

Pregnancy in the domestic cat after artificial insemination with previously frozen spermatozoa

C. C. Platz, D. E. Wildt and S. W. J. Seager

Institute of Comparative Medicine, Texas A & M University/Baylor College of Medicine, Texas Medical Center, Houston, Texas 77030, U.S.A.

Summary. Methods for collection, freeze preservation and artificial insemination of domestic cat semen were developed. Total sperm count and pre-freeze and post-thaw % motility were significantly greater ($P < 0.05$) for samples collected by an artificial vagina method than for those collected by electroejaculation. Although conception rate was low (10.6%), 6 pregnancies were achieved, 2 after hormonal induction of oestrus.

Introduction

There are no reports in the literature on electroejaculation and subsequent successful freeze preservation and artificial insemination (AI) of feline semen. The domestic cat is a valuable and extensively used laboratory model. It also can be considered a suitable model for studying methods of artificially controlling reproduction in non-domesticated species of Felidae, some of which are endangered (Seager, Demorest, Wildt & Platz, 1976). The present study was of the development of semen freezing and AI techniques of cats to (1) improve the reproductive potential of commercially bred cats and (2) provide knowledge pertaining to the breeding of non-domesticated felids for control or preservation purposes.

Materials and Methods

Adult male and female cats (2.5–4.5 kg) from various sources were housed in individual cages and fed a commercial cat food. Water was always available. The light cycle was 12 h light/24 h. Behavioural cyclicity was checked daily by placing each queen with one of three vasectomized males. A queen was considered to be oestrous when the behavioural signs typical of oestrus in this species were displayed (i.e. lowered forequarters, arched hindquarters, tail deflection to one side, treading movement of the hind legs and vocalization).

Semen collection and processing

Semen was collected from 6 male cats over a period of 6 months by electroejaculation and then for a period of 6 months by using an artificial vagina and manual stimulation. Electroejaculation was performed as described by Seager (1976). Each male was anaesthetized with ketamine hydrochloride (Ketaset; Bristol Laboratories, Syracuse, New York; 33.0 mg/kg body weight) and placed in a lateral recumbency position. A teflon rectal probe (1 cm diameter, 12 cm long) containing 3 raised longitudinal stainless steel electrodes (5 cm each in length) was lubricated with K-Y jelly (Johnson and Johnson, New Brunswick, New Jersey) and inserted approximately 9 cm into the rectum. The electroejaculator, designed and constructed in this laboratory and capable of monitoring voltage and amperage, utilized an isolated current of 120 AC with a transformer producing a maximum of 60 V and 1 A. A plastic collection vial was placed over the prepuce and gentle pressure was directed cranially to expose the penis. The electrical stimulus was administered in a 3-sec on 2-sec off series, with a continuous rise in voltage to maximum, returning then to 0. The voltage and amperage used during electroejaculation ranged from 2 to 8 volts and 5 to 220 mA. Semen could be routinely collected from each cat once/week by this method.

Semen collection by manual stimulation was performed by a modification of the method first described by Sojka, Jennings & Hamner (1970). Males were trained to mount oestrous females and ejaculate into an artificial vagina in response to manual manipulation of the genital region. A 2 ml rubber pipette bulb with the enlarged end cut off formed the penis contact area of the artificial vagina. The cut end was attached to a section of glass tubing (6 × 30 mm) which was heat sealed at the other end. Ejaculates could be routinely collected three times/week.

Immediately after collection by electroejaculation or manual stimulation, the semen was mixed with 200 µl sterile 0.9% NaCl solution and 200 µl diluent at room temperature (22–23°C). The saline was added to increase the total volume of ejaculate which is normally between 10 and 500 µl. The diluent consisted of 20% (v/v) egg yolk, 11% (w/v) lactose and 4% (v/v) glycerin in de-ionized water, 1000 µg streptomycin sulphate (Eli Lilly & Co., Indianapolis, Indiana)/ml and 1000 i.u. penicillin G potassium (Eli Lilly & Co.)/ml. The total diluted volume was measured; the actual ejaculate volume was calculated by subtracting the volume of 400 µl from the total. The ejaculate was subjectively analysed for % sperm motility. Spermatozoa were counted with a Coleman Junior II spectrophotometer or a haemocytometer. Based on the calculated ejaculate volume, a further volume of diluent was added at room temperature to ensure that at least a 1:1 semen-to-diluent (v/v) ratio was maintained during the first equilibration period. The sample was then equilibrated at 5°C for 20 min, the temperature of the sample declining at the rate of -1°C/min. At the end of this first equilibration period, 200 µl diluent (5°C) plus an amount of diluent equivalent to the calculated ejaculate volume were added and the sample allowed to equilibrate at 5°C for an additional 10 min. The sample was then frozen by the technique originally described for bull semen (Nagase & Niwa, 1964) and applied to dog and wolf semen (Seager, Platz & Fletcher, 1975; Seager, Platz & Hodge, 1975). This involved pipetting, from a disposable Pasteur pipette, single drops of the diluted semen into 3 × 4 mm indentations in a block of solid CO₂. The frozen pellets were subsequently deposited in a bath of liquid nitrogen, then transferred into labelled 5 or 8 ml Nalgene vials (Sybron Corporation, Rochester, New York) and stored in liquid nitrogen (-196°C).

Insemination

Rapid thawing was required to obtain high sperm recovery rate. A small volume (0.1 ml/pellet) of 0.154 M-NaCl was placed in a plastic bag in a 37°C waterbath. Frozen pellets were rapidly poured into the corner of the bag containing the saline and the bag massaged while immersed to speed thawing. The thawed sample was transferred to a 15 ml plastic graduated centrifuge tube and centrifuged for 5 min at 1500 *g* after removal of a portion for estimation of motility. From this post-thaw evaluation of % motility and the prefreeze sperm count, a value for the number of motile spermatozoa in the thawed sample was calculated. A total volume of 0.1 ml of the pellet and supernatant was left in the centrifuge tube and the remainder of the supernatant was discarded. The supernatant and sediment pellet were gently mixed with a pipette and the semen was gently aspirated into a 1 ml tuberculin syringe fitted with a 20 g lavage needle 9 cm in length. The thawed semen was inseminated into an oestrous queen within 5 min of centrifugation and within 20 min of thawing.

Queens in natural or gonadotrophin-induced oestrus were inseminated on Days 2 and 3 of oestrus with 50–100 × 10⁶ motile spermatozoa/insemination. To obtain this insemination dosage, thawed sperm samples were sometimes mixed from two ejaculates of an individual male. Behavioural oestrus and follicular development were induced in anoestrous queens by daily injections of 2 mg FSH (F.S.H.-P.: Armour Baldwin Laboratories, Omaha, Nebraska) in saline until oestrous behaviour was displayed (Kinney, Seager, Platz & Wildt, 1977). Ovulation was induced by mating with a vasectomized tom or by injection of 250–500 i.u. hCG (Pregnyl: Organon, Inc., W. Orange, New Jersey) on Days 1 and 2 of oestrus. Follicular development and ovulation were confirmed by laparoscopy on Day 1 of oestrus and again 96–168 h after mating or the first hCG injection. The details for laparoscopy techniques in the cat have been published elsewhere (Wildt, Kinney & Seager, 1977). In brief, this method involves anaesthesia of the queen and direct observation of the ovaries.

Each queen to be inseminated was tranquillized with a low dose of ketamine hydrochloride (6 mg/kg) and placed in dorsal recumbency with the hind quarters elevated. The lavage needle was

inserted into the anterior vaginal area and the semen slowly deposited. After deposition of the thawed semen, the queen's hindquarters were kept elevated for 20 min to minimize backflow of semen from the vaginal opening and allow the semen to pool at the cervical os.

Results

Table 1 gives data on ejaculates collected by electroejaculation and by the artificial vagina technique. Ejaculation volumes were low for both groups but the volume of electroejaculates was significantly greater, probably attributable to increased accessory gland secretions in response to electrical stimulation.

Table 1. Characteristics (mean \pm s.e.m.) of semen obtained from six domestic cats by electroejaculation or by artificial vagina

Technique	No. of males	No. of ejaculates/male	Ejaculate vol. (μ l)	Total no. of sperm/ejaculate ($\times 10^6$)	% motility*	
					Before freezing	After thawing
Electroejaculation	6	7.5 \pm 2.5	223.6 \pm 49.8	29.7 \pm 9.0	70.4 \pm 2.6	53.8 \pm 4.8
Artificial vagina	6	30.5 \pm 7.6	33.8 \pm 4.8	60.7 \pm 12.9	82.5 \pm 2.7	70.6 \pm 2.3
Significance†	—	—	$P < 0.01$	$P < 0.05$	$P < 0.01$	$P < 0.02$

* % Motility was determined from observation ($\times 100$) of fresh ejaculate maintained at 37°C. The accuracy was estimated as $\pm 5\%$.

† Assessed by one way analysis of variance methods.

Table 2. Pregnancy results of queens artificially inseminated with previously frozen spermatozoa

Queen	Oestrus	Ovulation induced with	Semen* collection	No of motile sperm./insemination	Period of storage (days)	Length of gestation (days)	No. of kittens born
1	Natural	Vasectomized male	E	50 $\times 10^6$	174	71	1
2	Natural	hCG	AV	100 $\times 10^6$	16	68	4
3	Natural	hCG	AV	50 $\times 10^6$	12	65	2
4	Natural	hCG	AV	50 $\times 10^6$	50	69	2
5	Induced	hCG	AV	50 $\times 10^6$	5	67	1
6	Induced	hCG	AV	50 $\times 10^6$	19	68	2

*E = electroejaculation, AV = artificial vagina.

To date, 6 pregnancies out of 56 attempts have resulted from the AI of queens with frozen-thawed semen (Table 2), and these are the first reported pregnancies for the domestic cat from previously frozen spermatozoa. Length of gestation, birth weights and development of all offspring were normal.

Discussion

The average litter size in the present study (2) was less than that expected from natural breeding (3–6) but was comparable to litter sizes resulting from artificial insemination with similar numbers of fresh spermatozoa (Sojka *et al.*, 1970).

The reduced pregnancy rate of 10.6% does not appear to be entirely related to the freezing and thawing procedures because post-thaw sperm motility was relatively high, particularly in ejaculates collected by the artificial vagina technique. Since the cat is an induced ovulator, conception rates may be improved by more accurate timing of the insemination with the expected onset of ovulation.

Projects are currently in progress to study alterations in sperm morphology due to freeze preservation and to determine the ideal time of insemination for optimum conception rates and litter size from frozen-thawed semen in the domestic cat.

This research was supported, in part, by a grant from the Ralston Purina Company, St Louis, Missouri. The authors acknowledge the invaluable assistance of Ms Sylvia Charman, Ms Gail Kinney, Ms Alexandra Zell and Mr Lawrence Denis.

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Received 28 June 1977