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High efficient and non-invasive collection of ejaculates from rats using penile vibratory stimulation

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ABSTRACT

Background: The rat is one of the most important experimental animals, which plays an indispensable role in biomedical research, particularly in reproduction. However, according to our best knowledge, there is no easy and efficient method available for semen collection from rats.
Results: In this study, we successfully collected semen through penile vibratory stimulation ejaculation (PVSE) from laboratory rats. This is an easier and more efficient method compared with rectal probe electro-ejaculation (RPE). We found that the ejaculation rate, volume, concentration and motility of semen collected with PVSE were substantially better than those of RPE. Although PVSE was time-consuming compared to RPE, the quality of semen was better; additionally, sperm concentration and motility of semen were significantly higher with a two-day interval between collections compared to a five-day interval. Moreover, we found that electrical stimulation, use of anesthesia and increased age of rats have a negative effect on sperm quality. In the last experiment, four fertile female rats were artificially inseminated with PVSE-collected semen, and healthy offspring were born.
Conclusion: Here, for the first time, we established the repeated collection of semen using the PVSE method in rats.

1. Introduction

Rats have many valuable features that highlight their potential as experimental animals in biomedical research, including their genetic, anatomical, and biomechanical similarities with humans [1]. Historically, the first attempt to investigate the reproductive biology of rats was described by Hewer in 1914 [2]. Recently, sperm collection, *in vitro* fertilization, embryo transplantation and gene knock-out in rats have been successfully reported [3–5]. The demand for a genetically modified rat model for biomedical and genetic research has been increasing exponentially [6,7]; however, the purchasing and transportation of genetically modified rats are very expensive.

It is well established that much time and energy has been spent on exploring female reproductive issues whereas male issues have not received much attention [8].Sperm plays an irreplaceable role in assisted reproductive techniques [9].Sperm collection via an artificial vagina, squeezing or slicing of the epididymis, and electro-ejaculation are the most commonly used methods in small animals [10,11].However, the application of these methods in rats is limited. The vaginal washing method is time consuming and tedious [12]. The epididymal sperm collection method is costly, and the same animal cannot be used for the next sperm collection [13]. Rectal probe electro-ejaculation (RPE) is a potential sperm collection method, but the quality of sperm significantly decreases due to use of electro-stimulation and anesthesia, which limits its application [14].Therefore, an efficient and repeatable sperm collection method from rats is still needed.

Recently, pharmacologically induced ejaculation has been successfully reported in different species, such as stallions [15]. However, this technique is not successful in rats yet. Therefore, it is very

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important to introduce some new valuable and possible sperm collection methods for rats. Currently, penile vibratory stimulation ejaculation (PVSE) method has been reported as a repeatable noninvasive sperm collection method in marmoset monkeys [16]. It has been reported that the PVSE method is superior compared to other methods because it produced significantly higher semen samples with high sperm motility [17]. Additionally, successful application of PVSE has been reported in patients with spinal cord injuries [18]. However, the application of PVSE in rats has not yet been reported. Therefore, the current study was designed to explore the application of the PVSE method in rats and its effect on sperm quantity, quality and motility.

2. Materials and methods

2.1. Animals

A total of 50 fertile adult male rats (age 18 or 60 weeks) and four adult females (*Rattus norvegicus*) were obtained from a breeding colony at Southern Medical University Center. All animals were kept in individual cages (320 mm × 180 mm × 160 mm) with controlled temperature (20–25 °C) and humidity (40–60%), a cycle of 12 h light and 12 h darkness, and free access to water and food. Before the start of the experiment, sperm quality of all animals was measured, and then the animals were randomly divided into different experiment groups. This study was carried out in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of South China Agricultural University. The protocol was approved by the Committee of the University on the Ethics of Animal Use in Experiments (protocol approved number: 2016019).

Protocol: 1. Sodium pentobarbital was injected intramuscularly only for the RPE method. 2. Animals were placed in a homemade holder. 3. The prepuce area was cleaned. 4. To arouse erection, external genitals were mildly rubbed for 1 min with wetted cotton ball. 5. Semen was collected with the VSE and RPE methods. 6. Semen was collected into 1.5 mL tubes and evaluated.

Experimental studies were performed under sodium pentobarbital anesthesia, and all efforts were made to minimize suffering. Anesthesia was used only for experiments 1 and 2.

2.2. Media preparation

All chemicals were obtained from Sigma Chemical Co. (St. Louis, MO, U.S.A.) unless otherwise indicated. The sperm diluent extender referred to as HTF contained NaCl 101.6 mM, KCl 4.70 mM, $CaCl_2$ 5.14 mM, KH_2PO_4 0.40 mM, $MgSO_4$ 0.20 mM, $NaHCO_3$ 25.0 mM, Sodium lactate 18.36 mM, Sodium pyruvate 0.34 mM, Glucose 2.78 mM, BSA (Fraction V) 4.0 mg/mL, Streptomycin sulfate 50 μg/mL, and Potassium penicillin 75 μg/mL, according to the method described by Aoto et al. [15]. All the extenders were kept in a 37 °C water bath before use.

2.3. Electrode used for Experiment 2

Homemade electrodes consisted of two 1.5 v batteries and a battery case lined with two sheets of aluminum.

2.4. Rectal probe electro-ejaculation

Rectal probe electro-ejaculation was performed via intramuscular injection of sodium pentobarbital anesthesia at a dose of 30–50 mg per kg of body weight. The electro-ejaculator (model AC-1, Beltron Instruments, Longmont, CO) with a custom probe with a diameter of 7.5 mm was used. The probe was lubricated with water-based non-spermicidal lubricant and inserted into the rectum at the level of the prepuce with the electrodes facing ventrally. Proper placement was verified by palpating the inguinal area. After the probe was inserted into its hub, the current was increased incrementally in a pulsatile fashion until an ejaculate was obtained or a maximum parameter was attained [19].

2.5. Penile vibratory stimulation ejaculation

Penile vibratory stimulation ejaculation was performed using a battery-operated FertiCareR personal vibrator (Multicept, Rungsted, Denmark). The basic modifications were similar to those described by Kuederling et al. [16]. Additionally, the plastic collecting 1.5 mL-EP-tube attached to the silicon rubber holder was 20 mm long with 5 mm internal diameter. This provided a better contact with the target and was more effective in achieving the required level of stimulation. Animals were placed in a homemade holder, as shown in Fig. 1a and b. Before we carried out sperm collection, the penis was gently washed with warmed saline by using sterile cotton. To arouse erection, external genitals were mildly rubbed for 1 min with wetted cotton ball. Then, the vibrating tube was placed on the genitals. Vibration parameters were set as follows: from an initial stimulation at 80 Hz and two mm amplitude, intensity was increased in 1 min. This stimulation protocol was repeated once (following a 2–3 min rest period) until ejaculation was completed. As an indicator of adequate stimulation, a rhythmic contraction and expansion of the penis occurred prior to ejaculation. The ejaculate could be seen as a clear droplet on the urogenital orifice, as show in Fig. 1c and d.

2.6. Semen evaluation assays

Semen samples collected with the RPE and PSE methodswere kept at 37 °C for 30 min to allow for liquefication. A 10-μLsperm sample was taken to examine sperm motility and acrosomal integrity as a non-frozen control. The motility of each sperm sample was evaluated by counting with an erythro-cytometer. A 5 μL sample of 100xdiluted semen with HTF was placed on the erythrocytometer and sperm cells were counted under light microscopy (Motic BA2000, Shanghai, China). Motility was assessed by two qualified technicians individually. Thawed sperm motility was evaluated immediately after thawing and washing. To minimize evaluator bias, the evaluator was blinded as to the identity of the samples.

2.7. Experiment 1: Comparison of ejaculation characteristics between the PVSE and RPE methods in mature rats

In this study, we sought to compare whether the PVSE method is better than the RPE method. Ten sexually mature male rats were randomly divided into two groups for PVSE and RPE. Successful ejaculation and the time taken for each rat were recorded. Semen samples were collected in 2-mL-EP tubes with 495 μLHTF. Thus, semen was incubated in a 37 °C in water-bath for 10 min before evaluating semen volume, concentration, motility and malformation.

2.8. Experiment 2: Effect of electrical stimulation and anesthesia on sperm motility

To determine the effect of electrical stimulation and anesthesia on sperm motility and to avoid semen contamination with urine during the process of ejaculation, 10 epididymal sperm samples were collected using a flushing method and incubated at 37 °C for 30 min, after which,5 mL semen samples were transferred into