

Historical Control Database of Cynomolgus Macaque Spermatozoa

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ABSTRACT

The assessment of sperm is useful to characterize potential adverse effects of pharmaceuticals on the reproductive system in males. Standard sperm assessments include motility, concentration, and morphology. Although the cynomolgus macaque is a common test species, in particular for biotechnology-derived pharmaceuticals, ICH S5(R2) highlights that historical background data is lacking for non-human primates (NHP) in this area. NHP semen is at this time evaluated based largely on the WHO Laboratory Manual for the Examination and Processing of Human Semen. Few publications exist describing normal cynomolgus macaque sperm, which coupled with the low numbers of animals typically used on studies, limit the translatability to humans of any potential effects observed in monkeys. Establishing robust criteria for normal NHP spermatozoa is crucial to accurately interpreting potential toxicological effects. To alleviate this gap, sperm concentration, motility, and morphology were collated from over 400 NHPs between 2007 and 2019 to generate an historical control database to assist in the interpretation of cynomolgus macaque sperm. All animals were either naïve or had been dosed with a control/sham article. Sperm was collected via direct penile electrostimulation. Ejaculate volume was recorded, and sperm motility and concentration were assessed using the TOX IVOS or TOX IVOS II computerized assisted sperm analysis (CASA) systems. Morphology was evaluated using light microscopy. Descriptive statistics (mean, SD, and quartile ranges) were generated to summarize the normal ranges for each parameter.

INTRODUCTION

Per ICH S5(R2), sperm motility, concentration, and morphology examinations with cynomolgus macaques can be used as screening for reproductive toxicology. However, little research has been published describing the normal characteristics and behavior of the macaque spermatozoa, leaving interpretation and identification of macaque sperm changes difficult to evaluate. Using over 10 years of historical macaque data, sperm motility, concentration, and morphology were analyzed to better describe the normal human spermatozoa for future toxicological research. Male reproductive procedures are typically based on human guidelines from the WHO. General comparisons were made to human values taken from an unscreened male population described by Cooper TG et al. (2010), which is currently used as the standard human reference range by the WHO.

METHODS AND MATERIALS

1. Animals and Animal Care

Animal model: Macaca fascicularis, male, 4-8 years old at time of collection

Source: Cambodia, China

Environmental conditions: Primary enclosure complies with the Animal Welfare Act and recommendations set forth in the Guide for the Care and Use of Laboratory Animals (National Research Council 2011). Animals were housed in a temperature and humidity-controlled environment with a target range of between 18 and 29 degrees C and 30 and 70%, respectively. A 12-hour light/dark cycle was set, and animals were kept in stainless steel metal cages.

Diet: PMI LabDiet® Fiber-Plus® Monkey Diet 5049 biscuits, and water was provided ad libitum. Treats were provided daily and included fresh produce, marshmallows, raisins, juice, etc.

2. Semen Collection

Samples were collected once from awake, conscious animals via direct penile stimulation prior to dosing on study, or after dosing with a control article.

3. Semen Analysis

Semen was analyzed with Tox IVOS™ or IVOS II™ CASA systems (Hamilton Thorne Research, Beverly, MA) using identical calculations. Sperm motility (%) and concentration (10^6/mL) were determined. Sperm motility was assessed as total motility.

- Calculation of total sperm count (10^6/ejaculate): Concentration X Ejaculate Weight
- Ejaculate density assumed to be 1 for volume calculation purposes

4. Sperm Morphological Analysis

Samples were placed on slides, stained, and examined under a light microscope. At least 200 sperm were counted per slide using a cell counter. If present, the number of each type of abnormality was documented.

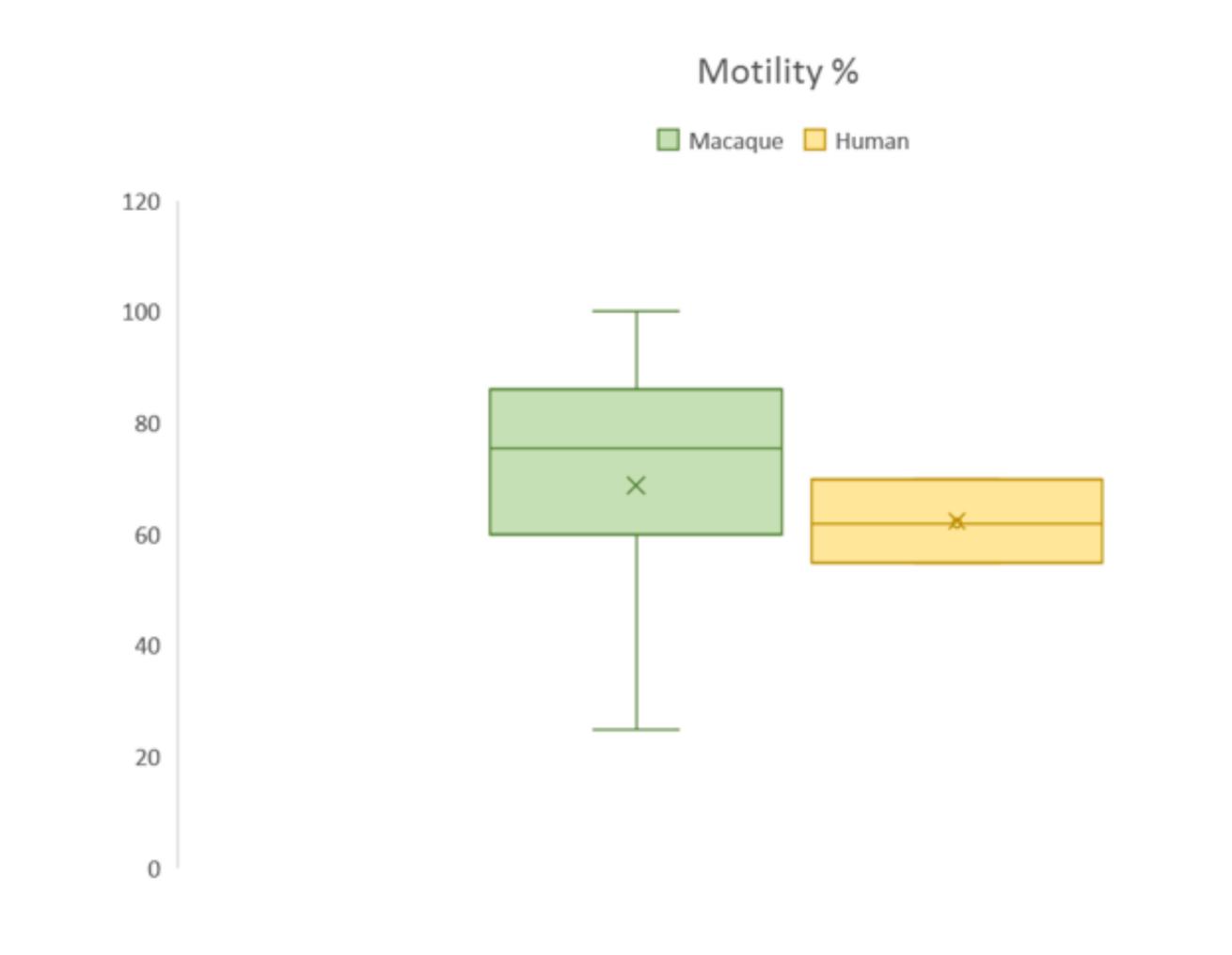
RESULTS

1. Motility

a. Motility data generated from 608 animals

b. Mean: 68.77%. Standard Deviation: 23.31%

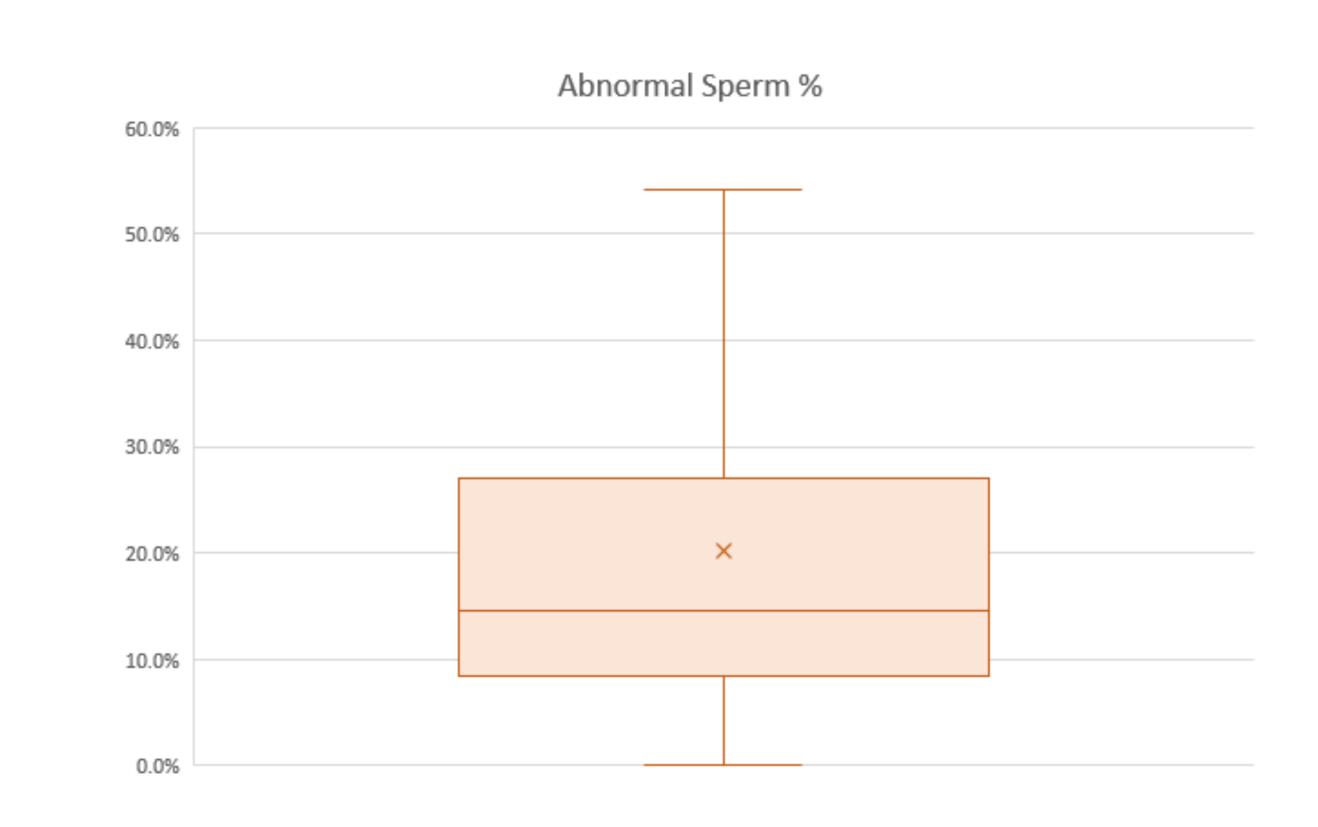
25th percentile: 60%, Median: 75.5%, 75th percentile: 86% c. Distribution:



distributed slightly higher than the human population (median: 62%), although the overall distribution for macaques may be wider than humans (25th percentile: 55%, 75th percentile: 70%), suggesting higher variability between macaques.

3. Sperm Morphology

- a. Abnormality occurrence
- Abnormality normal/abnormal ratio generated from 320 animals, displayed as percentage abnormal
- Mean: 20%. Standard Deviation: 17%
- 25th percentile: 8.5%, Median: 14.5%, 75th percentile: 27% b. Distribution:

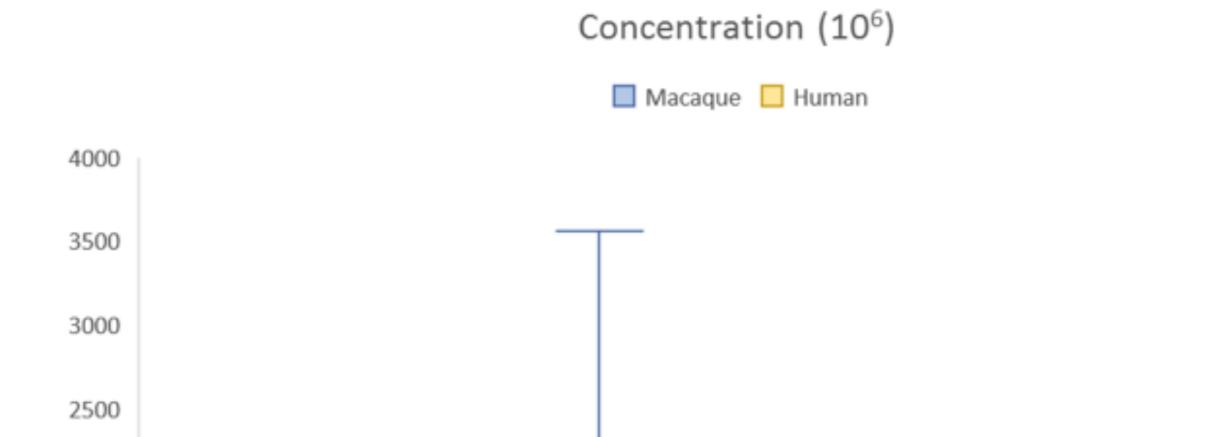


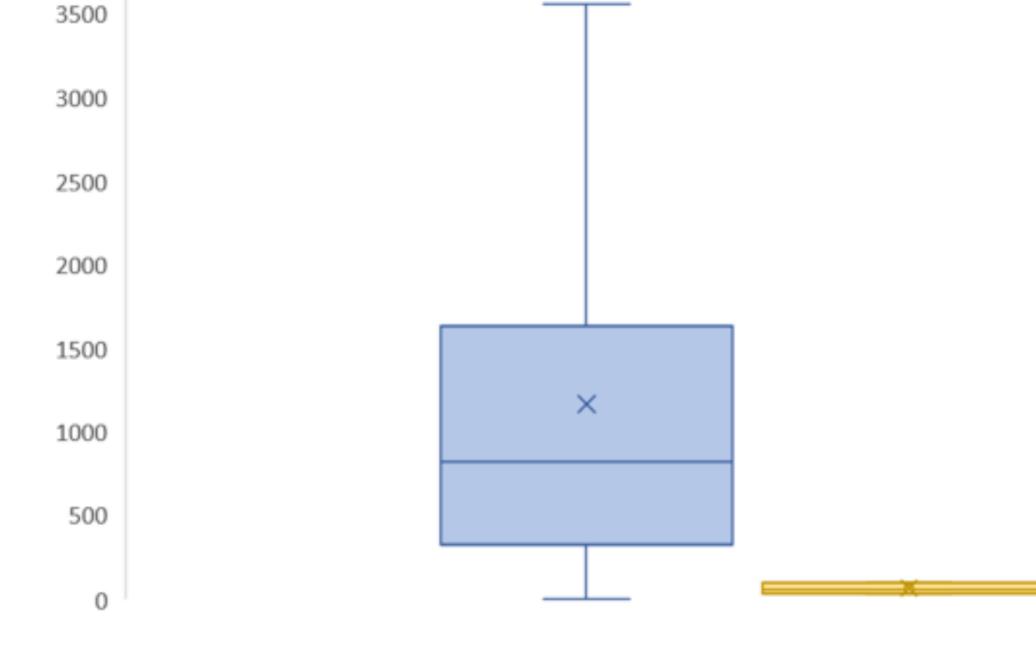
2. Concentration

a. Concentration data generated from 357 animals, reported in the millions (106)

b. Mean: 1166.36. Standard Deviation: 1181.13

25th percentile: 329.55, Median: 882.2, 75th percentile: 1633.4. c. Distribution:

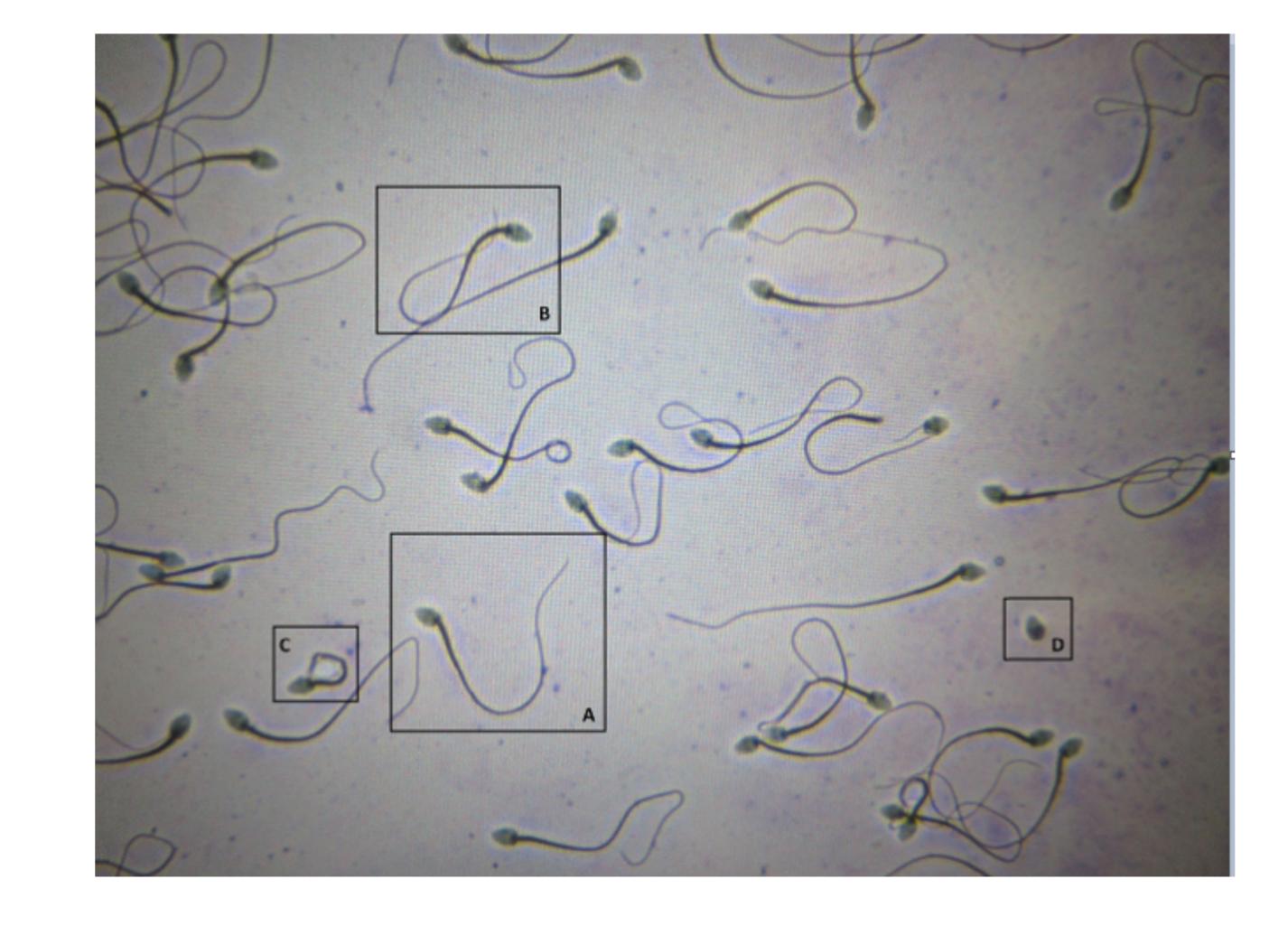




d.Results: The macaque population sampled may be d.Results: Macaque sperm concentrations were well beyond reported human concentrations (25th percentile: 36, median 62, 75th percentile: 100) However, similar to motility, the inter-animal variability was noted to be larger than humans as well. The 25th and 75th percentile concentrations were reported as -42% to 61% of the median value respectively, where the macaques ranged between -37 % to 85% of the median.

4. A stained slide of semen from a 6-year-old male Cambodian cynomolgus macaque. Viewed under a light microscope with 400x magnification

- A. A typical normal sperm
- B. A looped tail
- C. A coiled tail D: A head with no tail



5. Individual Abnormalities

a. Detailed sperm analysis performed on samples from 136 animals, with 200 cells counted for each.

MORPHOLOGY ABNORMALITIES PRESENT					
ABNORMALITY	% of sample population	Mean Count	Standard Deviation	Mean % of Abnormalities Present	Standard Deviation
LOOPED TAIL	100%	34.6	26.0	55%	22%
COILED TAIL	96%	21.6	21.6	31%	21%
BENT TAIL	65%	2.4	3.2	5%	6%
BENT MIDPIECE	47%	1.8	3.8	4%	7%
NO TAIL	24%	0.6	1.5	1%	4%
SHORT TAIL	17%	0.5	2.1	1%	4%
NO ACROSOME	7%	0.2	0.8	0%	2%
SMALL HEAD ^A	<1%	0.1	1.2	<1%	3%

A: NOTED IN ONE ANIMAL

RESULTS

For the purpose of reproductive toxicology studies, assessment of sperm morphology is typically much less strict than is seen with humans. Where actual measurements (e.g., tail length, head width) may be collected for the purpose of fertility testing in humans, toxicology studies typically aim to merely identify any general changes in the morphology over time. As such, the macaque data was not completed to the WHO standard, and acts as more of a screen than a true assessment. For toxicological research however, it should be noted that tail abnormalities occurred in all animals, and were the majority of abnormalities present (86%) with a high level of variability. Although large changes in tail structure should be noted, changes in non-tail abnormalities may be more useful in identifying toxicity.

CONCLUSIONS

- Intra-animal variability is quite large for both motility and concentration, so attention should be given when designing reproductive studies with small population sizes, with multiple acclimation/pretest collections taken to help ensure an accurate baseline.
- Further examination using repeated collections would be helpful in identifying if values remain consistent for each animal, or if other factors play a larger role.
- Although the WHO manual may provide a means to standardizing collection/analysis procedures, use of the macaque in toxicology research needs further data and evaluation to increase the accuracy of study data in regards due to the potential species differences.

1. Cooper TG et al. (2010). World Health Organization reference values for human semen characteristics. Human Reproduction Update, 16:231-245.

2. World Health Organization. WHO Laboratory Manual for the Examination and Processing of Human Semen 5th ed. (2010)