Viable spermatozoa in the bladder after electroejaculation of lion-tailed macaques (*Macaca silenus*)

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Summary. The bladder of 6 lion-tailed macaques was emptied and flushed with sterile saline. TALP-Hepes buffer was infused and the animals were electroejaculated. After electroejaculation, the semen quality was determined in the ejaculate and the bladder infusate. Of the 15 ejaculates analysed, a mean ($\pm$ s.e.m.) sperm count of $133.8 (\pm 30.7) \times 10^6$ with $69.5 (\pm 6.0)\%$ motility was obtained in the infusate as compared to the sperm count of $72.4 (\pm 38.6) \times 10^6$ with significantly lower ($47.7 \pm 5.8\%$) motility in the ejaculate.

Keywords: macaque; retroejaculation; ejaculation; sperm viability

Introduction

Rectal probe electroejaculation is routinely used to obtain semen from non-human primates. This procedure has been reported to induce retrograde flow of semen into the bladder in many species. This loss of spermatozoa to the bladder may have a significant effect on sperm count. This study attempts to determine whether retrograde flow of semen occurs following electroejaculation in lion-tailed macaques, and if so, to estimate the relative loss of spermatozoa to the bladder and to study the feasibility of recovering these spermatozoa.

Materials and Methods

Animals. Six healthy lion-tailed macaques were placed in lateral recumbancy after sedation with 25 mg ketamine HCl/kg (Ketaset, Bristol Laboratories, Syracuse, NY, USA) intramuscularly. The bladder was emptied of urine by using a size 8 french catheter and subsequently rinsed with 20 ml saline. Immediately, 20 ml TALP-Hepes (Bavister *et al.*, 1983) medium were infused into the bladder, the catheter removed and the animal electroejaculated.

Electroejaculation. A 2.5-cm diameter rectal probe with three 130×3 mm electrodes that were 0.7 cm apart was placed in the pelvic rectum with electrodes positioned ventrally. An electrical current of 20 Hz was delivered to the probe by a Lane P-3 Z stimulator (Lane Manufacturing Co., Denver, CO, USA). The pattern of electrical delivery consisted of rhythmic pulse stimulations at a constant voltage. The stimulus was applied in pulses of 3-sec duration with a 1-sec rest between pulses. Three series of 10 pulses each, took place at settings of 5, 8, 10 V. Stimuli were applied at a current density which did not exceed 0.25 mA/mm^2 (Gould *et al.*, 1978). The probes were adjusted inside the rectum to obtain maximal erection of the penis. After each series, the urethra was 'stripped' (massaged via the rectum) to remove seminal fluid from the urethra (Roussel & Austin, 1968).

After electroejaculation, the bladder was catheterized again and the contents emptied. The volume, sperm count and motility were determined in the bladder infusate and ejaculate after electroejaculation.

The mean and standard error of the mean were calculated and paired *t* tests were performed to assess the significant differences in means of sperm count and motility between the ejaculate and the bladder infusate.

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Results

No infections occurred after the procedure even though some attempts resulted in the presence of blood in the catheter. Spermatozoa were not found in the urine or saline lavages before electroejaculation in any of the animals. After electroejaculation, spermatozoa were found in all but one of the bladder infusates (Table 1). The volume of the bladder samples was significantly higher because of the inclusion of 20 ml medium. The lack of significant difference between sperm numbers in the ejaculate and the bladder infusate indicated that at least half of the spermatozoa flowed into the bladder during electroejaculation.

Table 1 also demonstrates a significant ($P < 0.05$) improvement in the motility of spermatozoa obtained from the bladder infusate. However, raw samples were incubated in seminal fluids for 30 min which may be detrimental to sperm viability and the TALP-Hepes medium may improve sperm motility.

Table 1. Semen parameters of ejaculate and bladder infusates obtained from 6 lion-tailed macaques after electroejaculation (15 samples)

	Volume (ml)	Sperm count ($\times 10^{-6}$)	Sperm motility (%)
Ejaculate	0.7 ± 0.2	72.4 ± 38.6	47.7 ± 5.8
Bladder infusate	$23.9 \pm 0.8^*$	133.8 ± 30.7	$69.5 \pm 6.0^*$

Values are mean $\pm$ s.e.m.

* $P < 0.05$ compared with value for ejaculate.

Discussion

Normal ejaculation is a neurologically sequenced event proceeding first with seminal emission into the posterior urethra and then with forceful propulsion of semen from the posterior urethra out of the distal penis by contraction of penile musculature (Rose, 1953; Kedia & Markland, 1976). During this process external and internal sphincter synchrony along with proper peristaltic flow is required to encourage semen in an antegrade direction (Schienen & Hupe, 1976; Newman *et al.*, 1982; Thomas, 1983) as demonstrated by radiological studies of rams (Hovell *et al.*, 1969) and marsupials (Rodger & Pollitt, 1981). The lack of synchronous flow therefore traps semen in the posterior urethra and allows it to flow back into the bladder when stimulation ceases. Men that can ejaculate, but are partly dysfunctional will also retroejaculate (Reiser, 1961; Rose, 1953; Okamura *et al.*, 1985). In addition, spermatozoa are also found in the bladders of paraplegic men after electroejaculation (Brindley, 1981; Sarkarati *et al.*, 1987). The neurological dys-synchronous stimulation occurring after injury, or in the process of electroejaculation procedures, causes physiological disruption of normal ejaculation events and semen flows retrogradely into the bladder.

Preliminary cystocentesis studies have suggested loss of 60–80% of the total spermatozoa to the bladders of macaques (Schaffer *et al.*, 1989). In the present study, on the average, 65% of the spermatozoa were lost to the bladder. Extensive loss of spermatozoa to the bladder during electroejaculation has been reported for many species of animals (Pineda *et al.*, 1987). Cystocentesis studies by Dooley *et al.* (1984, 1986) determined a 6–61% and a mean 21% loss of spermatozoa to the bladder of electroejaculated cats and bulls, respectively.

Reports on retrograde sperm loss in man rarely give sperm counts. Only a few men exhibit complete retroejaculation, while the large majority have both retrograde and antegrade ejaculation.

Analysis of available data (Halstead *et al.*, 1987) from three retroejaculatory patients reported loss of 3–50% total sperm numbers to the bladder.

In the present study, pushing of spermatozoa back into the bladder by the catheter was a possibility, but 'stripping' was used to clear the semen from the urethral tract before introducing the catheter (Roussel & Austin, 1968; Halstead *et al.*, 1987) and any residual semen in the tract could not have produced the significant numbers of spermatozoa found in the bladder. In addition, no spermatozoa were found in the bladder infusate of one collection that produced significant numbers of spermatozoa in the ejaculated sample, indicating that 'stripping' the urethra *per rectum* was an effective means of removing semen from the urethra.

Total sperm recovery is needed for proper fertility determination and to provide greater numbers of spermatozoa for artificial manipulations. Other researchers have made attempts to prevent retroejaculation during electrostimulation. Brindley (1981) suggests that more appropriate electrode placement facilitates antegrade flow, while Warner *et al.* (1986) unsuccessfully blocked the neck of the bladder with a foley catheter. Most reports advocate retrieving spermatozoa from the bladder after alleviating the harmful effects of bladder contents by alkalizing the urine (Garcea *et al.*, 1982; Cameron & Gillett, 1985; Brassesco *et al.*, 1988) or replacing it with medium. Bennett *et al.* (1987) replaced the bladder contents with a buffered medium before ejaculation and recovered bladder spermatozoa for artificial insemination. They were able to obtain successful pregnancies with this semen. With the lion-tailed macaque, replacing bladder contents with medium was used because of the difficulty in administering alkalizing drugs and the risk of drug interactions. Improved motility was seen in the spermatozoa in the TALP–Hepes medium recovered from the bladder in stimulated macaques. Spermatozoa recovered from the bladder have successfully fertilized eggs *in vitro*, and 2 pregnancies have resulted from bladder recovered spermatozoa (N. E. Schaffer, unpublished data).

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