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Analysis of follicular events in owl monkeys (*Aotus azarai infulatus*) using B-mode and Doppler ultrasound

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ABSTRACT

Ultrasound (B-mode) was used to analyze follicular events in 12 trained female owl monkeys (*Aotus azarai infulatus*). The animals were examined every 48 hours for over 90 days to measure and map follicular growth in both ovaries and to measure (using Doppler velocimetry) local hemodynamic changes during the peri-ovulatory stage. There were 44 follicular growth events, each with two or three follicular waves, and a mean ± SEM interval between events of 17 ± 1.13 days. There were various hemodynamic changes during follicular growth; both vascular resistance index and pulsatility index decreased during the time when the follicle diameter peaked. Thus, both B-mode and Doppler ultrasound were useful for monitoring ovarian follicular events in owl monkeys.

1. Introduction

Nonhuman primates (NHPs) are extremely important animals for biodiversity because they contribute to a variety of species in the ecosystem [1]. A primary goal of research in primate reproduction is preventing the disappearance of endangered NHP species. Studies on animals under controlled conditions provide information regarding the physiological basis for reproductive events.

The Brazilian National Primate Center (Centro Nacional de Primatas—CENP) at the Evandro Chagas Institute and the Health Surveillance Office (the Brazilian Ministry of Health) are responsible for performing biomedical studies in the Amazonian region and maintaining a colony of owl monkeys for reproductive studies. Mating this species in captivity has been considered difficult [2], but it can be enhanced by closely simulating the social structure of their natural habitat [3].

Studies on the reproduction of captive animals from the *Aotus* genus have been increased recently [4–11]. However, reproductive success in captivity is generally not accompanied by basic studies of the reproductive physiology of the genus. Although monitoring the estrous cycle is a basic procedure, performing this on wild animals can be more complex.

Ultrasound technology is an excellent tool for studying primate gynecology [7,9] as it allows the internal organs to be monitored noninvasively [12]. In addition to other technologies, such as Doppler velocimetry, which has been used for monitoring the gestational and estrous cycle in both domestic and wild animals [13], ultrasound can be used to monitor ovarian follicular events in owl monkeys.

The objective of this study was to monitor (B-mode and Doppler ultrasound) ovarian follicular events in owl

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monkeys and to increase our understanding of the reproductive physiology of this species.

2. Materials and methods

The project was carried out at the Brazilian National Primate Center (CENP/Ananindeua, PA) between April and August 2011 and was approved by the Ethical Committee for Animal Research at the Evandro Chagas Institute (Comitê de Ética de Pesquisa com Animais do Instituto Evandro Chagas—CEPAN/IEC, 036/2008).

Twelve adult females (six multiparous, four primiparous, and two nulliparous animals) from the owl monkey (*Aotus azarai infulatus*) reproductive colony at CENP were used. These females were isolated from males and were kept in individual brick pens with tiled walls and floors and metal screens covering the top, back, and front sides. The monkeys were maintained in a closed system in the mating facility number I and were not mated.

All animals were evaluated physically in the laboratory by using direct examinations: the flotation technique [14], the sedimentation technique [15], and the coproparasitological examinations.

Animals were fed various fruits, vegetables, roots, tubers, milk, eggs, and commercial feed for Cebidae (Cebidae P18 Megazoo, Megazoo Feed, Betim-MG, Brazil) in accordance with the care practices adopted by CENP. The animals also received daily supplements containing amino acids (0.5 g/kg of their body weight), vitamins, and macro- and microminerals (Aminomix Pet—Vetnil Indústria e Produtos Veterinários Ltda, Louveira-SP, Brazil) and had *ad libitum* access to water.

2.1. Training animals to be examined with ultrasound

Females were trained for ultrasound examinations using daily food rewards for over a 30-day period [10]. Females were later taken to the reproductive facility clinic at CENP in an animal transport cage, removed from the cage, and physically restrained using leather gloves to perform the examination. After the animals became acclimatized to being restrained, their training proceeded efficiently. The females were highly cooperative during the examinations. Vocalization, urination, and defecation only occurred during their capture in the enclosure and their removal from the transport carrier. Each ultrasound examination lasted for an average of 10 minutes.

2.2. Ultrasound analysis of follicular events

The B-mode and triplex Doppler ultrasounds were performed every 48 hours for over a 90-day period (45 times per animal) to monitor the ovarian cycle.

Females were restrained and placed in a supine position while their abdominal areas were shaved. A water-based gel was used to aid the ultrasound. During the examination, females were rewarded with several types of fruit, as described by Monteiro et al. [7,10].

A MyLab30 VET Gold (Esaote, Genoa Via A. Siffredi, 58 16153 Genova, Italy) ultrasound machine equipped with a linear multifrequency transducer (LA435, 6–18 MHz) was used to perform the ultrasounds. Images were blindly analyzed on a 15″ LCD high-resolution monitor by an experienced technician who located and evaluated the ovaries for size, shape, position, follicular growth pattern, ovulation, and corpus luteum (CL) formation.

Follicles were defined as structures that were filled with anechoic fluid and surrounded by a smooth outline in the ovarian stromal. Ovulation was considered to have been occurred when a follicle with large diameter was detected during one examination but was not observed during the following examination. The interval between the two follicular growth periods was defined as one cycle.

The hemodynamic characteristics of the right and left internal iliac arteries (RIIA and LIIA, respectively) were measured in triplex Doppler mode, and the arteries were identified and analyzed in a longitudinal section. The blood flow velocity waveforms were obtained by measuring a sample of the volume with colored Doppler, followed by spectral Doppler [16]. The equipment automatically calculated the vascular resistance index (RI) and pulsatility index (PI) following adequate measurements of the frozen image using calipers.

The results of the gynecological measurements were summarized in ultrasound examination reports that were saved in the equipment and were later recorded in a database on a portable external 250-GB hard drive (Samsung Eletrônica da Amazônia Ltda, Av. Itauba, BR 319 km 03, Manaus-AM, Brazil).

2.3. Statistical analysis

Basic descriptive statistics were calculated for all the analyzed variables, including the average duration of follicular events. An analysis of the Doppler ultrasound data was performed by comparing the averages of the periovulatory stage with the average of the whole cycle.

3. Results

3.1. Ultrasonography

Ultrasound provided an excellent visualization of ovaries, which are located cranial-laterally to the uterus, close to the internal iliac arteries and veins (Fig. 1). The ovaries were round in shape and slightly elongated with fine hypoechogenicity and homogeneous aspects. The mean ± SD volumes of the right and left ovaries were 0.32 ± 0.05 cm^3 and 0.38 ± 0.09 cm^3, respectively. The growing follicles were observed in various stages of development. The follicles were round, homogeneous, and finely anechoic and were located in the ovarian stromal (generally not extending beyond the organ's boundaries). The presence of a follicle made the ovarian stromal appear heterogeneous by ultrasound, which allowed differentiation of the stromal from the CL. Follicles were detected frequently with an average interval of 17 ± 1.13 days (average ± SEM). Forty-four events (22 in each ovary) that were compatible with follicular growth were observed. Two females had two events, four females had three events, two females had four events, and four had five events. Of these events, 21 (47%) had two or three follicular waves