STUDIES ON THE COLLECTION AND STORAGE OF SEMEN FROM THE AFRICAN ELEPHANT, *LOXODONTA AFRICANA*

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Abstract – Methods of semen collection following electrical stimulation are described. Semen was also collected from the male reproductive tract of slaughtered animals for several studies. Spermatozoa flushed from the male reproductive tract were immotile, but, dilution with buffered saline was sufficient to induce motility. It was found that the spermatozoa were sensitive to hypotonic solutions and rapid cooling to 5°C. Spermatozoa were frozen in egg yolk-citrate diluents. Dimethyl sulphoxide (DMSO) was a better protective agent than glycerol. Eight per cent v/v was the best concentration of DMSO when used alone, but better results were obtained using a combination of 7% v/v DMSO and 1% v/v glycerol. A semen bank was established using these concentrations of protective agents and thawed samples from the bank showed good viability in the laboratory.

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Introduction

The collection and storage of deep frozen elephant spermatozoa was studied in Kruger National Park, Republic of South Africa, during September and October 1972, as part of a research project at the Zoological Society of London on the artificial breeding of animals in captivity (Rowlands 1964, 1973). The elephant was of particular interest in this context as bulls have proved dangerous to handle and are usually not kept in zoos.

The problems of collecting and storing semen for artificial insemination were considered by Jones (1971). Two methods of semen collection were used in the present studies. The possibility of collecting semen following electrical stimulation with a rectal probe (electro-ejaculation) was examined as this would be the most desirable approach for future studies. However, to ensure that semen was available for the storage experiments, spermatozoa were recovered from the reproductive tracts of bull elephants culled during the Park's cropping programme.

Initially it was necessary to carry out some detailed studies on the epididymus of the elephant as the report of Short, Mann and Hays (1967) indicated that these ducts, which connect the vasa efferentia to the urethra, are relatively primitive tubes containing only immotile spermatozoa. Further, Short *et al* (1967) were unable to induce motility satisfactorily in spermatozoa collected from these tubes. The methods of processing and storing semen which were used in these studies were adapted from techniques used successfully to preserve semen from other species (Jones 1971) and which could be used under the prevailing field conditions.

Material and Methods

1. Electro-ejaculation

Bull elephants were immobilised with the central narcotic analgesic "Immobilon" (Reckitt & Coleman, Hull). The drug was administered by a rifleman, located in a helicopter, using darts which injected the solution on impact. Electrical stimulation was carried out with a rectal proble using a variable output square wave stimulator powered by a 12 volt wet cell battery (Figs. 1 and 2). The design of some of this apparatus was based on a description by Martin and Rees (1962). The main body of the rectal probe (Fig. 2) was manufactured from three lengths of tubular aluminium with a wall thickness of 0,75 centimetre. When assembled, the lengths and diameters respectively of the three tubes were 32 cm and 10 cm for the tube carrying the electrical contacts, 22 cm and 8 cm for the central portion and 4 cm and 5 cm for the end tube from which the electrical cord issued. The electrical contacts were sup-
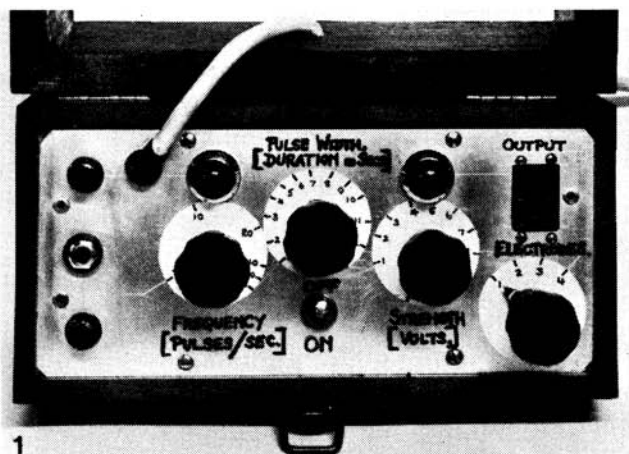
Fig. 1.   Square wave generator which provides the electrical stimuli for the rectal probe used for electro-ejaculation. Electrical circuit is shown in Fig. 3. Box length is 24 cm.
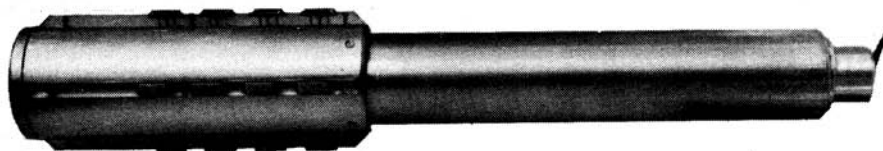

Fig. 2.   Rectal probe for applying electrical stimuli. Four strips of perspex (3 are shown) are equally spaced around the aluminium cylinder and each supports 4 brass electrical contact plates. The width of each plate is 1,0 cm.

ported 1 cm above the surface of the aluminium sleeve on four strips of perspex equally spaced around the circumference of the probe. Each of the four sets of contacts consisted of four brass plates with surface dimensions of 1,0 cm × 2,5 centimetre. Each set was wired so that a ring stimulus of band width 2,5 cm was obtained by bridging adjacent quadrants; each set could be selected and used independently (see electrode selector knob on stimulator shown in Fig. 1). The first set of contacts was 10 cm below the apex of the probe and positions 2, 3 and 4 were situated posteriorly at 2 cm intervals. Apart from the perspex strips and electrical contacts, the whole surface of the probe was painted with a waterproof varnish.

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The electrical generator for the rectal probe (Fig. 1) produced square pulses of variable duration, repetition frequency and strength (voltage). The circuit (Fig. 3 and Table 1) consisted of three sections: (a) a pulse
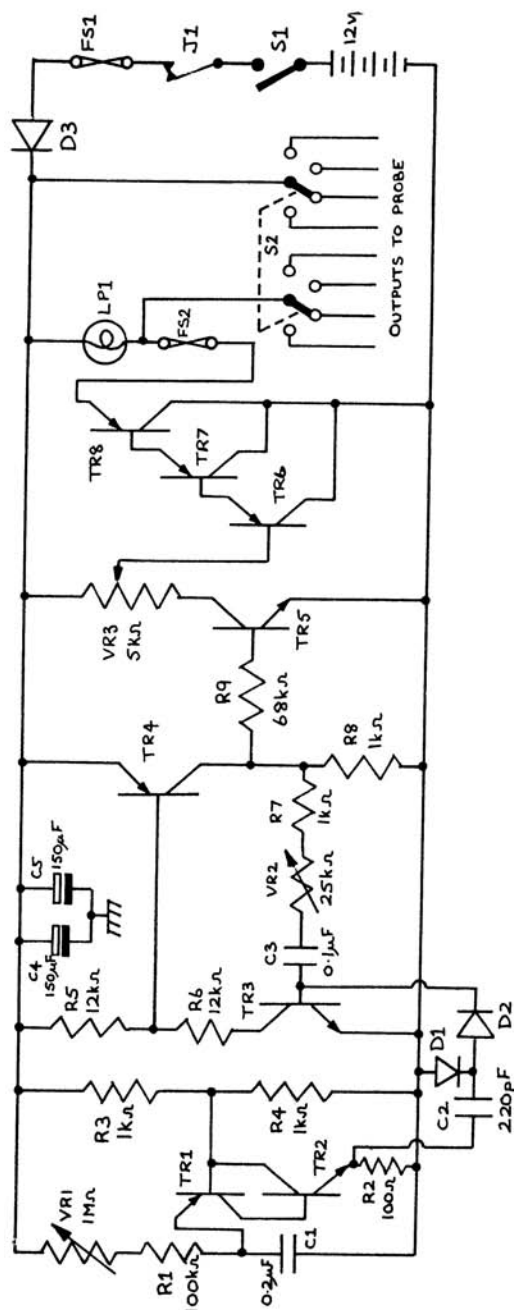

Fig. 3. Electrical circuit for the square wave generator used for electro-ejaculation. Details of the individual components are listed in Table 1.

Table 1

Electrical components of the circuit shown in figure 3

R1	100 kΩ	VR1	1 MΩ lin
R2	100 kΩ	VR2	25 kΩ lin
R3	1 kΩ	VR3	5 kΩ lin
R4	1 kΩ		
R5	12 kΩ	C1	0,2 uF (0,1 μF + 0,1 μF)
R6	12 kΩ	C2	220 μF
R7	1 kΩ	C3	0,1 μF
R8	1 kΩ	C4	50 μF 15v
R9	68 kΩ	C5	150 μF 15v

All ± 5% half watt carbon

TR1	ZT×500	TR5	ZT×300
TR2	ZT×300	TR6	ZT×500
TR3	ZT×300	TR7	ZT×500
TR4	ZT×500	TR8	AD 149

D1	1N914	FS1	2 AMP
D2	1N914	FS2	2 AMP
D3	any diode capable of handling at least 2 amps.		

repetition frequency generator (P. R. F.); (b) a pulse duration monostable; (c) a current amplifier. Each of these sections is described separately below:

(a) The P. R. F. generator is comprised of TR1, TR2, R1 to R4, VR1 and C1. With power supplied to the unit, the voltage on the base of TR1 is determined by the values of R3 and R4. C1 charges through VR1 and R1, the rate of charging being controlled by the setting of VR1 (P. R. F. control). Whilst the voltage across C1 is less than the voltage on the base of TR1, the base-emitter junction of TR1 is reverse biased; the input impedence to this transistor is therefore very high. When the voltage across C1 has risen to a sufficiently high value (approx. 0,6 volt greater than on the base of TR1) the base-emitter junction becomes forward biased. TR1 now begins to conduct drawing current through the base-emitter junction of TR2, causing TR2 to conduct. As TR2 conducts, the voltage on the base of TR1 falls, attempting an increase in the base-emitter voltage of TR1. This causes both TR1 and TR2 to turn hard on. The combined impedence of TR1 and TR2 falls to a very low value discharging C1 through R2, producing a voltage spike across R2. The sequence continues whilst power is supplied to the unit. The sequence is shown in Fig. 4. The pulse frequency could be varied from 0 to 50 hertz.

VOLTAGE ACROSS C1

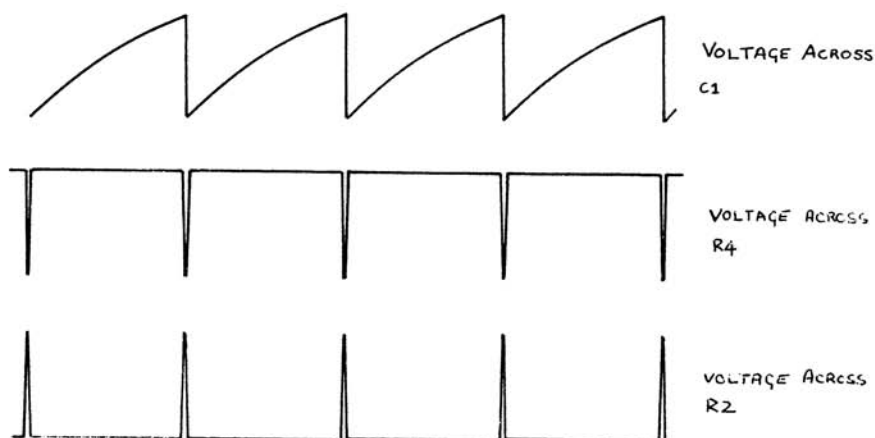VOLTAGE ACROSS R4

VOLTAGE ACROSS R2

Fig. 4.   Generation of pulse repetition frequency in square wave generator.

(b)   The monostable circuit is built around TR3 and TR4. In the stable state both TR3 and TR4 are switched off. A positive pulse applied to the base of TR3 switches the transistor on: the voltage at the junction of R5 and R6 falls below the rail voltage causing TR4 to conduct, causing the junction of R7, R8 and R9 to rise to rail voltage. C3 now charges through VR2 and R7, the charging rate being determined by the setting of VR2 (pulse duration control). As the charge on C3 increases, the current flowing through the emitter-base junction of TR3 falls, gradually switching off TR3 allowing the voltage at the junction of R5 and R6 to rise towards rail voltage. TR4 will eventually switch off causing a negative spike to be applied to the base of TR3; C3 then discharges via VR2, R7 and R8. This sequence is repeated whenever a positive pulse is applied to the base of TR3. This positive pulse is derived from the P. R. F. generator via C2, D1 and D2. D1 and D2 ensure that only positive pulses are applied to the base of TR3. This is important as negative pulses applied to the TR3 base will switch off TR3 causing the monostable to malfunction. The pulse duration could be varied from 0 to 14 milliseconds.

(c)   The above switching is carried out at low current levels; it is therefore necessary to amplify the output current. This is achieved by TR6, TR7 and TR8. TR5 inverts the output of the monostable, the amplitude output potentiometer (VR3) being the collector load. The voltage between the +12v supply line and the wiper of VR3 can be adjusted from 0

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to approximately 11 volts. The current amplifier consists of transistors TR6, TR7 and TR8 connected in a Darlington configuration. The combination of these transistors operate as an emitter follower, i.e. the voltage on the emitter follows the base voltage to within about 1 volt. As square wave pulses, variable between 0 - 11 volts, can be applied between the base of TR6 and the 12 volts supply-line, it follows that pulses of similar amplitude will be developed across the emitter load of TR8. This load consists of the indicator lamp (LP2) and the rectal probe. Switch S2 selects at which portion of the probe the pulses are applied. J1 is wired in series with the main supply so that remote control of the unit is possible. D3 protects the electronics in event of the supply being incorrectly connected.

2. Diluent Composition

egg-yolk-citrate: 50% v/v fresh egg yolk, 40 mM sodium citrate, 5M dibasic sodium phosphate and 5 mM sodium dihydrogen phosphate.

citrate-fructose: 69 mM sodium citrate, 140 mM fructose, 9 mM sodium dihydrogen phosphate and 9 mM disodium hydrogen phosphate. This solution also contained the protective agent at twice the final concentration used in the freezing solution so that when the samples of spermatozoa were diluted 1:1 the final concentration (described in the text) was achieved.

buffered saline: 149 mM sodium chloride, 2,5 mM disodium hydrogen phosphate and 2,5 mM sodium dihydrogen phosphate. In the experiment studying the induction of sperm motility (Table 2) this

Table 2

Motility of elephant spermatozoa* flushed with air from the distal portion of the epididymis, diluted 40-fold and stored for 3 hours at 30°C before microscopical examination (Results are means for samples from 3 elephants)

Diluent composition	Mean scores of:	
	Progressive motility	% Motile spermatozoa
Buffered saline	1,8	30
Buffered saline + KCl	2,3	40
Buffered saline + fructose	2,3	40
Buffered saline + KCl + fructose	3,3	50
Standard error of means†	0,6	4,7

* From Jones (1973)
† From residual mean square in analyses of variance.

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solution was used alone, or including 5 mM potassium chloride, 10 mM fructose or both substances.

Krebs'-Henseleit-Ringer: 110 mM sodium chloride, 5 mM potassium chloride, 1 mM potassium dihydrogen phosphate, 1 mM magnesium sulphate, 2 mM sodium bicarbonate, 10 mM disodium hydrogen phosphate, 10mM sodium dihydrogen phosphate and 10 mM fructose.

3. Recovery of sperm from the Reproductive Tract

Elephants from breeding and bachelor herds were immobilized with "Scoline" (Glaxo Laboratories Ltd), shot, bled from the jugular vein and eviscerated. The testes and excurrent ducts proximal to the ampullae were removed from bull elephants within 15 min of death. The excurrent ducts which were highly convoluted were dissected from the testes and the mesenteric fold in which they were supported and partly unravelled by cutting the connective tissue which enmeshed them. The ducts increased in external diameter from 0,06 cm adjacent to the vasa efferentia to 1,0 cm at the sphincter which opened into the ampullae. Spermatozoa from ducts of more than 0,15 cm external diameter were recovered for use in these experiments by inserting a blunted 23 G hypodermic needle, attached to a syringe, into the lumen. The ducts were then flushed posteriorly with either air (to obtain undiluted samples) or semen diluent. Care was taken to avoid exposing samples to sunlight or changes in temperature. Undiluted samples were immediately examined microscopically for motility. In studies of the physiology of semen, undiluted spermatozoa were added to diluents in 15 ml polyethylene tubes and these were loosely stoppered for transport in a thermos at 30°C to 37°C (or 5°C for one treatment) to a temporary laboratory at Letaba for further microscopical examination. Several stoppered plastic containers full of water were used to maintain a constant temperature in the thermoses.

4. Deep-freezing Spermatozoa

Spermatozoa were flushed from the ducts with *egg-yolk-citrate* (Blackshaw, Emmens, Martin and Heyting 1957) and then more solution was added to achieve a dilution rate of approximately 4-fold (approximately 1× 10^9 spermatozoa/ml). They were cooled in a thermos to 5°C over 2 - 3 hours during transport to Letaba. A further 1:1 dilution with *citrate-fructose* containing the protective agent, dimethyl sulphoxide (DMSO) and/or glycerol, was carried out at 5°C. The concentration of the protective agent(s) was varied between experiments.

After the addition of the diluent containing the protective agent, samples were stored at 5°C for 30 min (i.e. equilibrated) and loaded (Figs. 5 to 8) into 0,5 ml Cassou straws (Cassou 1964). The straws were
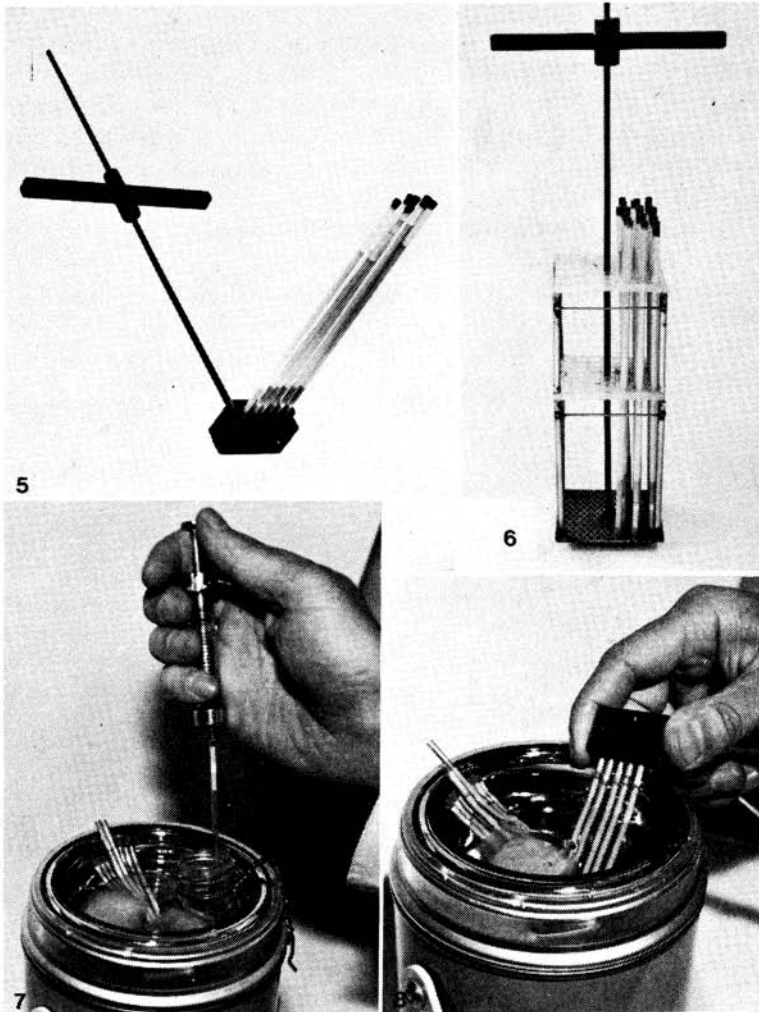
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Fig. 5. Brass rack for holding Cassou straws horizontally over the liquid in a liquid nitrogen refrigerator. The straws are held by pushing them into grooves in the brass jaws. The jaws are held by a threaded shaft that hangs vertically within the refrigerator. The height of the straws above the liquid nitrogen is adjusted by varying the location of the cross bar on the threaded shaft.

Fig. 6. Rack holding Cassou straws vertically in a liquid nitrogen refrigerator. The height above the liquid nitrogen is varied by adjusting the location of the cross bar on the vertical shaft.

Fig. 7. Loading samples into Cassou straws for freezing. The container holding the diluted spermatozoa was supported in an ice bath within a thermos, and straws were returned to the bath after filling and sealing the open end.

Fig. 8. Loading the Cassou straws into the support rack without warming them.

then mounted (Fig. 8) in the jaws of a rack (Fig. 5) which allowed them to be suspended horizontally 2 cm to 3 cm above liquid nitrogen (-196°C) in a LINDE LNR16 (Union Carbide) refrigerator. The period of equilibration in the protective agent varied from 0,5 hours to 2,0 hours depending upon the number of straws frozen from one day's collection. A small cold room (5°C) was available when semen from some of the bulls was frozen so that the vertical straw rack (Fig. 6) could be used to freeze larger numbers of straws at one time.

5. Scoring Viability

Viability of spermatozoa was assessed by examining samples as a thin film between a microscope slide and coverslip and scoring the rate of progressive motility (range: 0 = immotile, 4 = maximum motility; Emmens 1947) and percentage of motile spermatozoa. Eosin-nigrosin smears (Blackshaw 1955) were prepared when possible but for technical reasons some of the slides made in the field were unsuitable for accurate counting of spermatozoa.

At Letaba, frozen samples were stored for at least 24 hours before thawing in air. After the preliminary studies on freezing, samples of spermatozoa from the next seven bulls that were culled were frozen using the best diluent tested. These samples were transported in the liquid nitrogen refrigerator to London for storage at the Wellcome Institute of Comparative Physiology. After storage for one month, straws from each of the bulls were thawed in a water bath at 37°C and samples examined microscopically immediately, and after incubation for 1 hour and 2 hours at 30°C.

In the field, samples were examined microscopically at ambient temperature (20°C - 25°C) using a McArthur microscope (The Open University, Bucks.). At Letaba, samples were examined at ambient temperature with a Zeiss microscope equipped with phase contrast apparatus. A warm stage heated to 37°C was used on a Zeiss microscope equipped for phase contrast at the Wellcome Institute of Comparative Physiology.

Semen collected by electro-ejaculation was fixed and prepared for electron microscopy using the methods described by Jones (1973).

Results

1. Electro-ejaculation

Electro-ejaculation was attempted on two bull elephants. Initially, insufficient drug (4 mg) was given to the first elephant which remained mobile and responsive to herding by the helicopter for 30 minutes. An additional dose of 1 mg was administered and 20 min later the animal

became immobile in a standing position. He was lassoed, pulled to the ground and the rectal probe was inserted to its full length. Each set of ring electrodes was tested, and pulse duration, frequency and amplitude were each varied throughout their range starting with values which had previously been successful for electro-ejaculating rams. Best responses followed the use of ring electrodes 1 or 2, a pulse frequency of 30 Hz (repeated skeletal muscle contractions were induced by settings less than 10 Hz), an amplitude of 6 volts to 10 volts and a pulse duration of 12 milliseconds. Voltages above 6 volts caused some skeletal muscle reaction (Stiffening of limbs) and there was no noticable response to currents with pulse duration less than 12 milliseconds. An erection, 1 m - 1,25 m long, was obtained and 80 ml of a clear viscous fluid was collected during 20 min of stimulation. However, no spermatazoa were found in the fluid.

Subsequently, the automobile battery used to power the stimulator was suspect, so a new battery was submitted for use on the second elephant. The animal was immobilised with a dose of 9 mg of "Immobilon". After a latent period of several minutes, secretions were collected from the penis. The settings which gave the best response were much the same as for the first elephant: ring electrodes 1 or 2, amplitude 6 volts to 10 volts, pulse frequency of 30 Hz - 40 Hz and pulse duration of 12 milliseconds. Over 15 min, 250 ml of semen was collected and more could have been collected if stimulation had continued. A dilute suspension of spermatozoa was observed in the fluid when examined immediately under the microscope.

The elephant did not recover for 30 min after administration of Revivon (Reckitt and Coleman, Hull), the antidote for "Immobilon." During this period it urinated several times; and some of this fluid was collected. Samples of the semen and urine were then transferred to loosely stoppered tubes, cooled to 20°C in a thermos and transported to the field laboratory for further microscopical examination. Scores of viability for the semen were: rate of progressive motility of 3,5; 60 per cent motile spermatozoa; $4,8 \times 10^6$ spermatozoa/ml. Figs. 9 and 10 show electron micrographs of the spermatozoa and Fig. 11, a photomicrograph. Structurally they were similar to ungulate spermatozoa. The acrosome covered about two thirds of the nucleus, had a small apical ridge and very distinct equatorial segment. There was an accumulation of electron-dense material, the perforatorium, underneath the acrosome and the nucleus was condensed and homogeneous. The mitochondria were wound in a helix around the middle-piece and the axial bundle had the 2+9+9 arrangement of fibres which is typical of mamalian spermatozoa.

Approximately 4×10^5 spermatozoa/ml were found in the urine from the second elephant but none of the spermatozoa was motile.
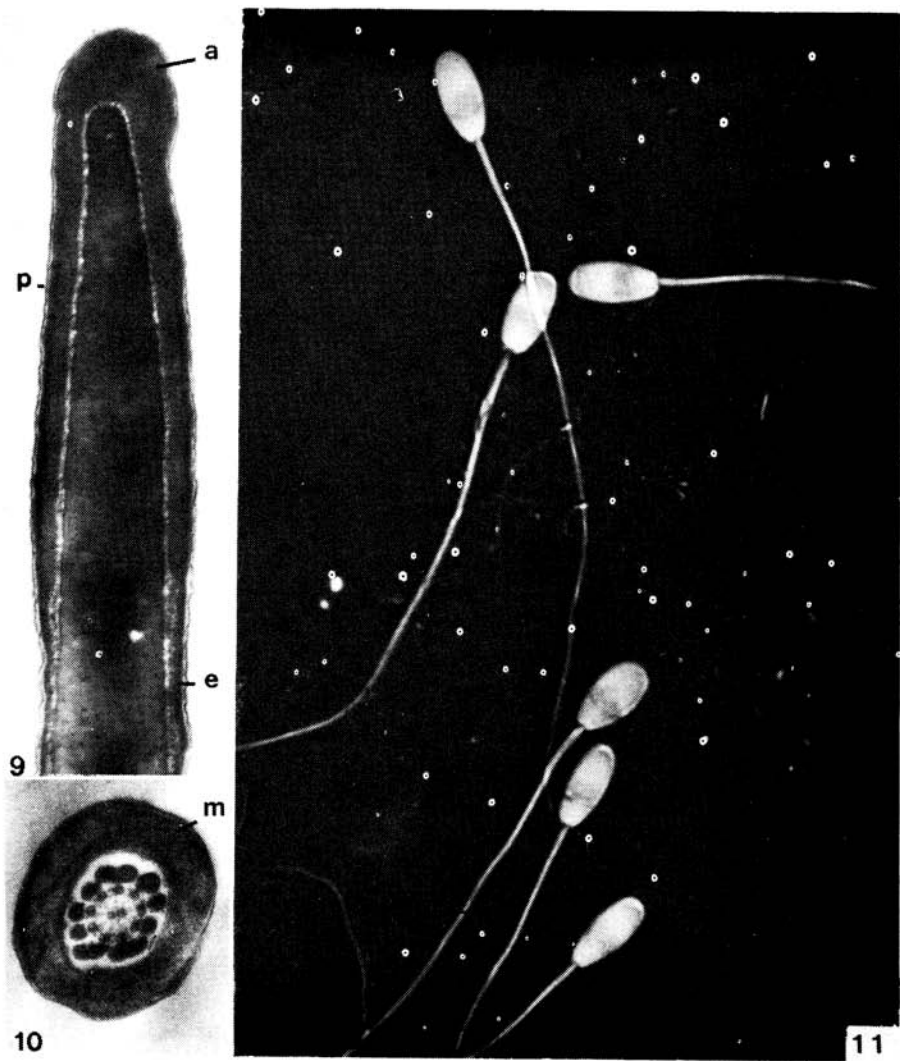
Fig. 9. Sagittal section of the anterior region of the head of an ejaculated elephant spermatozoon showing the plasma membrane (p), the anterior (a), and equatorial (e) segments of the acrosome over the nucleus. Note the flocculent electron dense perforatorium between the acrosome and nucleus. ×24 300.

Fig. 10. Cross-section of the middle-piece of an ejaculated spermatozoon showing the mitochondria (m) beneath the plasma membrane and the 2+9+9 arrangement of the fibrils. ×42 630.

Fig. 11. Light micrograph of ejaculated elephant spermatozoa in an eosin-nigrosin smear. Phase contrast. ×2 250.

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In the samples used in these and the storage studies, at least 85 per cent of spermatozoa had protoplasmic droplets attached to the middle piece adjacent to the principal piece (i.e. located distally). The droplets were at the proximal end of the middle piece in 5 per cent of spermatozoa and absent in 10 per cent.

Undiluted samples of spermatozoa flushed with air from the ducts of nine elephants were examined microscopically immediately after collection. In one sample it was judged that 15 per cent of spermatozoa showed signs of weak flagellation (motility score = 0,5) but in the other samples all of the spermatozoa were immotile. Undiluted samples (0,05 ml) from three elephants were diluted into 2,0 ml of buffered saline (including potassium chloride and fructose) in the combinations shown in Table 2. Some of the samples were examined immediately to confirm that the spermatozoa were motile. The tubes were then loosely stoppered and transferred, in a vacuum flask at 30°C to 37°C, to the field laboratory. Table 1 shows scores of viability after storage for 2 hours to 3 hours. Strong motility was observed in all samples and an analysis of variance of the responses showed that the addition to the diluent of potassium, fructose or both substances increased the proportion of motile spermatozoa ($P < 0,05$).

The effect of varying the osmotic pressure of a diluent was studied in an experiment in which Krebs'-Henseleit-Ringer solutions were prepared at 0,67, 1,00 and 1,33 times iso-osmotic concentrations. The mean scores shown in Table 3 indicate that the higher osmotic pressures were most favourable.

Table 3

The effects of changes in osmotic pressure of Krebs'-Henseleit-Ringer (KHR) on the motility of elephant spermatozoa after incubation for 3 hours at 30°C–37°C (Results are means for samples from 2 elephants)

Osmotic Strength relative to KHR	Osmolality (mOs/Kg)	Mean sources of:	
		Progressive motility	% Motile spermatozoa
0,67	188	0,5	10
1,00	282	2,5	28
1,33	376	1,8	40
Standard error of means*		0,39	12,8

* Calculated from the residual mean square in the analyses of variance.

The effect of cooling spermatozoa rapidly to 5°C (i.e. temperature shock) was studied in a small experiment in which one drop of an undiluted sample was diluted in 0,5 ml of buffered saline at 37°C or 5°C. The mean scores of survival shown in Table 4 indicate that cold shock depressed the viability of elephant spermatozoa.

Table 4

Effects of cold shock on the motility of elephant spermatozoa: one drop of undiluted spermatozoa was added to 0,5 ml buffered saline at 5°C or 37°C and stored for 3 hours (Results are means for samples from 2 elephants)

Temperature of diluent	Mean scores of:	
	Progressive motility	% Motile spermatozoa
5°C	2,0	23
37°C	3,5	65
Standard error of means*	0,35	8,8

* Calculated from the residual mean square in the analyses of variance.

Table 5

Motility of elephant spermatozoa stored overnight at 5°C in egg-yolk-citrate containing glycerol or DMSO

Concentration of Protective Agent	Number of replicates	Mean = S.E.* scores of:	
		Progressive motility	% Motile spermatozoa
2% v/v glycerol	2	1,8 ± 0,36	20 ± 8,3
4% v/v glycerol	5	1,6 ± 0,23	13 ± 5,2
8% v/v glycerol	5	0,9 ± 0,23	5 ± 5,2
16% v/v glycerol	2	0,3 ± 0,36	1 ± 8,3
2% v/v DMSO	2	1,3 ± 0,36	16 ± 8,3
4% v/v DMSO	5	2,3 ± 0,23	25 ± 5,2
8% v/v DMSO	5	2,4 ± 0,23	30 ± 5,2
16% v/v DMSO	1	0,0	0,0

* Calculated from pooled within treatment, between animal, sum of squares.

3. Deep-frozen Storage

In a preliminary experiment, the effects of diluent composition were examined in a 2×4+1 factorial design in which glycerol was compared with DMSO as a protective agent at concentrations of 2, 4, 8 and 16% v/v in an *egg-yolk-citrate* diluent. The whole experiment was replicated twice, then half of the experiment, which included the most suitable concentrations of protective agent (4% and 8% v/v), was replicated a further three times. With the last two replicates, a combination of 1% v/v glycerol and 7% v/v DMSO in *egg-yolk-citrate* was also tested.

Spermatozoa were stored overnight at 5°C to test the toxicity of the protective agents. Both compounds were toxic to spermatozoa (Table 5). However, as assessed by scores of motility ($P < 0,01$) and percentage motile ($P < 0,001$), DMSO was less toxic than glycerol.

Table 6 shows survival rates of spermatozoa after freezing and thawing. DMSO was a better protective agent than glycerol ($P < 0,001$) for both measures of response. However, survival improved when a combination of DMSO and glycerol was used ($P < 0,05$).

Table 6

Survival following freezing and thawing of elephant spermatozoa frozen in egg-yolk-citrate, *containing glycerol or DMSO or both*

Diluent composition	Number of replicates	Mean ± S.E. of scores of:	
		Progressive motility	% Motile spermatozoa
Egg-yolk-citrate:			
2% v/v glycerol[1]	2	0,0 ± 0,00	0 ± 0,0
4% v/v glycerol[1]	5	1,1 ± 0,24	1 ± 0,0
8% v/v glycerol[1]	5	0,9 ± 0,33	3 ± 1,1
16% v/v glycerol[1]	2	0.5 ± 0,50	1 ± 0,5
2% v/v DMSO[1]	2	2,0 ± 0,00	5 ± 0,0
4% v/v DMSO[1]	4	2,0 ± 0,00	10 ± 4,4
8% v/v DMSO[1]	5	1,9 ± 0,10	12 ± 3,2
16% v/v DMSO[1]	1	0,0	0,0
7% v/v DMSO + 1% v/v[1] glycerol	2[3]	2,5 ± 0,50	30 ± 10,0
7% v/v DMSO + 1% v/v[2] glycerol	2[3]	3,3 ± 0,25	55 ± 5,0

[1] Straws thawed in air at Letaba, Kruger National Park.
[2] Straws thawed in water (37°C) at Zoological Society of London.
[3] Elephants 907 en 931 (see Table 7).

Spermatozoa from bulls 907 and 931 (compare with Table 7) were frozen in the combination of DMSO and glycerol and a straw from each was thawed at Letaba and London. It is noteworthy that the mean scores of survival were higher for the samples thawed in London than Letaba (Table 6). However, counts of percentage unstained spermatozoa were much the same at 97±2 and 89±4 per cent (mean ± S. E.) for the samples thawed at Letaba and London respectively.

Table 7 shows the characteristics, prior to freezing and after thawing and incubation, of the sperm samples which were frozen in 7% v/v DMSO and 1% v/v glycerol in *egg-yolk-citrate* and transported to London as a semen bank for use for artificial insemination.

Table 7

[1]*Characteristics of elephant spermatozoa frozen and stored for artificial insemination showing motility after cooling to 5°C before freezing, viability immediately upon thawing and following incubation for 2 hours at 30°C, and concentration of spermatozoa in frozen samples*

Elephant identification	Mean scores of viability after:							
	[2]Cooling to 5°C		[3]Thawing			[3]Incubation		[3]Concentration (sperm per ml × 10^{-6})
	Motility	% motile	Motility	% motile	% un-stained	Motility	% motile	
907	2,0	30	3,5	60	94	2,5	40	—
915	2,0	60	1,0	10	68	0,5	10	13
931	2,0	30	3,0	50	85	2,5	40	54
942	2,5	50	3,0	50	88	2,0	35	533
943	1,0	10	3,5	50	93	2,0	30	790
944	2,5	30	3,0	30	95	1,5	15	753
945	2,5	50	3,5	60	90	3,0	50	290
Mean	2,07	37,1	2,93	44,3	87,6	2,0	31,4	405,5
Standard error	0,20	6,4	0,34	6,3	3,5	0,31	5,4	138,5

[1] Partly from Table 2 (Jones 1973).
[2] Evaluation made at Letaba, Kruger National Park.
[3] Evaluation made at the Zoological Society of London.

Discussion

Clearly, more work is required on the electro-ejaculation of the elephant. Nevertheless, the results of these studies are encouraging since

nothing was known about the design or dimensions of a rectal probe for such a large animal or whether "Immobilon" would affect the response of animals to stimulation. The failure to obtain spermatozoa from the first elephant tested may have been due to the long period of herding by the helicopter prior to ejaculation or a fault of the battery used as the power supply.

The study confirms observations by Short *et al* (1967) who concluded that spermatozoa are immotile when taken from the Wolffian ducts of the elephant. However, these studies showed that dilution with buffered saline was sufficient to induce good motility. Consequently, the addition of a metabolic substrate is not required to induce motility. Since the undiluted spermatozoa were exposed to oxygen during preparation for microscopical examination, it is unlikely that a change in oxygen tension caused the induction of motility. Possibly further studies on the epididymal fluid, or ionic composition of diluents may help to determine the factor(s) that are required to induce sperm motility.

It is not possible to carry out sufficient replicates of the experiments studying the effects of osmotic pressure and temperature shock to achieve statistically significant results. Nevertheless, the results do suggest that elephant spermatozoa respond in a similar fashion to these factors as spermatozoa from other mammals (Mann 1964), i.e. they are sensitive to rapid cooling and tolerate hyperosmotic solutions better than solutions that are hypo-osmotic to physiological saline.

Insufficient elephants were available to carry out extensive studies on methods of freezing spermatozoa. Nevertheless, by appropriate experimental design, using within animal comparisons, it was possible to quickly decide that DMSO was a better protective agent than glycerol, to determine the optimal levels of DMSO, and to establish that a combination of DMSO and glycerol was better than DMSO alone. The combination was chosen since the counts of percentage unstained spermatozoa were generally higher for the spermatozoa frozen in glycerol than DMSO, and this finding is in agreement with earlier findings with the ram (Jones 1965). Furthermore, earlier studies have shown that for freezing ram and rabbit spermatazoa a combination of the protective agents is better than either alone (Jones 1965; Stranzinger, Maurer and Paufler 1971).

Some evidence is provided in Table 6 to indicate that the conditions used to thaw and examine frozen spermatozoa were better in London than Letaba. It cannot be concluded whether this was due to the different thawing rates used or the different temperatures of the samples when examined, or both factors. It does indicate, however, that the responses shown in Table 6, and the earlier tables, would probably have been higher if samples were prepared and examined under better conditions.

Except for elephant 915, the viability of the spermatozoa thawed from the frozen semen bank (Table 7) is as good as samples of frozen bull

spermatozoa which are successfully used for inseminating cattle. This is considered encouraging since there was no selection of the seven bulls used for the preparation of the semen bank.

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