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Original article

A simple orchidometric method for the preliminary assessment of maturity status in male cynomolgus monkeys (*Macaca fascicularis*) used for nonclinical safety studies

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ABSTRACT

Introduction: The identification and use of mature male non-human primates in nonclinical toxicology studies could be important for evaluating candidate drugs for which the profile of toxicity may differ depending on sexual maturity. This investigation sought to establish operational criteria to complement the current standard of histological evaluation for defining sexual maturity in male cynomolgus monkeys *(Macaca fascicularis)* used for toxicology studies, and to identify a practical non-invasive measure to select mature males for study. **Method:** Retrospectively, the relationships between body weight, testicular weight and testis histology were established in control males ($n = 126$) used in previous toxicology studies. Prospectively, testicular volumes were measured in-life by orchidometry using comparative scrotal palpation ($n = 23$ males used for study), then compared to testicular weights measured at necropsy. **Results:** Consistent with previous literature, a weak relationship was observed between body weight and testicular weight. There was, however, a very good relationship between testicular weight and histological maturation level, which was based upon microscopic examination of testes, epididymides and prostates. Orchidometric measurement of testicular volume was found to be a reasonable predictor of testicular weight and served to rapidly select sexually mature males for study, and a total testicular volume (left and right combined) of >20 ml correlated with the histological appearance of maturity. **Conclusion:** Based upon this preliminary exploratory study, the initial simple measurement of testicular volume by orchidometry may provide a non-invasive alternative approach for assessing the sexual maturity of male cynomolgus monkeys in research colonies or during toxicology studies that will require more thorough validation.

1. Introduction

The selection of a non-human primate model for pharmaceutical nonclinical safety assessment finds importance in cases where metabolic and pharmacodynamic similarities to humans are a critical factor (Smith, Trennery, Farningham, & Klapwijk, 2001). The identification and use of mature male non-human primates could be important for evaluating candidate drugs for which the profile of toxicity may differ depending on sexual maturity (Smedley, Bailey, Perry, & O'Rourke, 2002). This is especially important for the evaluation of the reproductive organs as potential targets of toxicity. Accurate interpretation of study findings can be extremely difficult if there is uneven distribution of immature monkeys between treated and control groups. The interpretation is further complicated if there is a potential testicular toxicity concurrent with maturational variations of testicular histology (Dreef, Van Esch, & De Ruk, 2007).

Cynomolgus macaques (*Macaca fascicularis*) are the most common and preferred nonhuman primate model for safety assessment, consistent with their general use in biomedical research as an animal model for human diseases and its historical database and regulatory experience as a toxicology large animal species. In general, multiple characteristics are evaluated to definitively characterize sexual maturity in the male monkey. These include age, body weight, testicular size, histological evidence of spermatogenesis, presence of sperm ejaculate and various endocrine parameters (Honjo, Cho, & Terao, 1984; Steiner & Bremner, 1981; Meyer, Fitzsimmons, Hastings, & Chellman, 2006). Histologically, sexual maturation in male monkeys has been defined by the extent of spermatogenesis and relative completeness of spermiogenesis (Cho, Fujiwara, Honjo, & Imaizumi, 1973; Kluin, Kramer & de Rooij, 1983; Smedley et al., 2002; Dreef et al., 2007). In general, immature animals are defined by evidence of spermatogenesis but the absence of spermiogenesis (the production of haploid germ cells that eventually become sperm), while pubertal animals display the beginnings of spermiogenesis. Mature animals are

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identified by complete spermiogenesis, with the associated presence of epididymal sperm.

The sexual maturity status of male cynomolgus monkeys used for toxicity studies is generally assumed or estimated and often based on age, body weight, dentition and supplier records. The age of animals provided by suppliers if often inaccurate and therefore is considered an unreliable predictor of maturity. Testicular volume has proven to be a rough indicator for sexual maturity in humans and monkeys and is relatively easy to measure clinically using several methods (Behre, Nashan & Nieschlag, 1989; Fuse, Takahara, Ishii, Sumiya, & Shimazaki, 1990; Hamm & Fobbe, 1995; Korte, Vogel, Zühlke, Bee, & Hofmann, 1995; Chipkevitch, Nishimura, Tu, & Galea-Rojas, 1996; Diamond et al., 2000; Schiff, Li, & Goldstein, 2004; Meyer et al., 2006).

This investigation sought to further evaluate and compare diagnostic parameters to complement the current standard of histological evaluation for defining sexual maturity in male cynomolgus monkeys *(Macaca fascicularis)* used for toxicology studies. Specifically, we evaluated orchidometry as an initial practical, non-invasive method to select sexually mature males for routine toxicology study operations.

2. Methods

2.1. Retrospective study

A retrospective study was conducted to assess the relationship between terminal body weight, testicular weight and histological evidence of sexual maturity status in reproductive organs. An analysis of measurements performed and reported on Mauritian male cynomolgus monkeys serving as vehicle-treated controls for toxicology studies was conducted. The study used measurements from a sampling of 126 males housed in our AAALAC accredited facility over a 20 year period. There was no evidence of a compromise in microbiological status of animals based upon the histological appearance of selected tissues examined in the retrospective study.

Testicular (left and right) and terminal body weights were obtained and examined from issued study reports. Archived paraffin-embedded, hematoxylin and eosin stained histology slides of the weighed testis, epididymis and prostate were re-examined for the determination of maturity level. Each male was graded on sexual maturity level according to an established set of histological criteria (Table 1) and based upon previous literature (Cho, Fujiwara, Honjo, & Imaizumi, 1973; Kluin, Kramer & de Rooij, 1983; Smedley et al., 2002; Dreef et al., 2007). Immature animals (Grade 1) were characterized by the absence of spermiogenesis with prostates containing only small acini with cuboidal epithelium. Early adolescents (Grade 2) were differentiated from Grade 1 by evidence of spermiogenesis in scattered tubules. Adolescents (Grade 3) were differentiated from Grade 2 by the presence of a few epididymal spermatozoa mixed with sloughed nucleated cells and prostates containing acini with columnar epithelium and small amounts of colloid. Early adults (Grade 4) were characterized by spermiogenesis in the majority of tubules, moderate amounts of epididymal sperm and prostates containing acini with columnar epithelium and colloid. Mature adults (Grade 5) were characterized by complete spermatogenesis and spermiogenesis, with abundant epididymal sperm, and prostates with large acini lined by columnar epithelium. Histological maturity grades were evaluated for correlations with corresponding testicular weight.

Table 1
Maturity grades based on histology of testis, epididymis and prostate.

Maturity grade (number)	Testis	Epididymis	Prostate
Immature (1)	Early spermatogenesis, no evidence of spermiogenesis	No spermatozoa	Small acini with cuboidal epithelium
Early adolescent (2)	Spermatogenesis with evidence of spermiogenesis in scattered tubules	No spermatozoa	Small acini with cuboidal epithelium
Adolescent (3)	Spermatogenesis with evidence of spermiogenesis in scattered tubules	Few spermatozoa mixed with sloughed nucleated cells	Scattered acini with columnar epithelium and small amounts of colloid
Early adult (4)	Spermatogenesis with only scattered tubules lacking evidence of spermiogenesis	Moderate numbers of spermatozoa with occasional nucleated cells	Many acini with columnar epithelium and colloid
Adult (mature) (5)	Complete spermatogenesis and spermiogenesis	Abundant spermatozoa with rare nucleated cells	Large acini lined by columnar epithelium

2.2. Prospective study

A prospective study was conducted to assess the relationship between testicular volume and testicular weight in males awaiting study assignment and subsequently used in a 2 week toxicology study. Testicular volume measurements and body weights were collected on males ($n = 23$) in internal facility quarantine or awaiting study assignment. Animals used in the prospective study were screened and tested negative for antibody to Simian Retrovirus 1 (SRV) and Simian Herpes virus 1 (SHV-1).

For testicular volume measurements, animals were anaesthetized with ketamine-HCL (15 mg/kg) (Fort Dodge Animal Health, Fort Dodge, IA) by intramuscular injection administered to facilitate routine pre-study clinical examinations. Testicular (left and right) volumes were estimated using a Test-Size® Orchidometer (Accurate Surgical & Scientific Instruments Inc., Westbury NY) using comparative scrotal palpation. The orchidometer contains 12 numbered plastic reference ellipsoids of increasing size representing single testis volumes ranging from about 1 to 25 ml (in ellipsoid increments of 1, 2, 3, 4, 5, 6, 8, 10, 12, 15, 20 and 25 ml). Testis volume was estimated by palpating the scrotal testis and comparing it to the numbered plastic ellipsoid with the best fit. Testis volume was recorded as the closest numbered ellipsoid increment category in ml). The same investigator obtained all testicular volume measurements. Testicular weights in grams (without epididymis) were collected at necropsy upon study completion.

2.3. Husbandry

Monkeys were housed individually in stainless steel cages with visual contact with conspecifics in a single room dedicated to this study. Room design specifications for environmental conditions were: a minimum of 12 to 15 air changes per hour with air filtered through 80 to 85% efficiency filters then through HEPA filters, relative humidity of $50 \pm 10\%$, temperature of $70 \pm 5°F$ and a 12 h light:dark cycle. A standard diet of pelleted food supplemented with vegetable and fruit was provided daily and municipal drinking water, further purified by reverse osmosis, was provided *ad libitum*. The animals' general health was assessed daily. Animal care and use for animals for this study were reviewed and approved by the Pfizer Groton Institutional Animal Care & Use Committee.

2.4. Data analysis

Scatter plots were constructed for various data (body weight, testicular weight, histological maturity grade, testicular volume) and analyzed using first order linear regression analysis and calculation of correlation coefficients. Statistical analyses were performed using Student's paired *t*-test, with a significance level of $p \leq 0.05$.