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# Electroejaculation of chimeric rats

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## Abstract

With the advent of genetic engineering of rodents came the need to assess fertility and germline competency, especially in chimeric rodents generated using embryonic stem cells. Traditional methods rely on natural mating and progeny testing, which is time- and cost-intensive. Electroejaculation is a faster method of collecting sperm for genetic analysis and offers the additional benefit of using fewer animals. This column describes a refined electroejaculation technique for chimeric rats using light gas anesthesia and a custom-made platform for sperm collection.

Chimeric animals are typically produced by introducing embryonic stem cells (ESCs) of one strain, which often carry genetic manipulations, into blastocysts of another strain. In order for the genetic modifications to be successfully passed on to future generations, the ESCs must contribute to the germline of the chimera. Germline competency is most often assessed by breeding chimeras and confirming inheritance of the genetic modification in their offspring, a process that is time-consuming and uses many animals.

As an alternative to progeny testing, semen can be collected from chimeric rats and the sperm analyzed directly to assess germline competency. Electroejaculation has been successfully used to obtain sperm in a wide variety of animals. Electroejaculation and sperm collection in rats was first described in 1959 (ref. 1), and additional reports in the 1960s and 1970s refined the technique (refs. 2–4), but little has been published on the topic since then. All previous descriptions of electroejaculation in the rat included a crude restraint device for collection or intraperitoneal injection of barbiturates to achieve sedation prior to collection.

Here we describe a modified electroejaculation method using isoflurane anesthesia and a custom platform to collect semen from chimeric rats. This protocol was approved by the Animal Care and Use Committee of the University of Missouri and was carried out in strict accordance with the recommendations in the *Guide for the Care and Use of Laboratory Animals*[5].

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## ELECTROEJACULATION PROCEDURE

### Anesthesia and preparation for electrostimulation

In preparation for electroejaculation and semen collection, each rat was placed into a chamber (Fig. 1) containing isoflurane. When it became recumbent, the rat was removed from the chamber and placed on a plastic platform in dorsal recumbency. Anesthesia was maintained by administering 2–3% isoflurane via a nose cone (Fig. 2a). Rats were kept in a light plane of anesthesia as electroejaculation is not considered a painful procedure and can be performed in a conscious, acclimated animal. The plastic platform was fabricated from locally available supplies and incorporated a support ring for the nose cone (Fig. 1). Clips or small ties can be used to secure the rat's limbs to the platform if necessary to prevent movement during electrostimulation and ejaculation. Restraint is often not necessary, and in many cases, the procedure can be carried out by one person.

### Electrostimulation and semen collection

After the rat was placed on the platform, the rectum was palpated and any feces were gently removed with external digital pressure. The presence of feces in the rectum decreased conductivity of the probe, resulting in a poor collection. The electroejaculator (model AC-1, Beltron Instruments, Longmont, CO; Fig. 1) used a custom probe with a diameter of 0.125 in and length of 1.25 in (Fig. 1). The probe was lubricated with water-based non-permicidal lubricant (Priority Care, Elgin, IL; Fig. 1) and inserted into the rectum to the level of the prepuce with the electrodes facing ventrally (Fig. 2b). Proper placement could be verified by palpating the inguinal area. The electroejaculator was then turned on and the output was set to 2 V for 2–4 s then reduced to 0 V for 2–4 s. This cycle was repeated three times or until the penis was erect and exposed. For some rats, it was necessary to move the preputial skin cranially to allow for penis exposure. The voltage output was gradually increased in 2-V increments for cycles of 2–4 s until ejaculation occurred or until the voltage output reached a maximum of 10 V. Moving the probe slightly in all planes until erection facilitated faster and lower voltage ejaculation. Once erection occurred, the probe was held still and cycled until ejaculation. After the rat ejaculated, the probe was removed and the penis allowed to relax into the prepuce. The ejaculate could be seen as a clear droplet on the urogenital orifice (Fig. 3a). The prepuce was stabilized with one hand while the ejaculate was collected with a 1-ml sterile syringe. Ejaculate was then transferred to a 1.5-ml microfuge tube and either stored at −20 °C or used immediately for semen evaluation (Fig. 3b) followed by DNA extraction. The average amount of time needed to anesthetize, electrostimulate and collect semen from a rat was less than 5 min. After semen was collected, the rat was returned to its home cage for recovery and was given a yogurt drop treat (Bio-Serv, Frenchtown, NJ). Rats were monitored daily for adverse events.

### Semen evaluation

Semen was cooled or thawed for sperm counting. Samples were diluted in flushing and holding media prior to analysis[6]. A Hamilton Thorne Biosciences (Beverly, MA) Version 12 TOX IVOS Sperm Analysis System was used with the following settings: standard objective, 4×; minimum contrast, 80; minimum size, 7 pixels; default cell size, 25 pixels; default cell intensity, 80; slide chamber depth, 80 μm.

## PROCEDURAL SUCCESS RATE

We carried out electroejaculation and semen collection for 11 male chimeric rats derived from injection of F344-Tg.EC4011/Rrrc ESCs (Rat Resource and Research Center RRRC# 654, Columbia, MO) into dark agouti × Sprague-Dawley blastocysts[7]. Rats were approximately 9–10 months of age. All rats tolerated the procedure well, and there were no adverse events or signs of urogenital disease. During a period of 37 d, we carried out the procedure 56 times, and the rat successfully ejaculated in 52 cases, resulting in a 93% success rate with this technique. Approximately 30–60 μl of semen was obtained per collection. Initial collections were not analyzed for sperm concentration. Of the 34 ejaculates that were submitted for sperm counts, 19 (56%) had sperm concentrations high enough to be counted by the sperm analysis system. In comparison, an earlier publication reported collecting ejaculates with sperm concentrations high enough to be counted from 25 of 27 3-month-old rats (92.6%)[4]. Sperm concentrations in the samples we collected ranged from 1 million to 80 million cells per ml with an average of 27.6 million cells per ml.

## CONCLUSIONS

Electroejaculation under gas anesthesia has not been previously reported to our knowledge. Using the anesthesia chamber allowed for minimal handling of rats. No further restraint was needed while rats were anesthetized, avoiding potential injury. Electroejaculation of rats under light anesthesia is an easy and useful technique for collecting semen for evaluation of fertility, morphology and genetic analysis (e.g., to evaluate germline transmission in chimeric animals).

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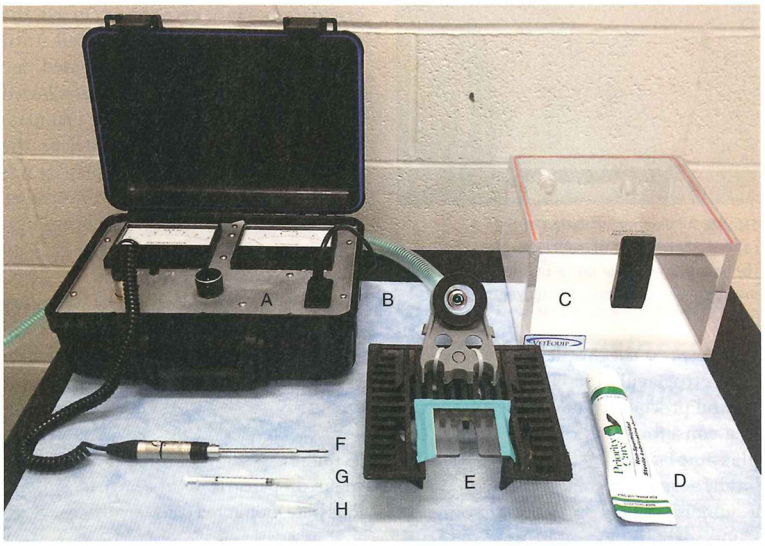


**FIGURE 1.**

Equipment for electroejaculation of rats. A, Beltron AC-1 electroejaculator; B, non-breathing circuit and nose cone for isoflurane anesthesia; C, anesthesia induction chamber; D, non-spermicidal water-based lubricant; E, platform for sperm collection with cutout for use in dorsal or ventral recumbency; F, stimulating probe; G, 1-ml syringe for aspirating ejaculate; H, 1.5-ml microfuge tube for semen storage.

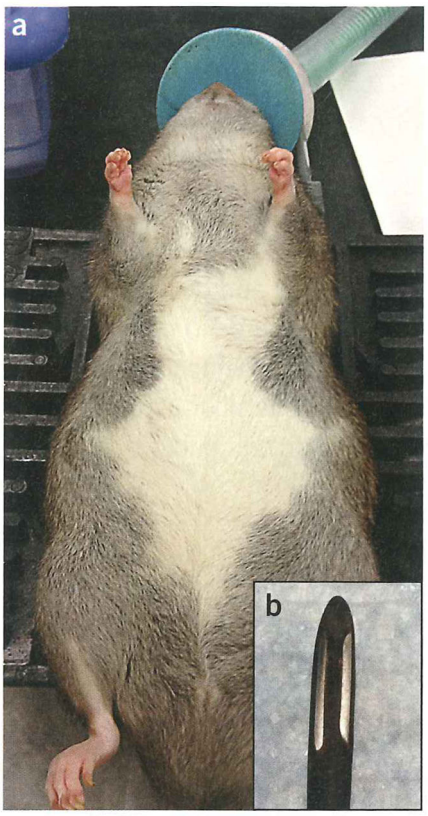


**FIGURE 2.**

Electroejaculation of the rat.

(**a**) Anesthetized rat on collection platform.

(**b**) Enlarged image of probe in orientation of insertion with electrodes facing ventrally.

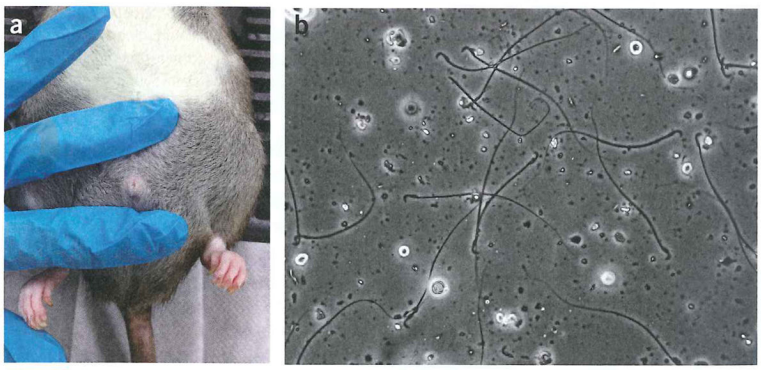


**FIGURE 3.**

Collection of ejaculate. (**a**) The ejaculate can be seen as a clear droplet on the urogenital orifice. The prepuce is stabilized with one hand. (**b**) A representative sperm sample (4× objective).