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Transcervical artificial insemination in the cat

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Abstract

Many studies have reported new reproductive techniques for reproduction in endangered non-domestic felids. Artificial insemination is an important tool for developing breeding programs. This manuscript reviews recent progress in feline artificial insemination, with particular emphasis on intrauterine sperm deposition. Intrauterine insemination of felids results in higher fertilization rates than intravaginal insemination, despite insemination with fewer sperm. Although surgical insemination was utilized historically, there are some reports of catheterization of the cervix of the queen. Recently, a new technique and catheter were proposed for transcervical insemination of fresh or frozen-thawed semen.

Keywords: Assisted reproductive technique; Artificial insemination; Intravaginal insemination; Intrauterine insemination; Domestic cat

1. Introduction

In the last three decades, there has been substantial progress in reproduction of the domestic cat. This animal serves as a laboratory model for several conditions in humans and for comparative studies of other felid species. In recent years, there is increasing interest in artificial insemination by cat breeders. Although successful artificial insemination of cats was first described more than 30 yrs ago, unfortunately this procedure is not routine in veterinary practice, even though other assisted reproductive techniques have been reported.

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Following the first successful pregnancies obtained with artificial insemination of fresh or thawed semen in the 1970s [1,2], there have been few further reports. In 2000, three reports [3–5] attempted to define the insemination dose for intravaginal or intrauterine insemination with fresh or frozen semen. Some authors [6–8] reported successful cannulation of the queen cervix and Zambelli andCastagnetti [9] reported successful fertilization (with fresh or frozen-thawed semen) after transcervical insemination, using a modified feline urinary catheter. This manuscript will describe the techniques and results for vaginal and intrauterine insemination in the cat.

2. Artificial intravaginal insemination (AIVI)

The first pregnancies after AIVI with fresh or frozen semen were reported by Sojka et al. [2] and Platz et al. [1]. Sojka et al. [2] obtained a conception rate of 42.9% using 5×10^6 fresh spermatozoa and Platz et al. [1] fertilized 6 of 56 queens with 50–100 $\times 10^6$ of motile previously frozen sperm, obtaining a low conception rate (10.6%). The apparent differences in conception rates between these studies may have been due to the experimental conditions, as well as sperm count and sperm quality, natural or induced estrus of the inseminated queens, and the site of sperm deposition. The sperm count to optimized pregnancy rates by AIVI was not clearly established in these reports. In 2000, Tanaka et al. [3] reported that insemination with 20×10^6, 40×10^6 and 80×10^6 fresh sperm produced conception rates of 6.6, 33.3 and 77.8%, respectively. The authors reported the higher insemination dose was approximately the number of sperm collected with two consecutive ejaculations [10]; their explanation for the number of sperm required to achieve higher conception rates was that cats copulate several times during estrus, presumably ensuring a large number of sperm for optimum fertilization [3]. Tsutsui et al. [4] reported a conception rate of 80.0% by inseminating 8×10^6 fresh sperm into one uterine horn. The ratio of 10 times the number of sperm needed for high fertilization rates for intravaginal (80×10^6) versus intrauterine (8×10^6) insemination is the same as reported for the dog [11,12].

The number of sperm required for insemination reported by Tanaka et al. [3] was greater than that reported by Sojka et al. [2]; perhaps the use of general anesthesia by Tanaka et al. [3] inhibited the entry of sperm into the uterus. The number of sperm inseminated and conception rates after AIVI are shown in Table 1. One study [2] used a 20 gauge by 9 cm long needle with a bulb, connected to a 0.25 mL syringe. Alternatively, a 1.5 mm diameter by 9 cm long nylon probe connected to a 1.0 mL syringe was used for insemination [3].

Table 1
Protocols for artificial intrauterine insemination (AIUI) in the domestic cat

Estrus	Induction of ovulation hCG (IU)	Method of insemination	Semen	Author
Induced (eCG)	75–100	Laparoscopy	Fresh	Howard et al. [13]
Natural	100 × 2 or 250	Laparotomy	Fresh	Tsutsui et al. [4]
Natural	100 × 2	Laparotomy	Frozen	Tsutsui et al. [5]

In all reports, anesthesia was induced with a combination of ketamine and acepromazine, and maintained with halothane.

Sperm were deposited in the anterior vagina or posterior cervix. General anesthesia or heavy sedation is usually recommended [1,2]. Frequently, the queen is maintained in dorsal recumbency with the hind quarters elevated for 15–20 min post-insemination to minimize the backflow of sperm [1,3].

3. Artificial intrauterine insemination (AIUI)

In 1992, conception after AIUI of 2.4–19.2 $\times$ 10^6 sperm was reported [13]. This was fewer sperm than previously [3] shown necessary for fertilization with AIVI. Numerous reports subsequently reported varying conception rates with varying doses of fresh or frozen semen [1,4,5,13,14].

Reports differ as to whether anesthesia affects ovulation [4,13]. Howard et al. [13] indicated that ovulation in cat is compromised by pre-ovulatory anesthesia. In contrast, Tsustui et al. [4] suggested that ovulation was not affected by anesthesia. In fact, ovulation in that study occurred in 90% of the animals anesthetized before ovulation. Additional variables in the literature include varying dosages of hCG and techniques of insemination (Table 1). Conception rates obtained after AIUI by Tsutsui et al. [4,5] and Howard et al. [13] are shown in Table 2.

All authors [4,5,13] performed surgical AIUI. Howard et al. [13] used laparoscopy. For this technique, a surgical plane of anesthesia was induced with a mixture of ketamine and acepromazine and maintained with halothane. Under laparoscopic observation, each uterine horn was elevated toward the ventral abdominal wall and cannulated, for deposition of semen, using a feline indwelling catheter (20 gauge, 5 cm long) inserted percutaneously into the proximal third of the uterine wall. After penetrating the lumen, a polyethylene tubing connected to a syringe containing 100 μL sperm suspension was inserted into the catheter. Tsutsui et al. [4,5] used a similar protocol for anesthesia and performed insemination after laparotomy. The semen was infused into the uterine horn with the greatest number of ovarian follicles or ovulations An 18 gauge needle was inserted into the central region of the uterine horn and the semen was infused at the tip of the horn with a 20 gauge indwelling catheter connected to a syringe.

Table 2
Conception rates in domestic cats inseminated with fresh or frozen-thawed semen

Estrus	Semen	No. sperm ($\times 10^6$)	Semen volume (μL)	Site of insemination	Conception rate (%)	Author
Natural	Fresh	5	100	Intravaginal	42.9	Sojka et al. [2]
Natural	Fresh	80	50–100	Intravaginal	77.8	Tanaka et al. [3]
Induced (FSH)	Frozen	50–100	100	Intravaginal	10.6	Platz et al. [1]
Induced (eCG)	Fresh	4.2–7.5	200	Bilateral intrauterine (surgical)	34.4	Howard et al. [13]
Natural	Fresh	8	30	Unilateral intrauterine (surgical)	80.0	Tsutsui et al. [4]
Natural	Frozen	50	30	Unilateral intrauterine (surgical)	57.1	Tsutsui et al. [5]

Intrauterine sperm deposition produces higher conception rates in cats, other carnivores and livestock [15–23]. Studies in the fox, ferret and sheep showed the importance of AIUI performed by surgical or non-surgical techniques [15–23]. The filtering role of the cervix and its secretions in preventing certain types of morphologically abnormal sperm from reaching the site of fertilization is well described [24]. Domestic and non-domestic felids [25–28] frequently produce ejaculates with low sperm concentration and a high percentage of morphologically abnormal sperm. Therefore, it may be reasonable to assume that conception rates may be lower with AIVI than with AIUI.

4. Transcervical catheterization and transcervical insemination

The first non-surgical cervical catheterization was reported by Hurlbut et al. [7]. Seventeen attempts to catheterize the cervix in eight queens resulted in 12 successful procedures, four unsuccessful attempts, and one penetration through the vaginal fornix. Difficulties with catheterization were associated with the dorsal medial fold, which prevented the catheter from entering the cervical os. Only three blastocysts were collected from one queen; no embryos were recovered from any other queens. Additionally, on average only 68% of the flush media was recovered. Various authors developed various catheters and techniques for transcervical embryo collection or insemination. The catheter designed by Hurlbut et al. [7] was a modified 18 gauge by 9.4 cm long ball-tip stainless steel needle. A 2 mm × 8 mm glass speculum was designed to aid catheter introduction. Like reported by Swanson et al. [8], the technique proposed by Hurlbut et al. [7] was inconsistent, and penetration of the cervix could not be ensured, differently from their technique that permits to obtain 7 out of 8 successful catheterization. Swanson and Godke [8] and Chatdarong et al. [6] used a modified polypropylene urinary catheter (2.7–2.8 mm in diameter) as a speculum and a 3.5 Fr tomcat catheter to enter the cervix.

Zambelli et al. [9] reported the first successful transcervical insemination in cats with fresh or frozen semen; there are no other similar reports in the literature. Zambelli et al. [9] obtained successful catheterization only in a few cases with the methods proposed by

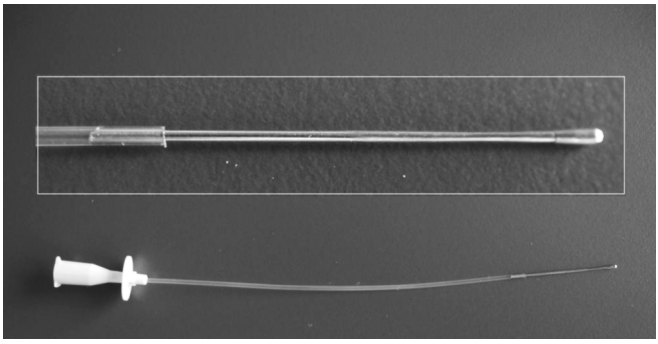

Fig. 1. Catheter proposed by Zambelli et al. [9] for transcervical insemination. The inset (at the top of the image) is a close-up of the tip.

Table 3
Quality of fresh and frozen-thawed semen used to inseminate cats by transcervical, intrauterine insemination

Semen quality (%)	Fresh	Frozen
Viability	98 ± 1.1	67.4 ± 3.6
Motility	90 ± 0.8	60.7 ± 1.7
Normal sperm	93 ± 1.6	77.2 ± 1.8

Swanson et al. [8] and Chatdarong et al. [6], due to a an excessively large speculum (outer diameter 2.7 or 2.8 mm). The catheter proposed by Zambelli et al. [9,29] was made from a 3 Fr tomcat catheter with a rounded tip needle (0.65 mm outer diameter by 30 mm long) inserted at the cut end (Fig. 1). The catheter is inserted through the vestibule and into the cranial vagina, until it was possible to palpate the catheter tip (in the vaginal fornix) and cervix with a forefinger in the rectum. The catheter was then withdrawn slightly (a few millimetres) in order to reach the cervical opening; with the aid of the finger in the rectum, it was then introduced into the cervix and passed through the cervical lumen into the uterine body. When appropriately placed, the catheter passed easily into the uterine body.

The presence of the catheter inside the uterus was confirmed with ultrasonography. The site of sperm deposition was approximately 2 cm cranial to the cervix. Successful transcervical catheterization was also verified when the operator had the sensation of touching cartilage and after passage through the cervix, the catheter passed without obstruction, and there was no sperm reflux. All queens used in this study were anesthetized and inseminated by transcervical, intrauterine insemination, 25–30 h after hCG injection (100 IUI). The insemination dose used was 200 μL of fresh or frozen-thawed semen containing 10×10^6 or 30×10^6 motile sperm, respectively (Table 3). The frozen semen used was frozen with a previously described protocol [30]. Transcervical insemination was possible in four of eight queens with induced estrus. A description of the success of insemination and the number of embryos obtained is shown in Table 4.

Table 4
Number of embryos collected after transcervical, intrauterine insemination of cats with frozen-thawed or fresh semen

Semen	Queen	No. embryos		
		Total	2–16 cells embryos	Morulae
Frozen	1	No ovulation		
	2	4	1	3
	3	Catheterization failure		
	4	6	2	4
	Mean	5.00	1.50	3.50
	S.D.	1.41	0.71	0.71
Fresh	5	11	2	9
	6	Catheterization failure		
	7	Catheterization failure		
	8	10	1	9
	Mean	10.50	1.50	9
	S.D.	0.71	0.71	0

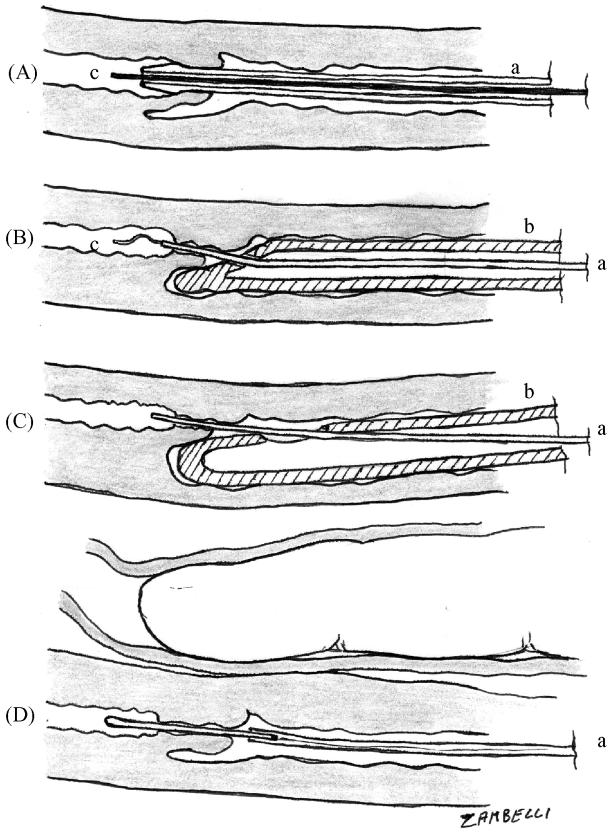

Fig. 2. Techniques proposed by Hurlbut et al. [7] (A), Swanson et al. [8] (B), Chatdarong et al. [6] (C) and Zambelli et al. [9] (C) for transcervical catheterization in the queen. Transcervical catheter (a), speculum (b) and catheter for embryo collection or transfer (c).

This new catheter is concurrently both rigid and flexible. The steel portion enables entry into the cervical os, whereas the plastic part gives some flexibility and minimizes accidental perforations [6,7]. Furthermore, transrectal palpation facilitates catheterization results (the operator feels the catheter pass) and concurrently decreases the perforation risk. The techniques for transcervical catheterization in the queen proposed by Hurlbut et al. [7], Swanson et al. [8], Chatdarong et al. [6] and Zambelli et al. [9] are shown in Fig. 2.

5. Discussion

Successful artificial insemination in the cat was first described more than 30 yr ago, but has not yet become a routine veterinary procedure. Fertilization in the cat is possible with both AIVI and AIUI. Comparing similar experimental conditions [3,4] approximately 10 times more fresh sperm are necessary with AIVI than with AIUI to obtain 80% conception rates in the cat. Additionally, approximately five times more frozen sperm were needed

compared to fresh sperm to achieve 57.1% conception with AIUI [5]. Although this manuscript has described a method of transcervical insemination, many authors still advocate surgical techniques for intrauterine insemination. The technique recommended by Zambelli et al. [9] reduces the potential for vaginal trauma does not always achieve cervical catheterization, although operator success improves with experience. A non-surgical procedure for artificial insemination is recommended to reduce surgical risks or complications, and to decrease the cost and time required for insemination.

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