Penile vibratory stimulation yields increased spermatozoa and accessory gland production compared with rectal electroejaculation in a neurologically intact primate (*Saimiri boliviensis*)

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Assisted reproductive techniques require an efficient semen collection procedure in cases of ejaculatory dysfunction. Anejaculation may be of psychogenic or neurogenic origin but can be overcome with stimulatory techniques. Penile vibratory stimulation (PVS) therapy for anejaculation has recently emerged as an alternative to rectal probe electro- ejaculation (RPE) and more invasive testicular procedures. Comparison of the stimulatory procedures in neurologically intact subjects is not ethically possible due to the discomfort involved with electroejaculation, and comparison in spinal cord injured men may be compromised due to the intricate effects of chronic denervation on semen quality. We have previously shown the efficacy of PVS in a non-human primate, the squirrel monkey. A cross-over study design comparing semen collected by PVS and RPE was employed during the breeding season in which 15 donor males were divided into two groups. One group received PVS and the other RPE, then, three days later, treatments were reversed. Twelve of 15 animals responded to PVS (80%), all with other RPE, then, three days later, treatments were reversed. During the breeding season in which 15 donor males were involved with electroejaculation, and comparison in spinal cord injured men may be compromised due to the intricate effects of chronic denervation on semen quality. We have previously shown the efficacy of PVS in a non-human primate, the squirrel monkey. A cross-over study design comparing semen collected by PVS and RPE was employed during the breeding season in which 15 donor males were divided into two groups. One group received PVS and the other RPE, then, three days later, treatments were reversed. Twelve of 15 animals responded to PVS (80%), all with other RPE, then, three days later, treatments were reversed.

Penile vibratory stimulation therapy (PVS) for anejaculation has recently emerged as an alternative to rectal probe electroejaculation (RPE) and invasive testicular procedures (Brackett *et al.*, 1997; Ohl *et al.*, 1997; Abdulla and Rizk, 1998). Successful PVS requires intact afferent–effenter neural connections below T10, thus some spinal cord injured men are unlikely candidates for vibrostimulation. In contrast, RPE relies upon direct stimulation and contraction of periprostatic structures to effect a release of adjacent stored fluid and spermatozoa (Ohl, 1994). When applicable, PVS has produced superior specimens in spinal cord injured men (Ohl *et al.*, 1997) possibly due to a more physiologically natural stimulation utilizing the reflexive sequence of emission and ejaculation. However, this difference in semen quality may be related to the intricate effects of chronic denervation rather than to the collection technique employed since spermatozoa experience senile degeneration with prolonged storage in seminal vesicles. Scrotal hyperthermia associated with paraplegia is a possible cause of asthenoteratozoospermia (Brindley, 1982; Beretta *et al.*, 1989). Therefore, a direct comparison of semen collected by these contrasting techniques is needed but has not been ethically possible in men due to the discomfort involved with electroejaculation in sensory intact individuals.

Recently, we reported that PVS is effective in the squirrel monkey (Yeoman *et al.*, 1997). The present study used the squirrel monkey as a spinal cord intact non-human primate model for direct comparison of PVS to RPE. A cross-over design experiment was performed allowing comparison of physical and biochemical characteristics of semen. In our

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**Introduction**

Assisted reproductive techniques require an efficient semen collection procedure in cases of ejaculatory dysfunction. An ejaculation may be of psychogenic origin in which descending cranial signals inhibit the spinal ejaculatory coordination centre. This situation can be especially stressful during assisted reproductive procedures that require spermatozoa in a timely manner. Additionally, anejaculation may be related to a disruption of the neuromuscular components of the ejaculatory reflex pathway resulting from spinal cord injury, or prostatic or bladder surgery. The ejaculatory reflex pathway in the primate involves penile afferent stimulation which enters the spinal cord via the pudendal nerve at S2–S4 and ascends to the ejaculatory coordination centre in T11–L2 where descending cranial input is integrated (Oates and Kasabian, 1994; Sonksen *et al.*, 1998). The efferent reflex output is relayed to the sympathetic chain at T10 to result in emission from the accessory glands which subsequently induces the ejaculatory contraction of the cavernous muscles.

Penile vibratory stimulation therapy (PVS) for anejaculation has recently emerged as an alternative to rectal probe electroejaculation (RPE) and invasive testicular procedures (Brackett *et al.*, 1997; Ohl *et al.*, 1997; Abdulla and Rizk, 1998). Successful PVS requires intact afferent–effenter neural connections below T10, thus some spinal cord injured men are unlikely candidates for vibrostimulation. In contrast, RPE relies upon direct stimulation and contraction of periprostatic structures to effect a release of adjacent stored fluid and spermatozoa (Ohl, 1994). When applicable, PVS has produced superior specimens in spinal cord injured men (Ohl *et al.*, 1997) possibly due to a more physiologically natural stimulation utilizing the reflexive sequence of emission and ejaculation. However, this difference in semen quality may be related to the intricate effects of chronic denervation rather than to the collection technique employed since spermatozoa experience senile degeneration with prolonged storage in seminal vesicles. Scrotal hyperthermia associated with paraplegia is a possible cause of asthenoteratozoospermia (Brindley, 1982; Beretta *et al.*, 1989). Therefore, a direct comparison of semen collected by these contrasting techniques is needed but has not been ethically possible in men due to the discomfort involved with electroejaculation in sensory intact individuals.

Recently, we reported that PVS is effective in the squirrel monkey (Yeoman *et al.*, 1997). The present study used the squirrel monkey as a spinal cord intact non-human primate model for direct comparison of PVS to RPE. A cross-over design experiment was performed allowing comparison of physical and biochemical characteristics of semen. In our

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**Key words:** accessory glands/electroejaculation/penile vibratory stimulation/semen/squirrel monkey

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squirrel monkey research, semen has previously been collected by RPE while under anaesthesia (Yeoman et al., 1994). A persistent factor was that the quality and quantity of spermatozoa has been low. Thus, the need to evaluate an alternative technique for obtaining quality spermatozoa prompted this investigation.

Materials and methods

Ten adult male squirrel monkeys (Saimiri boliviensis boliviensis) were utilized as semen donors during mid-breeding season (January and February). Animals were housed in breeding pens, isosexual pens or individually caged. They were then divided into two experimental groups with one group initially receiving PVS and the other RPE. Three days later, treatments of the groups were reversed. These treatments were repeated 4 weeks later. The cross-over design was intended to minimize the variance of prior unknown ejaculations due to either mating or masturbation. The PVS was conducted with a FertiCare® Clinic unit (Multicept, Copenhagen, Denmark) which was adapted with a 5 cm length of vinyl tubing to hold a 1.8 ml sterile microfuge tube (Yeoman et al., 1997). The microfuge tube moved vertically as an artificial vagina and contained 200 µl Biggers, Whitten, and Whittingham’s medium (BWV) (Irvine Scientific, Santa Anna, CA, USA) with 3 mg bovine serum albumin/ml. The tube and media were kept at 37°C until just prior to collection.

The collection procedure consisted of manually restraining the donors on a cloth sling stand in ventral recumbency. The animals were handled by experienced personnel to facilitate collection with minimum anxiety and delay. The collection was performed in a darkened room and grapes were frequently eaten by the monkeys during the procedure to help reduce the stress associated with manual restraint. Prior to collection, the penis was cleaned with a sterile gauze sponge moistened with warm saline water. The penis was then held by thumb and forefinger with sheath retracted as the vibrating artificial vagina was raised to surround the exposed portion of the head and shaft. Initial vibration parameters were 1 mm amplitude and 75 Hz for 1–3 min. After 1 min rest, further stimulation at 1.8 mm amplitude and 90 Hz for 1–3 min was given until ejaculation occurred or for up to four cycles of stimulation and rest. These stimulation parameters for PVS are much less than the 2 mm amplitude and 100 Hz recommended for spinal cord injured men (Sonksen et al., 1994).

Animals receiving RPE were captured and handled in a manner similar to animals receiving PVS except that they received anaesthesia with 3.5 mg/kg tiletamine–zolazepam HCl (Telazol®, Fort Dodge Lab, Fort Dodge, IA, USA) due to the discomfort associated with this procedure (Yeoman et al., 1994). Electroejaculation was done with a clinical unit (Model 12; G & S Instruments, Duncanville, Texas, USA) utilizing a 0.9 cm probe with two 35 mm longitudinal electrodes 180° apart. Between the electrodes was a temperature sensor which activated an alarm when the adjacent tissue reached 41°C in order to avoid harmful heating due to the electrical current. The probe was lubricated with KY Jelly® (Johnson and Johnson, Skillman, NJ, USA) and the electrodes were positioned ventrally to extend from 15 to 50 mm proximal to the anal sphincter for stimulation of the prostate and accessory glands. Stimulation consisted of 1 min trains of 1 s on, 1 s off in staircase increments up to 7.5 volts of 60 Hz sine waves. Stimulation was repeated with alternate 1 min rest periods for up to five cycles if needed.

All semen specimens collected from PVS or RPE were processed within 1 min by estimating the volume, diluting with 4 ml of warmed media and incubating at 37°C in 5% CO₂ for 40 min to allow spermatozoa to be released from the seminal coagulum. After incubation, the residual undissolved coagula were removed and the diluted samples centrifuged at 150 g for 20 min. The supernatant was reserved for biochemical analysis of accessory gland contributions. A 5 µl portion was diluted 1:20 with water for determination of concentration with a haemocytometer. The percentage of motile spermatozoa and grade of motility were evaluated with a direct wet mount on a slide with coverslip. The grade of motility followed the convention of 0 = no movement, 1 = slow forward progressive movement (twitching), 2 = slow forward progressive movement, 3 = good forward progressive movement, 4 = rapid forward progressive movement and 4.5 = hyperactivation characterized by rapid high amplitude whip-like tail movement and non-linear progression (Bavister and Andrews, 1988; Gorrill et al., 1991).

The supernatant from the swim-out was heated to 80°C for 15 min to stop enzymatic degradation of products and then stored at -20°C until used for evaluation of accessory gland secretions. Seminal vesicle gland contribution to the semen was determined by measuring the amount of d-fructose in the supernatant. Fructose was quantified by reaction with hexokinase and the stoichiometrically produced NADPH was measured by an increased absorbance at 340 nm (Boehringer-Mannheim, Indianapolis, IN, USA). The sensitivity was 0.087 mg/ml and the standard control differed by less than 2% from what was measured. Prostate gland contribution to the semen was determined by measuring the amount of citric acid in the supernatant. This measurement also utilized an enzymatic reaction and the reduction of NADH was evaluated with an absorbance change at 340 nm (Boehringer-Mannheim). The sensitivity was 0.046 mg/ml and the supplied standard control differed by less than 2% from what was measured. Creatinine was measured as a monitor of urine contamination by use of a single slide method that enzymatically produces hydrogen peroxide which reacts with a colorimetric dye (Vitros DTSC II, Kodak, Rochester, NY, USA). The sensitivity was 0.25 mg/dl. For comparison, urine from 10 other animals was measured for creatinine content.

Analysis of successful trials was done using Fisher’s exact test. The other numerical parameters were analysed using the paired t-test (SigmaStat®, Jandel Scientific, San Rafael, CA, USA).

Results

Twelve of the 15 trials with PVS resulted in stereotypical mating responses: arching of the back, ventroflexion of the pelvis, erection, emission, and rhythmic ejaculatory contractions with production of semen (Table I). The three failures with PVS were from three different animals that were clearly agitated during the stimulation and did not respond. Two of these animals had previously responded to PVS and have since responded in other studies. The anaesthetized monkeys rectally stimulated during RPE had spasmodic contraction of the hind limbs, modest penile erection which frequently faded and stimulated during RPE had spasmodic contraction of the hind limbs, modest penile erection which frequently faded and did not respond. Two of these animals had previously responded to PVS and have since responded in other studies. The anaesthetized monkeys rectally stimulated during RPE had spasmodic contraction of the hind limbs, modest penile erection which frequently faded and did not respond. Two of these animals had previously responded to PVS and have since responded in other studies.
of just 0.5 ± 0.3 \times 10^6 and concentration of 2.8 ± 1.7 \times 10^6/ml (P < 0.01). In those nine RPE collected specimens that actually contained spermatozoa, the sperm motility was less vigorous than the PVS specimens with only 44.1 ± 11.4% of the spermatozoa in these specimens showing movement (P < 0.05). By considering only this subgroup of nine RPE collected specimens, the total count (0.7 ± 0.4 \times 10^6) and concentration (4.7 ± 2.7 \times 10^6/ml) were only slightly better. The grade of motility was significantly higher in the PVS collected specimens (PVS = 3.8 ± 0.2 versus RPE = 2.8 ± 0.4, P < 0.05). The combination of increased volume, higher sperm concentration and greater motility in the PVS collected specimens resulted in a much greater number of motile spermatozoa available for study per specimen (28.3 ± 9.2 \times 10^6 compared to 0.4 ± 0.2 \times 10^6; P < 0.01).

Biochemical analysis for accessory gland contribution found comparable fructose concentrations in semen collected by PVS (7.68 ± 1.05 mg/ml, n = 12) and in semen collected by RPE (6.07 ± 1.56 mg/ml, n = 15) (Table II). However, when adjusted for ejaculate volume, significantly more fructose was found after PVS (2.88 ± 0.67 mg/ejaculate versus 1.22 ± 0.32 mg/ejaculate from RPE, P < 0.05). Likewise, the concentration of citric acid, an index of prostrate gland contribution to the ejaculate, was similar in specimens produced by PVS compared to those collected by RPE (PVS: 0.98 ± 0.17 mg/ml; RPE: 1.39 ± 0.30 mg/ml). However, when the total amount per ejaculate was calculated, significantly more citric acid was found after PVS (0.46 ± 0.14 mg/ejaculate versus 0.24 ± 0.04 mg/ejaculate from RPE, P < 0.05). Since urine contamination of the semen was a concern, creatinine was also measured. Creatinine concentrations in both PVS and RPE specimens (0.322 ± 0.036 mg/10 ml and 0.260 ± 0.027 mg/10 ml, respectively) were similar to background (0.2–0.3 mg/10 ml) and much lower than in urine (7.9 ± 1.1 mg/10 ml, n = 10).

### Discussion

The present successful application of PVS to alert, spinal intact squirrel monkeys establishes the first animal model of this semen collection technique to our knowledge. An intact animal model is important to evaluate PVS clinical treatment for anejaculation since prior comparisons to RPE were able to utilize only spinal cord injured men for ethical reasons. However, factors such as the unknown extent of assessor gland neural dysfunctions, effects of chronic scrotal hyperthermia, and prolonged storage confound the semen analysis of human spinal cord injured individuals leaving the results difficult to compare (Brindley, 1982; Beretta et al., 1989; Engh et al., 1993).

Ideally, comparisons of PVS and RPE would be done in the same intact subjects as was done in the present comparison which utilized sexually active male squirrel monkeys for semen collection in a cross-over paradigm. Our results showed that the ability to stimulate production of a semen specimen was comparable between techniques with 100% of RPE trials producing specimens compared to 80% with PVS. The three failures with PVS involved monkeys that were clearly agitated during the test procedure and two of these have readily produced spermatozoa in other PVS studies. In examining the semen collected in the present trials, all the PVS specimens contained motile spermatozoa while only 60% of the RPE produced specimens containing motile spermatozoa. Thus, while there may be a trend for fewer of the PVS trials to induce ejaculation, more animals may yield spermatozoa. Rectal electroejaculation has long been the standard in many non-human primate species (Kuehl and Dukelow, 1974; Gould and Mann, 1988). However, results have been varied as shown in a recent report from another New World monkey, the marmoset, noting that only 30% of RPE trials produced suitable spermatozoa (Morrell et al., 1996). In comparison, successful responses to PVS in human spinal cord injured patients have ranged from 57–91% of the trials (Brindley, 1982; Oates and Kasabian, 1994; Sonksen et al., 1997; OHL, et al., 1997). Responses to RPE in similar patients showed comparable success (Brindley, 1984; Bennett et al., 1987; OHL, 1994; Abdulla and Rizk, 1998).

Of importance in the present squirrel monkey trials is the observation that penile vibratory stimulation produced much higher numbers of motile spermatozoa than did RPE. This enhanced yield with PVS stimulation was due to a greater semen volume, a higher concentration of spermatozoa and a higher percentage and grade of motility than found in specimens collected by RPE. In the spinal cord injured human, PVS has

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### Table I. Collection results from penile vibrostimulation (PVS) and rectal probe electroejaculation (RPE)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>PVS (n = 12)</th>
<th>RPE (n = 15)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Volume (µl)</td>
<td>436 ± 90</td>
<td>205 ± 25</td>
<td>&lt;0.05b</td>
</tr>
<tr>
<td>Concentration (×10^6/ml)</td>
<td>77.1 ± 20.4</td>
<td>2.8 ± 1.7</td>
<td>&lt;0.01b</td>
</tr>
<tr>
<td>Total spermatozoa (×10^6)</td>
<td>32.8 ± 10.2</td>
<td>0.5 ± 0.3</td>
<td>&lt;0.01b</td>
</tr>
<tr>
<td>Ejaculate with motile spermatozoa (100%)</td>
<td>12/12</td>
<td>9/15</td>
<td>&lt;0.05a</td>
</tr>
<tr>
<td>Percent motile</td>
<td>80.6 ± 4.3</td>
<td>44.1 ± 11.4</td>
<td>&lt;0.01b</td>
</tr>
<tr>
<td>Motility grade</td>
<td>3.8 ± 0.2</td>
<td>2.8 ± 0.4</td>
<td>&lt;0.05b</td>
</tr>
<tr>
<td>Motile spermatozoa (×10^3)/ejaculate</td>
<td>28.3 ± 9.2</td>
<td>0.4 ± 0.2</td>
<td>&lt;0.01b</td>
</tr>
</tbody>
</table>

aFisher’s exact test.
bPaired t-test.

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### Table II. Biochemical analysis of semen collected by penile vibrostimulation (PVS) or rectal probe electroejaculation (RPE)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>PVS (n = 12)</th>
<th>RPE (n = 15)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fructose</td>
<td>7.68 ± 1.05</td>
<td>6.07 ± 1.56</td>
</tr>
<tr>
<td>Citric acid</td>
<td>0.98 ± 0.17</td>
<td>1.39 ± 0.30</td>
</tr>
</tbody>
</table>

Note: *P* < 0.05 with paired t-test.
also been shown to produce greater numbers of spermatozoa than RPE in the antegrade sample (Brackett et al., 1997; Ohl et al., 1997). However, additional retrieval of the retrograde fraction from the bladder of RPE produced a total number of spermatozoa similar to PVS specimens but those spermatozoa had reduced motility. Retrograde ejaculation during PVS has also been found in macaque monkeys (Schaffer et al., 1989) and may be due to loss of coordinated sphincter control related to the anaesthesia or the electrical surge. However, in the present study, the reduced number of spermatozoa found in the RPE stimulated specimens could only partially be due to retrograde ejaculation since the specimen volume was only half of the PVS volume, yet the number of spermatozoa was less than one twentieth in number.

Clinically, ejaculations in some cases of spinal cord injury, diabetes, bladder neck surgery, and retroperitoneal lymphadenectomy have been associated with bladder sphincter failure and retrograde ejaculation (Brindley, 1984; Sigman, 1994). An unfortunate complication is that the quality of spermatozoa produced by spinal cord injured men whether retrieved by PVS or RPE ranges from 10–48% motility compared with a much higher level of motile spermatozoa from intact men (Beckerman et al., 1993; Ohl et al., 1997). In these patients, motility has been shown to improve with repeated sampling over time, suggesting prolonged storage is a major problem (Beretta et al., 1989; Engh et al., 1993). The electrical current used for electroejaculation has been suspected of physically damaging the spermatozoa either directly or via oxidative free radicals. However, some studies have not found any effect on sperm function (Linsenmeyer et al., 1989; Witt et al., 1992; Ohl et al., 1994; Rajasekaran et al., 1994; Hovav et al., 1996).

In matched PVS versus masturbation trials in intact humans, semen analyses have shown comparable characteristics (Toussaint et al., 1993) suggesting that vibratory penile stimulation itself is not harmful to the spermatozoa. Additionally, in intact individuals, PVS and masturbation are likely to involve a similar emission and ejaculatory sequence since the quality of spermatozoa is similar. However, the concentration of spermatozoa is still less than that found after natural intercourse (Purvis et al., 1986; Sofikitis and Miyagawa, 1993).

In order to better understand the stimulatory processes of PVS and RPE, accessory gland markers present in the semen were compared. In the squirrel monkey, the seminal vesical contribution of fructose to the semen was comparable in concentration between the two techniques. However, with the greater volume of semen collected by PVS, twice as much total fructose was found, indicating a greater collection of seminal vesicle secretions. Semen collected by masturbation in humans and chimpanzees has shown greater seminal vesicle marker concentration than when collected by RPE, indicating a comparatively reduced seminal vesicle stimulation with RPE in these species (Hirsch et al., 1991; Young et al., 1995). In the present experiments, fructose content from RPE was slightly higher than that found in a previous brief report of RPE in the squirrel monkey (Ackerman and Roussel, 1968) but was still significantly less than that obtained with PVS.

Prostate gland contribution to the semen, as indexed by citric acid, was also comparatively evaluated in PVS and RPE collected specimens. Semen citric acid concentrations from both methods were similar in the present squirrel monkey studies, yet the increased ejaculate volume with PVS resulted in a greater total amount of citric acid indicating a greater collection of prostate products. Prostate secretion studies in the human found that the masturbation method of semen collection in spinal intact men produced higher levels of alkaline phosphatase compared to those produced by RPE collection in spinal cord injured subjects (Hirsch et al., 1991). However, differences between the subjects can confound the results. Another study using intact humans compared PVS with masturbation and found equivalent prostate stimulation as judged by total alkaline phosphate (Toussaint et al., 1993). In contrast to the above, studies in the chimpanzee using citric acid as a prostate stimulation marker found that specimens collected by RPE contained similar total amounts of citric acid to specimens collected by masturbation (Young et al., 1995).

Thus, the differences in prostate contributions between methods are not clear, but may reflect the position of the stimulating probe within the rectum relative to the adjacent prostate.

Other studies have noted increased epididymal marker concentrations associated with increased numbers of spermatozoa in the ejaculates of humans and chimpanzees collected by masturbation (Soufir et al., 1981; Marson et al., 1988). In a chimpanzee study, a comparison between RPE and artificial vagina collection also found an association between the higher concentration of the epididymal marker glucosidase and total spermatozoa in specimens collected by artificial vagina (Young et al., 1995). Thus, there is a strong suggestion that an increased number of spermatozoa are recruited from the epididymis when sensory stimulation is involved in ejaculation. Sexual stimulation prior to ejaculation in domestic bulls can dramatically increase the yield of spermatozoa (Hafs et al., 1962). This increase may relate to the pre-ejaculatory transport of spermatozoa from the storage site in the tail of the epididymis into the more distal genital ducts (Prins and Zaneveld, 1979).

The present squirrel monkey study found that PVS induced ejaculates contained much more spermatozoa, a higher grade sperm motility, and more accessory gland secretions in comparison with RPE ejaculates. These findings were not complicated by the effects of spinal cord injury as has been the case in other comparative studies. Additionally, the relative ease of specimen retrieval makes PVS clearly the preferable semen collection method in this species. Whether the difference in yield is due to a greater epididymal stimulation involved with PVS or to the loss of a major portion of the ejaculate into the bladder with RPE requires further study.

In human assisted reproductive procedures with spinal cord injured patients, spermatozoa collected by both PVS and RPE have been successfully used for intrauterine insemination, in-vitro fertilization and intracytoplasmic sperm injection (Tournaye et al., 1995; Denil et al., 1996; Nehra et al., 1996; Brinsden et al., 1997). The present results would support the choice of PVS in the human although its use may not always be possible depending on the extent of spinal injury in the individual patient (Ohl et al., 1998; Sonksen et al., 1998).
Penile vibratory stimulation and electroejaculation

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