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Non-invasive Collection of Ejaculates From the Common Marmoset (*Callithrix jacchus*) Using Penile Vibrostimulation

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Penile vibrostimulation (PVS), a noninvasive repeatable method, has been shown in the squirrel monkey to yield semen of higher quality than rectal probe electro-ejaculation (RPE). The present study aimed at establishing the conditions for PVS to collect ejaculates from marmoset monkeys. Ten adult males were trained on the appropriate handling before each was subject to six to 12 PVS tests. Ejaculation was stimulated using a FertiCare® personal vibrator fitted with a 2 cm × 0.5 cm i.d. glass tube. The stimulus was repeatedly applied over a frequency of 75–95 Hz and amplitude of 1–2 mm for up to 20 min. Ejaculates were analyzed for volume, total sperm number, sperm concentration, and proportion of living and motile sperm. Ejaculates were obtained in 31 of 88 PVS tests; 87.1% of the ejaculations occurred at 80–85 Hz frequency and 1–1.5 mm amplitude. In 18 tests ejaculates were produced within 49.7 seconds. Ejaculates were characterized by (mean values): volume 31.9 µl, total sperm number 34.2×10^6/ejaculate, concentration $1{,}154.2 \times 10^6$ sperm/ml, live sperm 74.6%, motile sperm 59.6%. Total number and concentration of spermatozoa were significantly enhanced in singly living males. PVS yielded three to four times more spermatozoa than comparable previously published values for RPE. Enhancing the success rate by preselecting males for responsiveness may render PVS the sperm collection method of choice in marmoset monkeys. Am. J. Primatol. 52:149–154, 2000.

Key words: penile vibrostimulation; marmoset; ejaculate; noninvasive

INTRODUCTION

In the marmoset monkey *Callithrix jacchus*, a small New World primate, repeated collection of semen from the same individual is usually performed using rectal probe electro-ejaculation (RPE) or vaginal washing after natural copulation (VW) [Morrell & Hodges, 1998]. Both methods have disadvantages: the first is invasive and therefore requires anesthesia; the second yields ejaculates inevitably contaminated with mucus from the female genital tract.

Recently penile vibrostimulation (PVS) has been described as a repeatable noninvasive method of semen sampling in another small New World primate, the squirrel monkey [*Saimiri boliviensis*, Yeoman et al., 1998]. The method was

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found to be superior to RPE in this species as it produced significantly larger semen samples with an enhanced yield of motile spermatozoa.

The objective of the present study was to examine the conditions required to perform PVS for sperm collection in marmoset monkeys, which are used in our laboratory as a model species for the development of assisted reproductive techniques. Tests were carried out on males that were housed with a female and males that were living singly for several weeks, as copulations among pairs might influence the reliability of response to PVS. The analysis of ejaculate volume as well as total number, concentration, and proportion of live and motile spermatozoa provides preliminary information on the quality of semen samples obtained by PVS.

MATERIAL AND METHODS

Animals

Ten adult male marmosets from the callitrichid colony of the German Primate Center, eight of which were proven fertile, were randomly selected as test animals. One male (Mo) lived together with an intact female; the others were paired with ovarectomized adult females. Details of housing conditions are described in Kuederling et al. [1996]. Each male was habituated to test-specific handling for 15 min at least eight times before the experiment started.

Five males were tested while being paired and also during a period of several weeks when they were kept singly. For reasons of colony management, three males were tested only while paired and two only while living singly. Six tests were performed on each male in each living condition at intervals of one week or more. Paired males were separated from their females for one to three days prior to testing. Tests were performed in a dimmed room. Animals were rewarded with preferred food items during and after the tests.

PVS Conditions

Vibratory stimulation of the penis was carried out using a battery-charged FertiCare® personal vibrator (Multicept ApS, Rungsted, Denmark) with a frequency range of 70–110 Hz and amplitude of 0.5–3.5 mm. The vibrating shaft was fitted with a 4-cm-long piece of vinyl tubing into which a 5-mm i.d. × 20-mm empty glass tube was inserted to serve as an artificial vagina.

The unsedated, manually restrained males were placed in a prone position on a sling stretched over a stand with the genital area exposed. The hind legs were manually held slightly bent similar to the posture during natural mating. For stimulation, the vibrating glass tube was gently held against the preputial orifice. Stimulation was started with 75–80 Hz and 1 mm amplitude for 2–3 min. If no ejaculation was achieved the animals were given a rest of 1–2 min. Stimulation cycles were repeated with increasing stimulus intensity (maximally 95 Hz and 2 mm amplitude) until ejaculation occurred. Stimulation was discontinued if after a total test duration of 20 min no ejaculate was produced.

Analysis of Ejaculates

The seminal volume of each sample was determined by volumetric pipette. Then the ejaculates were mixed with 50 μl of TALP medium [Gilchrist et al., 1997] at 37.5°C. The suspensions were left to equilibrate at 37.5°C for 20–30 min to allow the sperm to swim out of the coagulum. For determination of sperm

concentration, an aliquot of each sample was diluted with distilled water and the number of free sperm was counted using a Neu-bauer cell counting chamber [World Health Organization, 1992]. The proportion of living sperm was assessed by staining an aliquot of the suspension with Eosin Y 1% and counting the number of live and dead spermatozoa [World Health Organization, 1992]. The proportion of motile sperm was assessed by computer aided sperm analysis (Hobson sperm tracker, Hobson Tracking Systems Ltd, Sheffield , UK).

Statistical Analysis

Statistical analysis was carried out using the Jandel Scientific SigmaStat software package. Comparison between single data from paired and singly living animals was made by applying a paired *t*-test.

RESULTS

Success Rate of Penile Vibrostimulation

A total of 88 PVS tests was performed: 47 tests on paired males and 41 on males living singly. Table I shows the success rates for each individual. Ejaculations were achieved in 31 (35.2%) tests with 16 ejaculates from paired and 15 from singly living males. The success rate varied largely among individuals, with 9 of the 10 males ejaculating in fewer than 50% of the tests, while in one male (Do) all tests (100%) resulted in ejaculation.

The number of ejaculates obtained from paired males was not significantly different from that produced by singly living males (paired: mean 2.3 ± 0.7 SEM, single: 2.1 ± 0.7 SEM; t = 0.232, df = 4, $P = 0.8$). A training effect due to repeated testing was not observed; a Spearman correlation between the ordinal number of each trial and the rank of the number of ejaculates obtained on each trial was not significant (n = 12; r = –0.29; $P = 34$)

The majority of ejaculations (87.1%) occurred at a vibration frequency between 80 and 85 Hz and an amplitude between 1 and 1.5 mm, while 12.9% of the ejaculations were achieved with a stronger stimulus (frequency 90–95 Hz, amplitude 1–1.5 mm). In 18 tests ejaculation occurred on the first stimulus. The la-

TABLE I. Number of Ejaculates Obtained From Individual Males

Male	Paired	Single	Total
Am	0 (6)	–	0 (6)
Dn	1 (5)*	1 (6)	2 (11)
Do	6 (6)	5 (5)*	11 (11)
Ha	1 (6)	–	1 (6)
Hi	–	2 (6)	2 (6)
Lo°	1 (6)	0 (6)	1 (12)
Ma	–	2 (6)	2 (6)
Mo	3 (6)	–	3 (6)
Se	1 (6)	4 (6)	5 (12)
Sn°	3 (6)	1 (6)	4 (12)
Σ	16 (47)	15 (41)	31 (88)
	= 34.0%	= 36.6%	= 35.2%

( ), number of tests performed per male; *, for technical reasons only 5 tests were performed, °, fertility not proven.

tency from start of the stimulus to ejaculation was 49.7 ± 8.0 sec (mean ± SEM, range 20–120 sec).

Analysis of Ejaculates

Table II shows the volume, total sperm number, and sperm concentration of the ejaculates as well as the percentage of motile and live spermatozoa in the sperm suspensions for each male. For all ejaculate parameters the inter- and intra-individual variation was considerable. Volumes varied between 10 µl and 79 µl. Ranges for total number of sperm/ejaculate were 4.4–138.4 $\times 10^6$, while those for sperm concentration were 210–2,760 $\times 10^6$ sperm/ml. On average, 74.6% of spermatozoa collected were live, of which 59.6% were motile.

The ejaculates from paired males contained significantly fewer spermatozoa than those from singly living males. Total sperm number and sperm concentration in the "paired" group were reduced to 37.2% and 51.7%, respectively, of values obtained in the "single" group (total sperm: mean 19.1 ± 3.0 $\times 10^6$ SEM vs 51.5 ± 10.1 $\times 10^6$ SEM, t = 3.048, df = 11, $P = 0.011$; concentration: mean 805.2 $\times 10^6$/ml ± 167.4 SEM vs. 1,556.8 $\times 10^6$/ml ± 192.2 SEM; t = 2.411, df = 11, $P = 0.035$). No statistical difference was observed between the two groups for volume (paired: mean 29.8 µl ± 4.9 SEM, single: 34.2 µl ± 5.6 SEM, t = 0.593, df = 11, $P = 0.565$), and proportion of live (paired: 79.4% ± 3.2 SEM, single: 69.5% ± 5.6 SEM, t = 1.295, df = 12, $P = 0.22$), or motile sperm (paired: 68.1% ± 4.0 SEM, single: 41.2% ± 7.1 SEM, t = 1.409, df = 8, $P = 0.197$).

DISCUSSION

The present study describes the first application of PVS in marmoset monkeys. The major advantages of this method are that it is noninvasive, repeatable, and provides uncontaminated ejaculates. In comparison, RPE requires anesthesia and is invasive. Vaginal washing (VW), the other noninvasive method of sperm sampling from marmosets, yields samples contaminated with mucus from the female genital tract, which may influence sperm movement [Schaffer et al., 1989].

The reliability of PVS, with 35.2% of the tests yielding an ejaculate, was lower than that reported for squirrel monkeys [80%, Yeoman et al., 1998]. An improvement of the present results may be achieved by preselecting males for

TABLE II. Ejaculate Characteristics of Individual Males

Male	Volume (µl)	Total sperm ($\times 10^6$)	Sperm/ml ($\times 10^6$)	Live sperm (%)	Motility (%)
Dn	19.0; 25.0	4.4; 63.8	229.2; 2550.0	67.0; 73.5	25.0; 73.0
Do	15.7 ± 1.3 (9)*	18.3 ± 1.8 (9)*	1201.2 ± 109.0 (9)*	77.4 ± 4.6 (10)*	63.4 ± 4.4 (9)*
Ha	25.0	7.7	306.8	87.0	70.0
Hi	35.0; 20.0	58.7; 14.3	1677.2; 714.0	50.0; 31.5	25.0*
Lo	78.0	32.0	410.3	73.0	69.0
Ma	49.0*	30.4*	621.3*	72.0*	*
Mo	34.7 ± 11.7 (3)	14.0 ± 4.9 (3)	491.7 ± 171.2 (3)	88.8 ± 2.1 (3)	82.0 ± 4.7 (3)
Se	39.0 ± 7.2 (5)	82.9 ± 15.9 (5)	2225.1 ± 262.1 (5)	80.5 ± 4.1 (5)	46.3 ± 8.6 (4)*
Sn	50.3 ± 10.3 (4)	31.1 ± 8.1 (4)	599.0 ± 61.3 (4)	67.0 ± 14.7 (4)	56.3 ± 11.3 (4)
Σ	31.9 ± 3.7 (28)	33.7 ± 5.8 (28)	1154.2 ± 143.6 (28)	74.6 ± 3.3 (29)	59.6 ± 3.9 (25)

Data are given as mean values ± SEM, single values are separated by semicolons. ( ), number of analyzed ejaculates; *, number of analyzed ejaculates is less than number of ejaculates obtained.

responsiveness. This is suggested by studies on VW where preselection of marmoset males enhanced the reliability from 44.9% [Kuederling et al., 1996] to 80% [Morrell et al., 1996].

The stimulus strength used for squirrel monkeys [Yeoman et al., 1998] appeared to be appropriate for marmosets, and most ejaculations (87.1%) occurred at a frequency of 80–85 Hz and an amplitude of 1–1.5 mm. Habituation to the stimulation seems not to be necessary as the success rate did not increase with the number of tests performed.

The variability in ejaculate characteristics observed in the present study and in that from Yeoman et al. [1998] is not inherent to PVS. High inter- and intraindividual variability in ejaculate characteristics has been reported for all primate species, independent of the collection method [Martin & Gould, 1981]. In our study, only sexual abstinence had an effect on total number and concentration of marmoset sperm, while other parameters were not influenced. The same is known from chimpanzees and man [Marson et al., 1989].

The volumes of the ejaculates from PVS and the proportions of motile and living sperm were in the same range as those reported for samples obtained by RPE [Cui et al., 1991]. However, the total number and concentration of sperm in the samples from PVS were three to four times higher than in those from RPE. Similarly, in the cross-over study in squirrel monkeys, sperm yield from PVS was much higher than from RPE [Yeoman et al., 1998]. These findings suggest that at least sperm yield is better using the physiologically more natural stimulation as provided by PVS. This assumption is corroborated by a comparative study in chimpanzees where sperm concentration in naturally produced ejaculates was much higher than in samples obtained by RPE [Young et al., 1995].

Taken together, the present study has shown that PVS can be performed without difficulties in marmoset monkeys. It provides semen samples of a better quality than the other two repeatable methods. The considerable advantages of being noninvasive and providing uncontaminated samples may outweigh the drawback of having to preselect males to achieve a higher success rate. PVS may then become a superior alternative to RPE and VW for sperm collection from marmosets and other small monkeys.

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