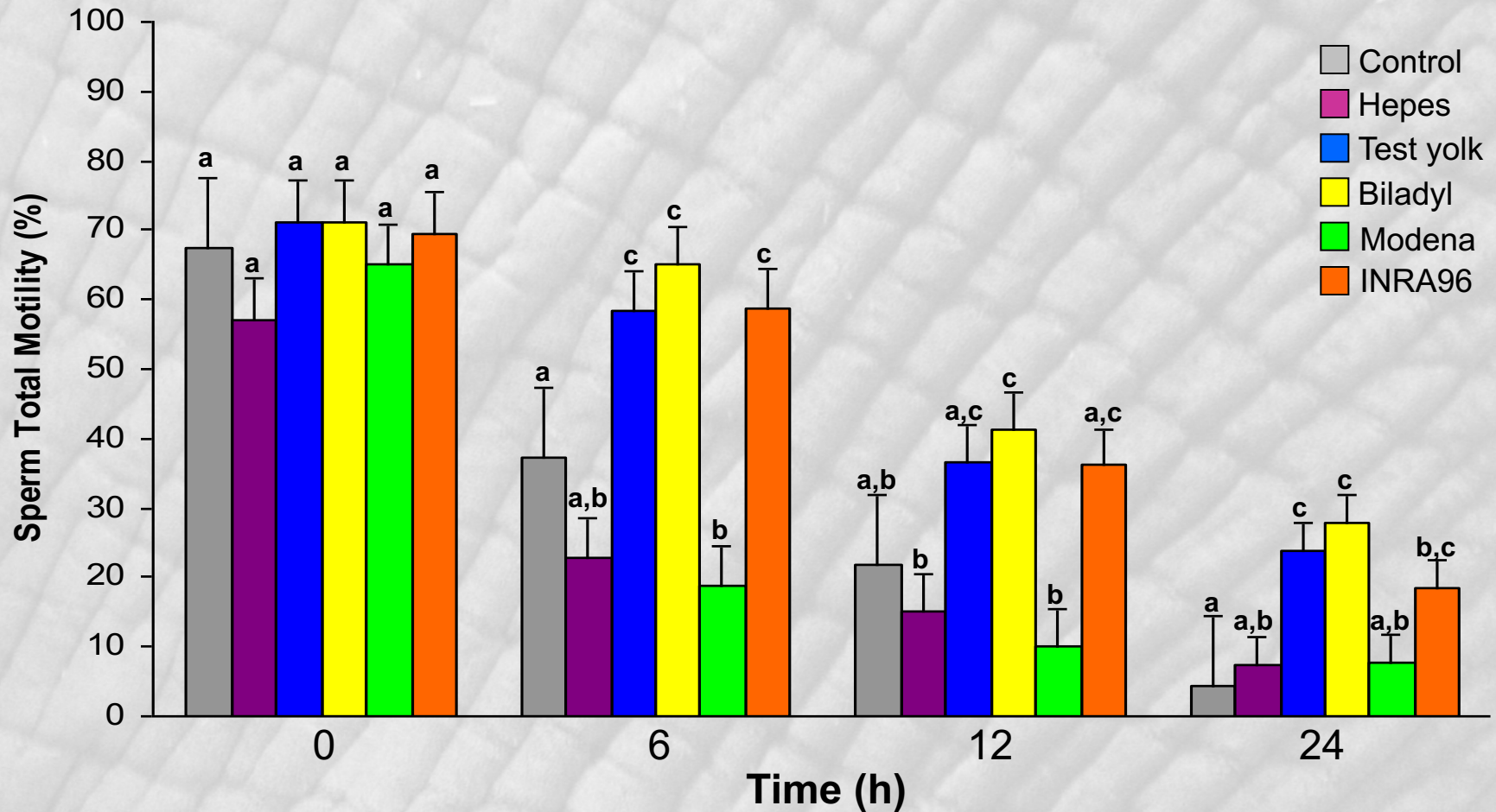
* Samples collected from Jan 2006 – Present

EXTENDER STUDY

ASIAN ELEPHANTS (n = 4 Bulls; 1 ejaculate/bull)

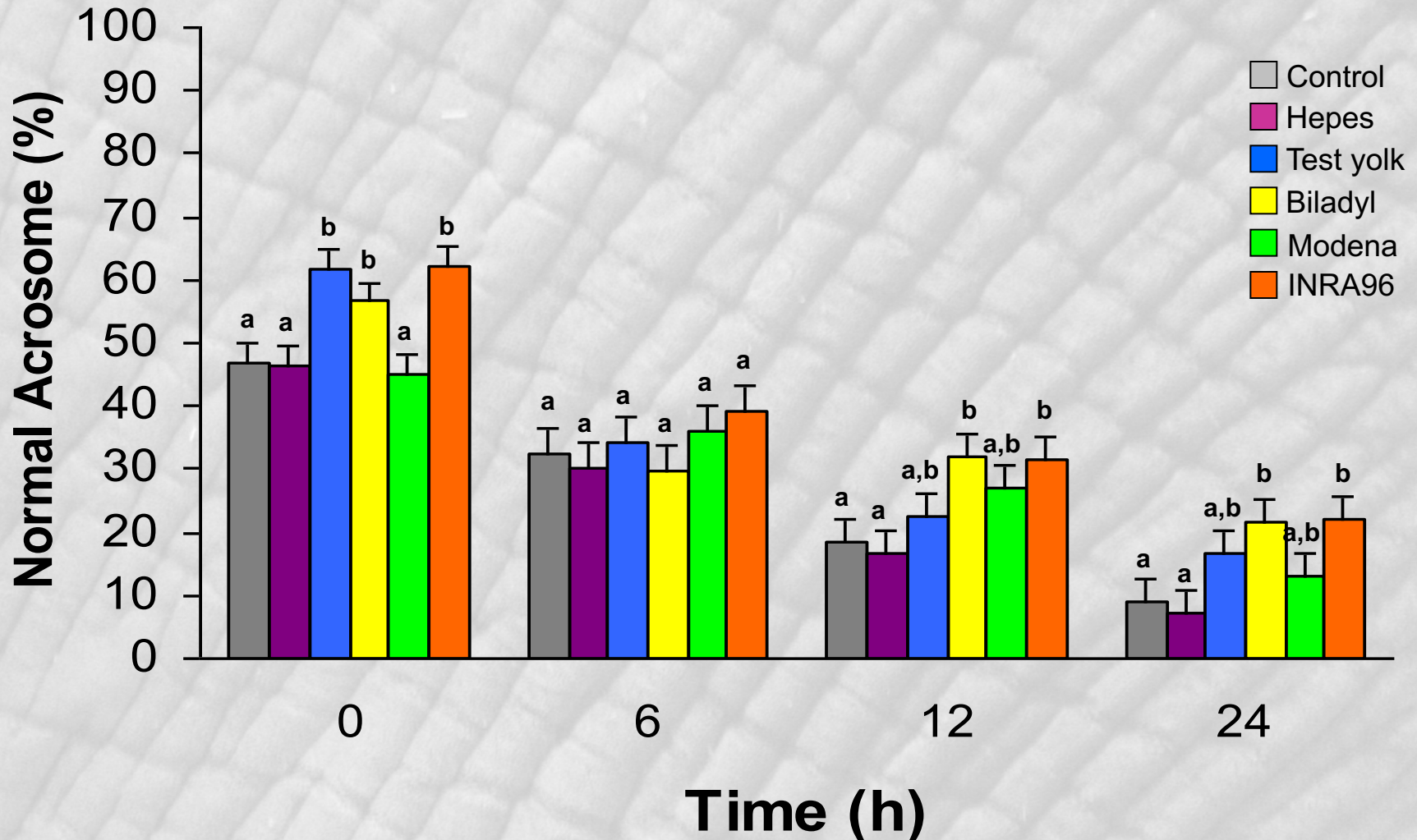
Bull	<u>EM 1</u>	<u>EM 2</u>	<u>EM 3</u>	<u>EM 4</u>
Volume (ml)	102	119	108	14
Concentration (x 10⁹ sperm/ml)	0.01	0.3	0.5	2.0
Ejaculate concentration (x 10⁹ sperm/ml)	1.0	12.3	18.6	28.0
Total motility (%)	90	90	75	75
Progressive motility (%)	90	90	70	74
Normal spermatozoa (%)	78	78	59	47
Acrosome intact sperm (%)	63	70	38	24
Viability (%)	53	46	63	21
Osmolality (mOsm)	283	269	255	274
pH	7.3	6.6	8.3	6.0

Effect of Storage Time on Sperm Motility



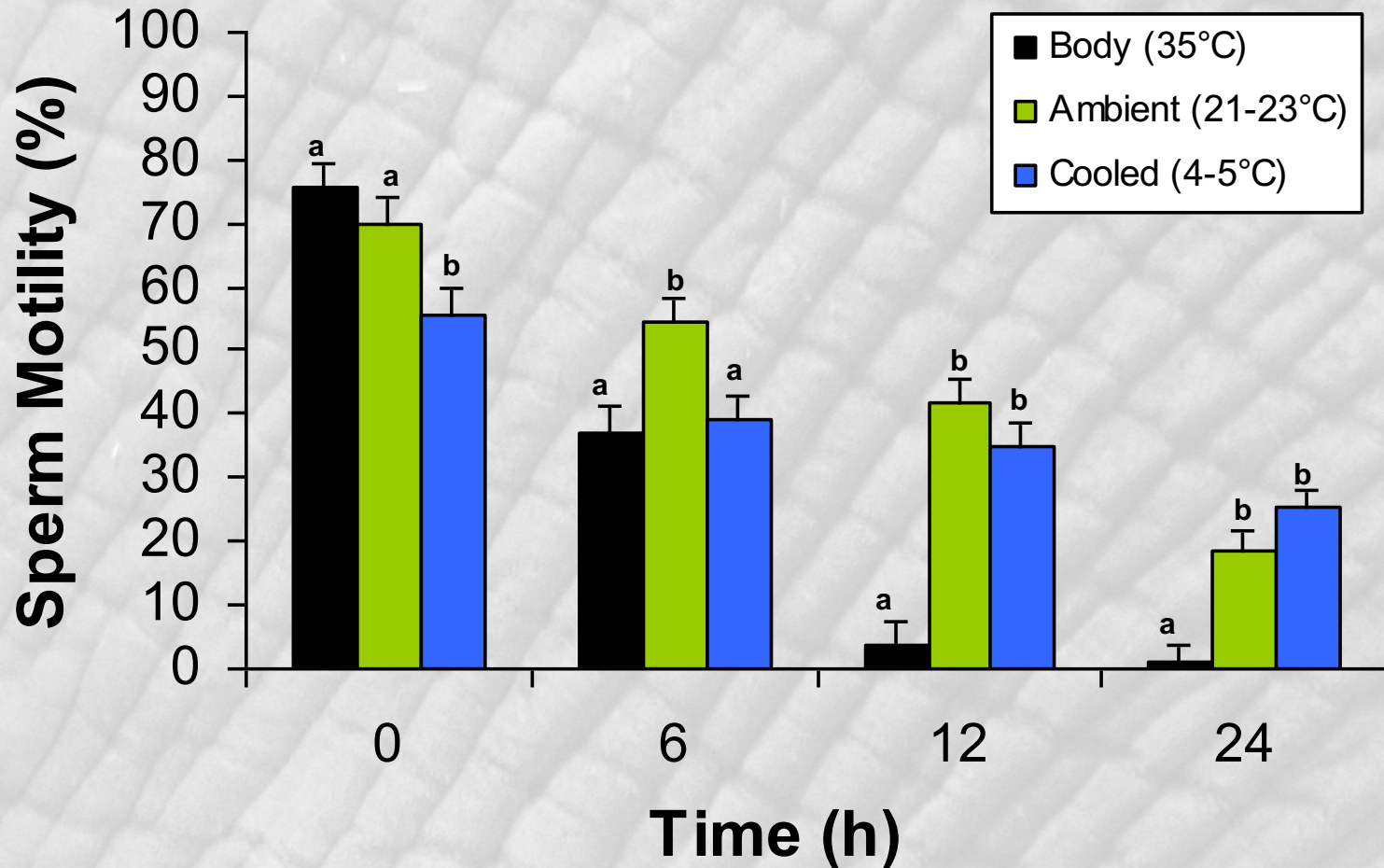
a,b,c Within a time interval, values with different superscript are different ($P < 0.01$).
Values are Mean $\pm$ SEM.

Effect of Storage Time on Acrosome Status



a,b,c Within a time interval, values with different superscript are different ($P < 0.01$).
Values are Mean $\pm$ SEM.

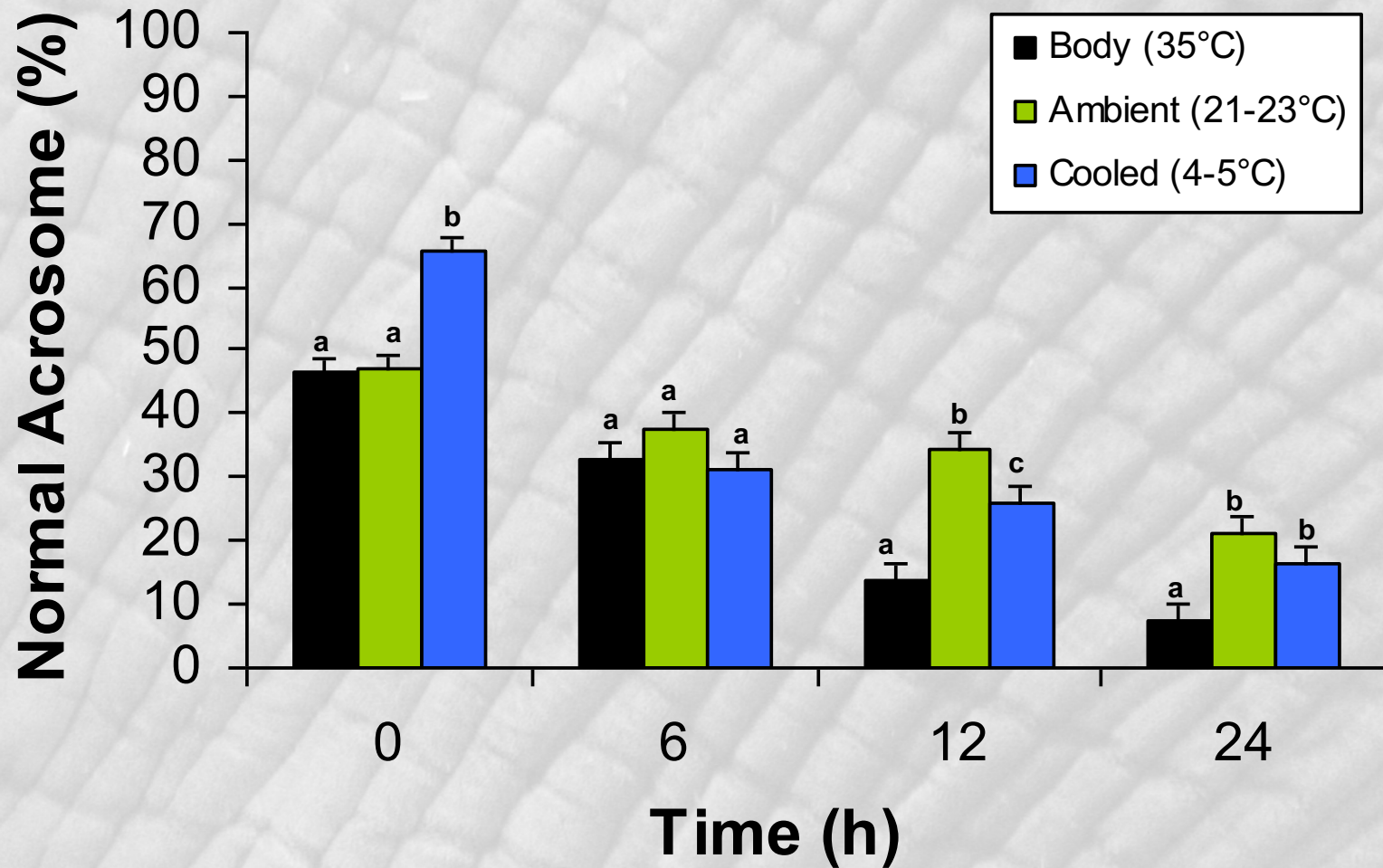
Effect of Storage Temperature on Sperm Motility



a,b,c Within a time interval, values with different superscript are different ($P < 0.01$).

Values are Mean $\pm$ SEM.

Effect of Storage Temperature on Acrosome Status



a,b,c Within a time interval, values with different superscript are different ($P < 0.01$).
Values are Mean $\pm$ SEM.

CONCLUSIONS

- More bulls and samples must be assessed
- Only 18% of samples expressed >50% total motility
- Asian elephant spermatozoa (i.e. sperm motility and acrosome) exhibit a preference for egg-yolk and skim milk extenders
- Storage below body temperature appears to be beneficial for maintaining sperm motility and acrosome status
- Semen shipment <12h at ambient temperature appears to be best at maintaining both sperm motility and acrosome status

ACKNOWLEDGMENTS

- Participating Institutions:

- Ringling Bros. Center for Elephant Conservation
- Riddle's Elephant Sanctuary
- Fort Worth Zoo
- Tulsa Zoo
- Columbus Zoo
- African Lion Safari
- Rosamond Gifford Zoo

- Funding provided by:

- Feld Entertainment, Inc.
- AZA-Conservation Endowment Fund
- International Elephant Foundation (IEF)
- Indianapolis Zoo
- Friends of the National Zoo (FONZ)



ANY QUESTIONS?

