

THE PRESENCE OF SPERMATOZOA IN THE ORAL CAVITY OF SEXUALLY ACTIVE MALE RATS

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ABSTRACT

Many Ayurvedic Physicians in Sri Lanka claim that rat semen contains toxin/s which by gaining entry to the human body through a rat bite or a scratch could cause vitiligo. The presence of semen in the oral cavity and in the claws of rats is a prerequisite for such a mode of entry. The aim of this study was to investigate the presence of sperm (as an indicator for semen) in the oral cavity and in claws of the forelimbs of both male and female rats. The oral cavities and claws of fore limbs of the following groups of rats were washed with 0.25ml of isotonic saline and the sperm numbers in the washouts were estimated: (a) males and hormonally primed receptive females, 2-5 min and 60 min of ejaculation; (b) males and dioestrous females 60 min following pairing; c) unpaired males after 1.0 h observation period; and d) unpaired males with no observation period. The results show that sperm are found in moderate numbers in the mouth wash of males paired with receptive females both immediately (mean \pm SEM: 0.59 ± 0.11 million ml^{-1} ; range : 0.2-1.35 million ml^{-1}) and 60min (mean: 0.38 ± 0.09 million ml^{-1} range : 0.2 - 1.0 million ml^{-1}) after ejaculation. Furthermore, at 60 min the mean sperms count was lower than that immediately after ejaculation although not significant. On the other hand, no sperms were detected in the mouth wash of males paired with dioestrous females, in unpaired males and, in receptive and dioestrous females upto 1h of pairing. Except in one male, which was paired with a receptive female, no sperms were detected in the claw wash of any rat of either sex. Thus, a strong possibility exists that rat semen could infiltrate the human body via a bite of male rat following ejaculation.

Key words : semen, spermatozoa, oral cavity, claws and rat.

INTRODUCTION

In Sri Lanka, many Ayurvedic physicians claim that rat bite is one of the possible causes of human vitiligo. This claim appears to have its roots from Susrutha, the Indian Maha Rishi and a pioneer of the Ayurvedic system of medicine who proposed, 2500 years ago, that the rat semen contains toxin/s which by gaining entry to the human body through a bite or a scratch of a rat causes a variety of diseases (Buddadasa, 1962). The presence of semen in the oral cavity and in the claws of sexually active rats is a prerequisite for such a mode of entry into the human body. However, to our knowledge, presence of sperm in the oral cavity or in the claws of rats has not been reported to date although genital autogrooming and pawgrooming (Hart et al, 1990) occur during their copulatory behaviour. The aim of this preliminary study was to investigate the presence of sperm (as an indicator of the presence of semen) in the oral cavity and in the claws of fore limbs of post-copulatory rats, in an attempt to test the claim made by Susrutha, toxins that toxins in semen can enter the human body though a bite or scratch.

MATERIALS AND METHODS

Mixed-bred healthy adult rats from the colony at the Department of Zoology, University of Colombo were used (male weighing 225-275g and females weighing 200-250g). The animals were kept under standardised animal house conditions (temperature: 28-31°C; photoperiod : 12h light and 12h dark; relative humidity : 50-55%) with free access to pelleted chow (Oils & Fats Co. Ltd., Sceduwa, Sri Lanka) green vegetables and tap water. Male rats were isolated from females for at least 3 weeks prior to commencement of the experiment.

12 randomly selected male rats were individually placed in a wire-meshed cage with two side glasses (observation cage) and were left for 15 min to get acclimatized to the novel environment. These rats were presented with a stimulatory female brought into oestros by sequential treatment with oestradiol benzoate (Sigma, St. Louis, USA), 12.5µg per rat, subcutaneously, in olive oil, 54h before pairing) and progesterone (Sigma, St Louis, USA) (0.5g per rat, subcutaneously in olive oil, 6h before pairing) (Ahlcnias *et al*, 1990). The sexual behaviour of these male rats was recorded for 60min.

Following ejaculation (2-5min), each male was removed from the observation cage and sperm numbers in the mouth wash and claw wash of the fore limbs were estimated in the following manner. Using a glass pipette, 0.25ml of isotonic saline (0.9% NaCl w/v) was administered into the oral cavity of the rat after keeping a watch glass immediately under the mouth (to collect any fluid falling from the mouth). The solution was retracted into the pipette and reintroduced to the oral cavity. This procedure was performed five times. Any fluid accumulated in the watch glass was collected and mixed with the contents collected in the pipette and was examined for the presence of sperm under phase contrast microscopy (x 400). If sperms were present, their number was estimated in duplicate by using an improved Neubauer haemocytometer (America Optical Corporation, Buffalo, USA). The claws of each fore limb of these males were washed 5 times using 0.25ml isotonic saline solution using a glass pipette as described earlier after keeping a watch glass immediately under each fore limb. The sperm number in the claw wash was then estimated as described earlier. Sperm numbers were then estimated in the mouth wash and in the claw wash of the paired females. Mouth and claw washes were made again from the male rats 1.6h following ejaculation and sperm numbers were estimated.

Another set of 12 males were randomly selected and paired in the observation cage individually with dioestrous females (n=12) selected by vaginal smearing. The sexual behaviour of the paired males was observed as in the previous experiment for 60 min. Mouth wash and claw wash of the fore limbs were obtained as described earlier and then examined for the presence of spermatozoa. If sperms were present their numbers were estimated.

12 males were selected randomly and were placed individually in the observation cage. Each rat was observed for 1.0h for the presence of any auto - genital licking activity. Mouth wash and claw wash of the fore limbs of these rats were taken and examined for the presence of sperm.

Another set of 24 male rats were randomly selected at 8.00h and mouth wash and claw wash were taken and examined for the presence of sperm.

RESULTS

Males exhibited genital licking of the paired females both in receptive and non receptive (dioestrous) condition. However, all the males (100%) paired with receptive females displayed several mounts and intromissions and 11 out of 12 rats (92%) finally ejaculated. In contrast, 2 out of 12 (17%) males paired with dioestrous females showed mounts and intromission (1-2) and non (100%) ejaculated. Intromissions and ejaculations were always followed by self genital licking.

Sperm counts in the mouth wash of males following ejaculations are shown in Table 1. Sperm counts in the mouth wash of males immediately following ejaculation varied between 0.2 and 1.35 million ml⁻¹, (mean \pm SEM 0.59 \pm 0.11 million ml⁻¹). The mean sperm number in the mouth wash 60 min after ejaculation, was 0.38 \pm 0.09 million ml⁻¹ and the range 0.2 - 1.0 million ml⁻¹. However, the difference in sperm numbers in mouth wash on the two occasions was not statistically significant ($P > 0.05$, Mann - Whitney U-test). Interestingly, 60 min following ejaculation, in 2 adult rats (18%), sperm counts in the mouth were higher (by 1.3 - 1.5 fold) than that on the previous occasion. This was an unexpected finding. On the other hand, sperm count in the mouth wash of 9 out of 11 rats (82%), in the second occasion was lower (2-450 fold) than at the first occasion. In complete contrast, sperms, in countable numbers, were not found in the mouth wash of 2 out of 11 rats (18%) 60 min following ejaculation although sperms were detected in the mouth wash on the first occasion. The mouth wash of all these males, in both occasions, had mild to moderate numbers of oestrous cells (not quantified).

The mouth wash of males paired with dioestrous females, never showed the presence of sperms though small numbers of leucocyte were detected (not quantified). Similarly no sperms were detected in the mouth wash, either in receptive females or in dioestrous females.

Except in one male (3-5 sperms per microscope field) paired with a receptive female, no sperms were detected in the claw wash of males or females paired together.

Of the 12 male rats observed without pairing (for 60min) 5 out of 12 (42%) exhibited self genital licking. However, no sperms were detected in their mouth wash and claw wash. Similarly mouth and claw wash of the 2 randomly selected male rats were found to be devoid of any sperm when checked at 8.00h

DISCUSSION

According to a recent report 1-4% of the world population is affected by vitiligo which is a disorder characterized by the disappearance of epidermal and/or follicular melanocytes by unknown mechanisms (Ortonne and Bose, 1993). Many Ayurvedic Physicians in Sri Lanka strongly claim that infiltration of some toxin (yet unidentified), present in the semen of sexually active rats, in man via their bites and /or scratches is one of the possible causes of vitiligo. In this respect, it is noteworthy that a pioneer of the Ayurvedic system of medicine, Susrutha, has suggested 2500 years ago the presence of toxins in rat semen and the possibility of rat semen contaminating the human body via scratch and/or bite (Buddadasa, 1962) The recent discovery of an epididymal protein with remarkable sequence similarity to snake venom haemorrhagic peptide in the epididymal secretion of rat (Perry *et al*, 1992) is equally interesting. If the claim of Ayurvedic Physicians in Sri Lanka is correct then semen should be present in the oral cavity and/

or claws of sexually active rats in order to gain entry in to the human body. Using sperm as an index of semen we tested this hypothesis.

The results of this study, show the presence of mild to moderate numbers of sperm in the mouth wash of male rats immediately following ejaculation. Sperms were also detected in the mouth wash one hour later although the numbers were considerably less. Subsequent studies carried out by us indicate the presence of sperm in mouth wash 24h following ejaculation (unpublished observation). Although, post-copulatory genital licking is described as a stereotyped behavioural response in rats (Hart et al, 1990) this is the first study to show the presence of sperms in the count in the mouth wash of post-copulatory male rats. The mean sperm count in the mouth wash immediately following ejaculation was 0.59 million ml⁻¹. The recovery rate of sperm by the method used in this study is 5-10% (Palathiratne, 1996). Thus, the actual sperm number in the oral cavity of male rats following ejaculation should be much higher than that recorded here. On the other hand, no sperms were detected in the mouth wash of unpaired male rats, male rats paired with dioestrous females and in female paired with males. Spontaneous ejaculations are reported in rats (Kihlstrom, 1996). Thus, a possibility exists that sperms could be present in the mouth wash of unpaired male rats due genital to auto licking although we did not find any. Except in one case no sperms were detected in the claw wash of fore limbs of rats of either sex. Usually, rats make scratches on objects with their fore claws.

These observations strongly indicate that infiltration of rat semen into the human body is only possible via a bite of a male rat following ejaculation. Thus, the suggestion made by the Indian Maha Rishi Susrutha, and the claim of some of our Ayurvedic Physician in Sri Lanka seems to be scientifically plausible. However, cause and effect relationship between rat bite and /or scratch and human vitiligo remains to be seen. Experiments are underway to test this hypothesis.

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TABLE 1 - Sperm count in mouth wash of male rats following ejaculation

<i>RAT NUMBER</i>	<i>2-5 MINUTES AFTER EJACULATION</i> (x10 ⁶ /ml)	<i>60MINUTES AFTER EJACULATION</i> (x10 ⁶ /ml)
1	0.35	0.45
2	1.25	1.00
3	1.20	0.60
4	0.85	0.70
5	0.35	0.20
6	NE	NE
7	0.40	0.60
8	0.30	0.20
9	0.20	0.00
10	0.60	0.20
11	0.45	0.00
12	0.55	0.25

NE = not ejaculated up to 60 min.