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Association of Primate Veterinarians Guideline for Semen Collection in Nonhuman Primates in Biomedical Research

Background

Collection of semen samples from nonhuman primates (NHP) is needed for a variety of purposes. Examples include reproductive research, species conservation efforts, infectious disease research,[12] and assessment of reproductive safety during drug development. The purpose of this guideline is to provide a review of appropriate approaches for the collection of semen samples and their applicability in select nonhuman primate species. This guideline is intended to present an overview of commonly used nonhuman primate practices (e.g., preliminary assessment of maturity status in males or evaluation of male reproductive endpoints during in-life study). Other semen collection techniques are available and may be considered when feasible and appropriate.

Semen Collection Approaches and Applicability

A number of approaches for semen collection are available. Semen collection by vaginal washing after copulation[20] and by manual manipulation (masturbation)[10] have been described but are not commonly used in biomedical research. The most common approaches are electroejaculation and penile vibratory stimulation. Electroejaculation is the process of using an electric current to produce an ejaculate. The most frequently used electroejaculation procedural approaches are rectal probe electrostimulation and penile electrostimulation.

Rectal probe electrostimulation is generally reported to produce samples with lower sperm concentration than penile electrostimulation.[4,8,24] It is therefore generally regarded as less well suited to quantitative evaluation of semen samples but is highly effective for qualitative analysis such as detecting presence of sperm in the ejaculate to confirm sexual maturity[16] and to assess the morphology of sperm cells[1]. Penile electrostimulation is most frequently used for collection of sperm for quantitative analysis due to higher semen volumes (e.g., determination of sperm counts and/or concentration)[15,17,18,22,23,24,26] and is the recommended semen collection method for in vitro fertilization (IVF) or intracytoplasmic sperm injection.[15,21] Most semen parameters yield low statistical power for evaluating reproductive function[18]; only sperm motility and to lesser extent sperm morphology have higher statistical power (between 70% and 90%), thereby rendering them more useful in the evaluation of reproductive function.

In smaller species such as squirrel monkeys and marmosets, penile vibratory stimulation has been shown to be the preferred method over electrostimulation.[6,25,30]

Rectal Probe Electrostimulation (RPE)

Description

The RPE procedure is the historical method for semen collection in NHP.[6,7,9] The mechanism of ejaculation is by direct stimulation of peripheral neuromuscular junctions, resulting in the contraction and relaxation of pelvic organs. This stimulation results in emission of the content of those organs.

The procedure requires sedation to light anesthesia but does not require training of the animal. However, operator skill and training are integral to the success of the procedure. Once lightly anesthetized, the animal is placed in lateral recumbency. Alternatively, a sling can be used to suspend the animal in ventral recumbency, this provides good access to the penis and improves the positioning for semen collection. The size of the probe is species dependent. It should fit easily into the rectum, and the probe electrode footprint should be oriented directly over the prostate. Prior to use, the electrodes are lightly sanded, rinsed, and sanitized. The penis is gently exteriorized from the prepuce and cleaned to remove particulate matter if necessary (e.g., sterile saline). The penis is held with powder-free gloves during the collection procedure. The prostate is gently palpated to determine depth and to facilitate placement of the probe electrodes directly over the prostate. A thin layer of sterile water-soluble lubricating gel is applied to the probe prior to insertion. To ensure contact of the electrodes with the prostate, the tip of the probe is gently pressed downward by lifting the other end of the probe. The probe is activated by slowly increasing the voltage until the animal responds to the stimulus by leg extension with or without clasping. The voltage setting that the animal responds to becomes the animal's voltage set point (the lowest voltage that will elicit a response). Each stimulus is comprised of a slow increasing voltage to a response, holding it briefly (~5 seconds maximum) and then decreasing the voltage. As the stimulus is applied the penis is observed for extension, rigidity, engorgement, and pre-ejaculate. Multiple repetitions comprise a set of stimuli. On average, three sets of stimulations will produce an ejaculation. During the entire course of stimulation, the tip of the penis is held over a pre-warmed glass beaker for reproductive purposes or other collection receptacle (e.g., 15 ml conical tube).

Animal Welfare Considerations

This procedure is more invasive than other techniques, and it is important to check the animal and the probe regularly to ensure that no blood is present. The rectum is gently manually palpated to check for evidence of blood and to determine if there is an increase in internal temperature in the tissue overlying the prostate. If blood is present or there is a subjectively increased temperature above the prostate, the procedure is immediately stopped, and the animal allowed to recover. Post-procedure care is up to veterinary judgment. The procedure should be limited to no more than twice weekly collection.

Penile Electrostimulation Procedure (PEP)

Description

The penile electrostimulation procedure (PEP) consists of stimulation of afferent nerve endings in the penis, with subsequent anterior transmission of stimuli and stimulation of the efferent pathways associated with penile erection and ejaculation.

Traditional restraint methods for this procedure involve placing the animal in an open restraint chair using the pole and collar

technique for conscious sample collections. However, recent studies demonstrate that training the animal to enter directly from its home cage into a closed box chair is a viable alternative to open chair restraint, potentially representing a significant refinement for animal welfare, semen quality (increased sample volume and sperm concentration)[11], and training time, so it may be a preferred method of restraint. Additional guidelines for NHP restraint are available for reference.[2]

To begin, the penis is gently extricated, gel defibrillator pads with lubricating gel are wrapped around the penis, and electrodes are attached to the gel pads and then connected to the electroejaculation stimulator device. A stimulating current is administered, the first pulsation given at low voltage (appropriate for the species and size of the animal) for no more than approximately 10 to 15 seconds, with slow incremental increases up to a predetermined maximum. Generally, no more than 3 trials are attempted at one session (depending on the specific procedure and the animal response). Species-specific voltages vary widely based on a number of variables and are described in the literature.[22,23] For example, in cynomolgus monkeys, a trial may start at 10-30 volts, increasing in 5-10-volt increments if needed, to a maximum of ~50 volts. At each stimulation, the animal is carefully observed for physiologic response and ejaculation. The animal is stimulated until an ejaculate is obtained or the last trial attempt has been reached. A collection tube is placed at the tip of the penis to collect the ejaculated semen sample, which is then processed for evaluation Semen collection can be performed multiple times in a row over days/weeks without ill effects. Sperm numbers are maintained through several electroejaculations[28] but will eventually decrease with multiple collections. In consideration of both animal welfare and sperm production rates, the procedure is usually not attempted more frequently than weekly (e.g., during a 2-week pre-study period for baseline determinations) and monthly/quarterly (e.g., during the dosing phase of a study). If sampling is conducted over longer durations for quantitative assessment, sampling at more frequent intervals (e.g., every other week) may be appropriate. The frequency of collection may increase depending on the study requirements. Animals collected for gamete preservation, IVF/ICSI, or for preservation of genetics should be given approximately 48 hours rest between samplings. Once animals are trained and experienced with the procedure, they should be observed carefully for possible spontaneous ejaculation prior to electrostimulation being applied.

Most reports of direct penile electrostimulations indicate higher numbers of sperm per ejaculate compared to the rectal probe method,[8,22,24] and higher sperm quality based on the ability of sperm to fertilize oocytes in vitro.[15,21] The direct penile method has been proven to achieve better stimulation of the entire reproductive tract and have fewer problems with urine contamination and semen sample dilution due to the lack of anesthesia. Also, the potential for retrograde ejaculation into the bladder, which has been associated with the rectal probe method, is minimized with penile electrostimulation.[24] This noninvasive technique does require the animal to be trained and the experimental environment to be controlled (e.g., quiet room, staff familiar to the animals). It may also take longer to obtain a sample with this method than with the rectal probe electroejaculation technique, but generally no longer than several minutes. A potential drawback of penile electrostimulation is that some animals do not respond to penile stimulation and will not provide a semen sample.[11] Additionally, because this method involves working with conscious, chair-restrained animals, animal health status, such as endogenous and exogenous viral status, and the occupational risk therein should be taken into consideration.

Animal Welfare Considerations

Penile electrostimulation is a noninvasive procedure. Use of defibrillator pads and gel for application of stimulating current to the penis increases the animal's comfort during the procedure and eliminates issues associated with older methods such as metal foil electrodes, which can lead to dermal trauma.[23] Animals are not anesthetized for the procedure, but rather acclimated and trained for restraint. The stimulating current is administered in a conservative and carefully controlled manner. Even for animals that have experienced the procedure, no more than three attempts to collect an ejaculate should be attempted at one session on a given day. The animal is rewarded for its participation regardless of the outcome of the procedure.

Penile Vibratory Stimulation (PVS)

Description

Penile vibratory stimulation is a procedure involving the natural reflex sequence resulting in a normal emission and ejaculation response, yielding a natural ejaculate.[25] This technique uses a modified human vibratory device equipped with a collection vessel. The glass collecting tube has an internal diameter appropriate to achieve good contact with the penis and the required level of stimulation. It is commonly used with the animal awake and chair-restrained with novel rewards provided to encourage participation while working in a darkened room to reduce excitement of the animal. For collection of sperm, the penis is gently extruded from the preputial sheath, and the vibrating glass tube is turned over the tip of the penis and held against the preputial orifice.[25] Vibratory parameters used depend on the response of the animal to stimulation, which is increased gradually with allocation for rest periods. If ejaculation is not achieved within 15-20 minutes, stimulation is discontinued. In marmosets, erection itself is not a good indicator of stimulation outcome. As an indicator of adequate stimulation, animals push their pelvis forward prior to ejaculation.[25]

Animal Welfare Considerations

PVS may be the preferred method for semen collection in species such as squirrel monkeys and marmosets. In these species, PEP is possible, but can be challenging because the small animal size makes direct placement of electrodes for penile stimulation difficult. RPE is also less suitable (in terms of reliability and safety) because the reproductive and digestive anatomy of these smaller NHP species is accordingly of reduced proportions. Accurate placement of the rectal probe (especially during contractions) and delivery of a reproducible stimulus are difficult to achieve.[25] Studies in squirrel monkeys and marmosets found significant increases in spermatozoa and accessory gland production in semen samples collected by vibratory stimulation compared to samples collected by RPE.[25,30] PVS yields ejaculates of enhanced quality and combines the advantage of not requiring sedation or light anesthesia, causing little to no discomfort, and producing a natural ejaculate free from contamination. From a practical point of view, the enhanced yield of motile sperm achieved with PVS in the marmoset is of particular significance in a species in which sperm numbers are naturally limited by the extremely small size of the ejaculate.[25]

Animal Selection

Regardless of semen collection method, animals used for this procedure should be carefully considered. Confirmation of spermatogenesis (by detection of sperm in semen) is the most reliable way to confirm sexual maturity in NHP.[16] Seasonality should be taken into consideration for timing of semen collection in some NHP species.[29] Prior to such confirmation, selection of animals can be optimized by applying conventional selection criteria, such as age, body weight, and/or testicular volume.[13,14,19,27,29] Testosterone is not a reliable indicator due to its pulsatile/circadian fluctuations and large inter-animal variability.[19] None of these parameters are unequivocally predictive of sexual maturity, and specific criteria for maturity are dependent upon the species and even the geographical source of the animals. For example, cynomolgus monkeys from Mauritius have been demonstrated to be sexually mature (sperm in semen) from between 3-4 years of age, whereas those from mainland Asia required an additional year (>4 years old) to reach maturity.[16] Literature looking at cynomolgus and rhesus macaques indicates that animals less than approximately 4 years old and 4 kg body weight are not likely to be sexually mature.[19,27]

A physical exam of the animal prior to the procedure includes close examination of the rectum for abnormalities such as scarring, stricture, or hemorrhoids. Additionally, animals with behavioral characteristics such as nervousness or anxiety that could cause duress to the animal and/or interfere with the success of the procedure are not selected for use. Animals that do not adapt to chair restraint or placement of electrodes should also not be used.

The chosen collection method should be matched to the size of the animal, in that small animals, such as New World primates (squirrel monkeys and marmosets), are more suited to PVS as opposed to the other two methods discussed[25,30]; there are no reports of success of this technique in macaques; however, data on pubertal maturation of male marmosets and squirrel monkeys are scant and conflicting.[3,5]

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