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Comparison of efficiency between two artificial insemination methods using fresh semen in domestic cat (*Felis Catus)*

Comparação da eficiência entre dois métodos de inseminação artificial utilizando sêmen fresco em gata doméstica (Felis Catus)

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ABSTRACT

This study aimed to compare domestic cats' pregnancy rates using fresh semen for the bilateral intrauterine insemination (BIUI) method and the novel uterine body insemination (UBI) method. Queens received a single injection of equine chorionic gonadotropin (eCG) (200 IU; IM) to induce ovarian follicular development and, after 83 h, an injection of human chorionic gonadotropin (hCG) (100 IU; IM) for final oocyte maturation and ovulation induction. Thirty-four hours after hCG administration, 3 × 10^6 fresh spermatozoa were used for insemination by the BIUI (n = 8 queens) or by the UBI (n = 7 queens) techniques, respectively. Pregnancy rates were 75.00% (6/8) by BIUI and 42.85% (3/7) by the UBI method. The mean litter size was 3.0 ± 0.86 for the BIUI, and 2.0 ± 1.0 for the UBI method. Spontaneous abortion occurred on day 35 of pregnancy in one queen following the UBI method. Our findings showed that the BIUI of queens with fresh semen resulted in higher pregnancy rates than the novel UBI method; also, acceptable pregnancy rates were achieved following BIUI with fresh semen in the domestic cat.

KEYWORDS: intrauterine insemination; assisted reproductive techniques; fresh semen.

RESUMO

O objetivo deste estudo foi comparar as taxas de prenhez em gatas domésticas usando sêmen fresco para o método de inseminação intrauterina bilateral (BIUI) e o novo método de inseminação do corpo uterino (UBI). As gatas receberam uma única injeção de gonadotrofina coriônica equina (eCG) (200 UI; IM) para induzir o desenvolvimento folicular ovariano e, após 83 h, uma injeção de gonadotrofina coriônica humana (hCG) (100 UI; IM) para maturação final do oócito e ovulação indução. Trinta e quatro horas após a administração de hCG, 3 × 106 espermatozoides frescos foram utilizados para inseminação pelas técnicas de BIUI (n = 8 gatas) ou UBI (n = 7 gatas), respectivamente. As taxas de gravidez foram de 75,00% (6/8) pela BIUI e 42,85% (3/7) pelo método UBI. O tamanho médio da ninhada foi de 3,0 ± 0,86 para o método BIUI e 2,0 ± 1,0 para o método UBI. Aborto espontâneo ocorreu no dia 35 de gestação em uma gata seguindo o método UBI. Nossos achados mostraram que a BIUI de gatas com sêmen fresco resultou em maiores taxas de prenhez do que o novo método UBI; também, taxas de prenhez aceitáveis foram alcançadas após BIUI com sêmen fresco no gato doméstico.

PALAVRAS-CHAVE: inseminação intra-uterina; técnicas de reprodução assistida; sêmen fresco.

INTRODUCTION

Since most feline species are endangered (WILDT 1991), and the domestic cat is a useful biomodel for wild felids research (FARSTAD 2000), many studies on assisted reproductive technologies (ARTs) have been done to save these threatened species. In domestic cats, follicular development can be stimulated with exogenous follicle-stimulating hormone (FSH) or equine chorionic gonadotropin (eCG), and ovulation can be induced with gonadotropin-releasing hormone (GnRH) or human chorionic gonadotropin (hCG) (ROTH et al. 1997). In the case of non-domestic felids, eCG followed by hCG has become the regimen of choice to prevent animal stress associated with several FSH injections (ROTH et al. 1997). The interval between hCG

administration and ovulation in domestic cat is 25–27 h (SOJKA et al. 1970).

There are different reports on the effects of anesthesia on ovulation. HOWARD et al. (1992) reported that anesthesia before ovulation compromised ovulation. However, TSUTSUI et al. (2000) reported that anesthesia did not affect ovulation. Therefore, it is debatable whether to induce anesthesia before or following ovulation.

The first artificial insemination (AI) in the domestic cat was conducted more than 50 years ago using 10-50 × 10^6 fresh spermatozoa inseminated intravaginally in natural estrus queens (SOJKA et al. 1970). Intravaginal insemination (IVI) is the best approach for cases in which a sufficient amount of fresh semen is available; however, the requirement for high sperm numbers has placed limitations on the practical use of the intravaginal method (SWANSON 2019).

There are cases for which a sufficient volume of fresh semen is available. However, when the volume of semen is limited due to the high number of queens ready to be inseminated, intravaginal insemination may not be an optimal option for the success of AI and conception rate.

ZAMBELLI & CASTAGNETTI (2001) reported the first successful transcervical insemination in domestic cats. Although it was a non-surgical technique, it is not routinely practiced (ZAMBELLI et al. 2015a). Catheterization of the cervix is difficult due to the narrowness of the reproductive tract, especially in the cranial vagina which is not distensible in nature; furthermore, the catheterization success rate is lower in the follicular phase (ZAMBELLI et al. 2015a, ZAMBELLI et al. 2004). Finally, to increase the success rate, this technique demands experience and proper operator training (ZAMBELLI et al. 2004).

Endoscopic transcervical insemination in the domestic cat was first described by ZAMBELLI et al. (2015a) According to them, it can be easily performed regardless of the estrous cycle stage. However, even though they performed catheterization relatively quickly (in 13.08 ± 7.27 min), it cannot be applicable to all queens (success rate: 85.71%) due to individual anatomical limitations.

Laparoscopic oviductal insemination is a new and promising method, and it is becoming more available, especially for non-domestic cats (CONFORTI et al. 2013, SWANSON 2012). Laparoscopic oviductal insemination is the preferred approach when the amount of collected sperm is low (AZUMANO et al. 2022) Laparoscopic oviductal insemination has circumvented many obstacles in feline AI, including cervical barriers, low semen quality, low sperm number, etc. (SWANSON 2019, CONFORTI et al. 2013, WILDT et al. 1977). In addition, this method has led to a pregnancy with much lower sperm (1 × 10^6 motile spermatozoa) compared to intrauterine, intravaginal, and transcervical insemination; however, the main disadvantage of this technique is the requirement for specialized equipment (JOHNSON 2018).

It is reported that intrauterine insemination (laparoscopic or laparotomic) requires lower spermatozoa than intravaginal or transcervical insemination to achieve a comparable pregnancy rate (HOWARD et al. 1992, TSUTSUI et al. 2000, ZAMBELLI & CASTAGNETTI 2001). This issue is especially important when the sperm is frozen-thawed or the quality of the semen is poor. TSUTSUI et al. (2000) achieved a pregnancy rate of 80% by inseminating 8 × 10^6 fresh spermatozoa into one uterine horn. HOWARD et al. (1992) achieved a pregnancy rate of 50% by inseminating 2.4-19.2 × 10^6 fresh spermatozoa into both uterine horns. To achieve a comparable pregnancy rate, 50-80 × 10^6 and 10-30 × 10^6 fresh spermatozoa are typically required for intravaginal and transcervical insemination, respectively (TANAKA et al. 2000). In contrast to transcervical insemination, intrauterine insemination is easy to perform and applicable to all queens (ZAMBELLI et al. 2015a, ZAMBELLI et al. 2004).

Because anesthesia can negatively affect smooth muscle contraction, it compromises sperm transport during intravaginal and transcervical insemination (SWANSON 2019). One advantage of intrauterine insemination over intravaginal or transcervical insemination is that it already bypasses the cervix so that sperm can be deposited closer to the site of fertilization (ampulla) (SWANSON 2019). However, there may still be a problem with sperm transport via the uterotubal junction and into the oviducts (SWANSON 2019).

Artificial insemination with frozen semen has had limited success, typically due to the need for high sperm counts (PLATZ et al. 1978, CHATDARONG et al. 2007). Additionally, cryopreservation procedures usually lead to reduced sperm motility and acrosomal integrity. Therefore, using frozen-thawed semen in cats has remained less satisfactory, and more frozen–thawed sperm may be required for AI (PLATZ et al. 1978, PUKAZHENTHI & WILDT 2003, TSUTSUI et al. 2000).

Semen collection is more difficult in cats when compared to domestic animals: either the cat is anesthetized for electroejaculation, or an estrous queen or phantom is needed as a teaser for the artificial vagina (AV) method (CHATDARONG et al. 2007, ACKERMANN & LOPES 2020). Furthermore, collection via AV is not very practical in clinical practice due to tom cat behavior and temperament (LINDE-FORSBERG 2005, RIJSSELAERE & VAN SOOM 2010). Urethral catheterization (UC) of tom cats after treatment with an

alpha-adrenergic agonist has recently been developed as an alternative method of semen collection; this approach has allowed the recovery of high sperm number in domestic cats without causing erection or ejaculation (ZAMBELLI et al. 2008). The main advantages of the UC method is that it can be repeated in the same animal, and no special equipment or expertise is required (PROCHOWSKA et al. 2015).

Since prior findings demonstrate significant discrepancies in the procedure, such as inseminating dose, fresh semen quality, time of AI, and so on, it is difficult to make reliable comparisons between AI methods for domestic cats. Despite ZAMBELLI et al. 2015a,b and DAŞKIN et al. 2022), there is not much data on the efficiency of the UC-collected sperm in terms of the pregnancy rate of queens, which made us even more obliged to test the efficiency of semen in two different deposition sites (BIUI and UBI). As a result, we applied the same collection and insemination procedure to compare the two AI approaches based on sperm deposition sites (BIUI and UBI) with fresh semen to improve AI protocol in domestic cats. The objective of the present study was to determine pregnancy rates following BIUI and UBI with fresh semen collected via the UC technique.

MATERIAL AND METHODS

Cats

Fifteen mixed breed queens between one and three years of age and a mean body weight of 3 ± 0.4 kg were used in this experiment. These queens were kept in cages (1.5 m × 1.5 m × 1.5 m) with two or three in each cage. A 4-y old mixed-breed tom cat with a body weight of 4.5 kg, coitus capability, and normal semen quality was used as the source of semen. The tom cat was kept in a 2 × 2 m room in the same hall as the queens were kept. All animals in this study were captured by a locally made domestic cat trap (between November and March) and were exposed to 12 hours of artificial fluorescent light (from 8 AM to 8 PM) as soon as they arrived on our private property. Around 30 to 40 days after the onset of artificial lightening, behavioral estrous was noticed. Cats were maintained at 22 °C to 23 °C during the seven months of the study period (between November and May). The animals were given commercial cat food (Fidar®, Iran) twice daily and had ad libitum access to water. All institutional and national guidelines for the care and use of laboratory animals were followed. This study was approved by Islamic Azad University- Babol Branch (Biomedical Research Ethics Committee) on 2021-05-25, with the approval id: IR.IAU.BABOL.REC.1400.004.

Semen collection

General anesthesia was induced in the tom cat with medetomidine (150 µg/kg; IM) (Dorbene vet®, Royan Daru Co, Tehran, Iran), and semen was collected each time just before the AI procedure by UC technique, as previously described by ZAMBELLI et al. (2008). In summary, the penis was extruded from the prepuce and cleaned. An open-ended sterile 3Fr tom cat urinary catheter (Baayen®, China) lightly lubricated (Salem® sterile lubricating jelly, Abzar Darman Co., Iran) was inserted into the urethra for approximately 9 cm. After 30 s, the catheter was removed from the urethra and the semen sample was sucked into the catheter by capillary forces and placed with slight air pressure into an Eppendorf tube containing prewarmed Ham's F10 with HEPES *(*BIO-IDEA®, Iran) medium. The diluted sample from the tom cat was maintained at room temperature and protected from light for 1-2 h until used for AI. Following semen collection, tom cat received atipamezole (0.125 mg/kg; IM) (Alzane®, Royan Daru Co, Tehran, Iran) for anesthesia reversal. A minimum of a one-week interval was allowed between consecutive semen collections.

Evaluation of semen quality

An aliquot (*10 µ*L) of semen was diluted with Ham's F10 (with HEPES) and evaluated to determine the total number of sperm using a hemocytometer chamber, and dilution was done at room temperature. Sperm motility and progressive motility were assessed using a *light* microscope at ×100 magnification. To evaluate subjective motility, 10 *µ*L of a sperm sample was placed on a slide prewarmed to 35 °C, and the percentage of motile spermatozoa was subjectively estimated under a *light* microscope at ×100 magnification, and the mean value was calculated. Viability and morphology were assessed on eosin-nigrosine stained slides, as previously described by PROCHOWSKA et al. (2015). The percentage of live spermatozoa was estimated by counting 200 spermatozoa, then another 200 spermatozoa were assessed morphologically. Immediately before insemination, the sample was evaluated for total volume, sperm concentration, sperm total motility, and progressive motility.

Induction of estrus and ovulation

Queens received a single injection of eCG (200 IU; IM) (Gonaser® 5000 Hipra laboratories Co, Amer Spain) to induce estrus and, after 80 h, an injection of hCG (100 IU; IM) (PD Preg® 5000, Pooyesh Darou Biopharmaceutical Co., Ltd., Tehran, Iran) to induce ovulation. Before insemination, ovaries were exposed

via ventral midline laparotomy to visually examine the ovaries and determine whether ovulation had occurred. Follicular development was confirmed by visualization of preovulatory follicles (2 to 4 mm in diameter, clear in appearance, and generally flattened or only slightly raised above the ovarian surface) or postovulatory corpora lutea (CLs) (each approximately 4 mm in diameter, dark red and distinctively raised 2-3 mm above the ovarian surface) as described by WILDT & SEAGER (1978). Queens with at least one fresh CL were classified as post-ovulatory phase, regardless of the number of follicles present. Females with preovulatory (3 mm in diameter) follicles and no CL (n=4) were given a second injection of 100 IU hCG just after surgery. When the queens showed both behavioral and cytological estrous signs, as well as medium-to-large ovarian follicles or fresh CLs at the time of insemination, AI timing was considered appropriate.

Vaginal cytology

To verify estrus, vaginal cytology was performed in all queens 34 h after the hCG treatment. A swab wetted with saline was used to collect exfoliated vaginal cells; the swab was gently rolled onto a clean and dry glass slide, and the slide was then stained with Wright-Giemsa (Asia Pajhohesh®, Tehran, Iran). Detection of more than 60% cornified superficial cells indicated follicular development and estrus.

Estrous behaviors

Queens were observed for estrous behaviors after pharmacological induction of estrus with eCG. Estrous behaviors were defined as vocalization, rubbing against soft objects, rolling, and crouching with tail deviation.

Anesthesia

General anesthesia was induced in the queens 34 h after the hCG treatment with xylazine (3 mg/kg; IM) (Xyla® Interchemie Werken "De Adelaar" BV, Metaalweg 8, Venray, The Netherlands) and ketamine (10 mg/kg; IM) (Bremer Pharma GMBH Warburg Germany), anesthesia was maintained by i.v injections of ketamine (5 mg/kg). Immediately after AI, queens received atipamezole (0.125 mg/kg; IM) (Alzane®, Royan Daru Co, Tehran, Iran) for anesthesia reversal. Before anesthesia, food and water were withheld from queens for 12 h and 2 h, respectively.

AI

Insemination was performed 34 h after the hCG treatment. Semen was stored at 27 °C, and after determining semen motility, viability and percentage of morphologically abnormal sperm, it was used for AI. A ventral midline laparotomy was done and the ovaries were exposed, females with positive ovarian response (presence of preovulatory follicles or fresh CLs) were inseminated. An insulin needle was inserted either into the luminal region of the uterine body (bifurcation region) (UBI) or into the luminal region of both uterine horns (BIUI), and 3×10^6 spermatozoa were injected into the tip of both uterine horns or body, respectively. After insemination, bleeding was controlled by placing a gauze pad over the insemination site and applying direct pressure. In the BIUI group, half of the semen was inseminated into each horn.

Pregnancy diagnosis

Cats were examined with a real-time, B-mode ultrasonographic scanner (Emperor - V9 EV 5 MHz, EMP Co., China) 30 days after AI. The number of kittens was counted on the delivery day.

Statistical analysis

Continuous dependent variables, including gestation length and litter size, were analyzed using the GLM procedure. Binary dependent variables, including conception rates, were analyzed using logistic regression analysis by GENMOD procedure considering function link logit in the model. All analyses were performed in SAS version 9.4 (SAS Institute Inc., Carry, NC, USA). Differences were considered significant at $p<0.05$.

RESULTS

Evaluation of vaginal cytology and estrous behavior

Signs of behavioral estrous were observed in 11 out of 15 queens. Moreover, all queens in both groups showed more than 60% of superficial cells and were considered to be in estrus.

Induction of ovulation

In most inseminated cats, evidence for ovulation was observed at the time of exploratory surgery and the total ovulation rate was 73.33% (11/15) after a single injection of hCG (Figure 1). The female cats that were diagnosed in a preovulatory state at the time of insemination (n=4) were subjected to a second administration of 100 IU hCG to induce ovulation.

Semen quality

Characteristics of the semen were comparable between the two groups. Table 1 presents the results of semen evaluation at both AI groups.

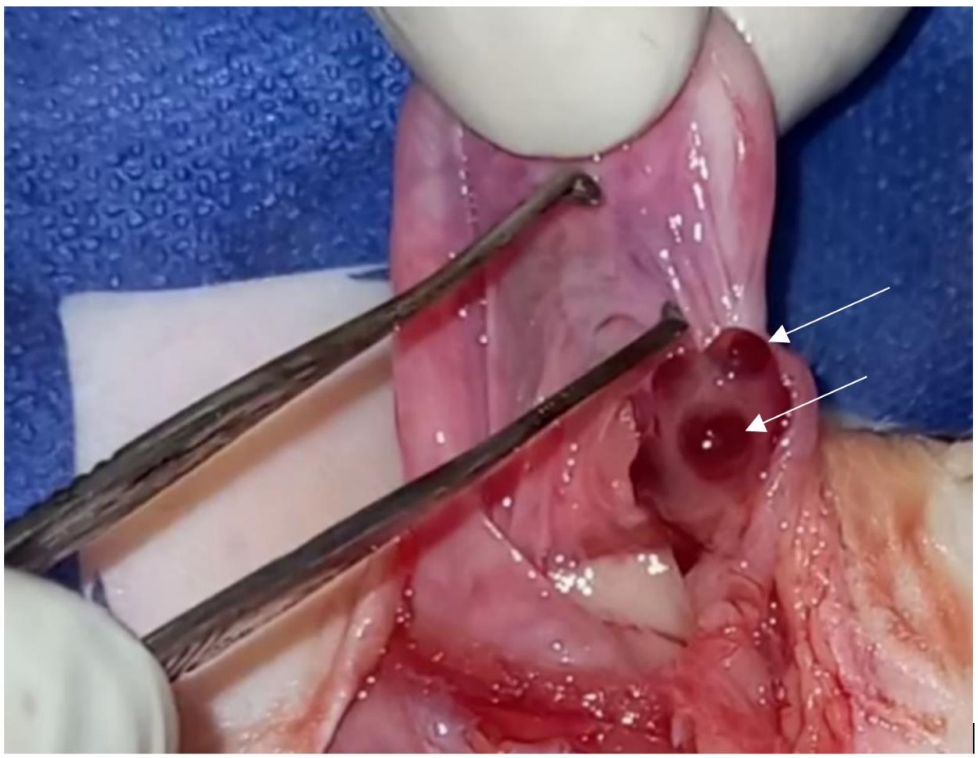

Figure 1. Ovarian response 34 h after hCG injection. Arrows indicate the fresh CLs.

Table 1. Semen characteristics between BIUI and UBI group.

Insemination site	Volume(µl)	Total Motility (%)	Progressive Motility (%)	Concentration (×10⁶)/ml	Total sperm (×10⁶)	Normal Morphology (%)	Viability (%)
BIUI	25	72	60	1296	32.4	70	67
UBI	21	70	60	1428	30.3	68	64

Conception rate

Results of insemination between the two techniques are presented in Table 2. An average of 3×10^6 fresh spermatozoa were inseminated in each queen by BIUI or UBI methods. BIUI resulted in a conception rate of 75.00% (6/8). The pregnant queens (n=6) in this group gave birth to 1 to 6 kittens (Mean±SE, 3.00 ± 0.86 kittens) after a gestation period of 63 to 66 days (Mean±SE, 64.50 ± 0.43 days). UBI resulted in a conception rate of 42.85% (3/7). The pregnant queens (n=2) in this group gave birth to 1 to 3 kittens (Mean±SE, 2.00 ± 1.00 kittens) following a gestation period of 65 to 67 days (Mean±SE, 66.00 ± 1.00 days). Queen number 11 from the UBI group spontaneously aborted 35 days after AI, and two mummified fetuses were observed in queen number 1 from the BIUI group (Figure 2). Conception rate tended to be higher in BIUI group as compared with UBI group (odds ratio = 7.500, 95% confidence interval = 0.759-74.154; P = 0.085) (Table 2). However, litter size and gestation length were not different between the two experimental groups (p>0.05; Table 2).

Table 2. Results of Insemination between BIUI and UBI group.

Insemination site	Queen No.	Sperm dose (×10^6)	Gestation length (d)	Litter size	Conception rate (%)
Uterine horns	1*	3 × 10^6 spermatozoa (1.5 × 10^6 for each horn)	63	1	75.00% (6/8)
	2		64	5	
	3		64	3	
	4		65	6	
	5		66	2	
	6**		65	1	
	7		0	0	
	8		0	0	
	Mean±SE		64.50±0.43	3.00±0.86	
Uterine body	9	3 × 10^6 spermatozoa	67	1	42.85% (3/7)
	10		65	3	
	11**		0	Aborted	
	12**		0	0	
	13**		0	0	
	14		0	0	
	15		0	0	
	Mean ±SE		66.00±1.00	2.00±1.00	

* Queen No. 1 had two mummified fetus,
**Queen No. 6,11,12 and 13 received the second dose of hCG.

Figure 2. Mummified fetuses in queen No. 1

DISCUSSION

Most populations of captive wild felid species and domestic cats used as biomodel could benefit from ARTs, particularly in terms of male and female behavioral issues, gene transfer across institutions, and physical limitations of animal transfer. Assisted reproductive technologies may help to improve reproductive success and genetic diversity by bringing new genes into isolated wild felid populations (SWANSON 2006, LERMEN et al. 2009).

In order to minimize potential bias, semen was obtained from the same tom cat for both AI groups. Semen quality was confirmed upon male selection. In this study, semen was collected by UC, and this technique is appropriate in practice since no expensive equipment, and specific permissions are required (ZAMBELLI et al. 2008, LUEDERS et al. 2012). It is easy to perform, and no animal training is required; furthermore, UC samples are free from urine contamination, which may sometimes occur during electroejaculation (EE) (ZAMBELLI et al. 2008, LUEDERS et al. 2012). Moreover, UC circumvents the tom cats' poor libido issues in AV collection (either using estrous queens or phantoms) and can be less time-consuming when compared to the EE method (ACKERMANN & LOPES 2020, FILLIERS et al. 2010). When

urethral method of semen collection is implemented, the accessory sex glands are not as stimulated as when semen collection by electroejaculator is performed. As a result, the semen collected using UC method has less seminal plasma than that collected by EE, and consequently has less volume and higher sperm concentration, which make the semen sample more suitable for intrauterine insemination (ZAMBELLI et al. 2008, LUEDERS et al. 2012).

Irrespective of the method used for AI in female cats, it is worth noting that semen collection was carried out through the introduction of the catheter into the urethra in the present study, which was a promising method for semen collection in tom cats. The preliminary microscopic evaluations revealed that the collected semen samples had adequate concentration, total and progressive motility, normal morphology, and viability for artificial insemination. More importantly, these microscopic findings were further substantiated by the successful pregnancy of inseminated queens. Accordingly, this method requires further evaluation in other feline species.

The sperm doses required for conception depend on the techniques of insemination. Vaginal AI typically requires 50–80 $\times 10^6$ fresh spermatozoa for consistent conception rates (SOJKA et al. 1970, TANAKA et al. 2000), whereas transcervical AI requires 10–30 $\times 10^6$ fresh spermatozoa to obtain similar fertility (ZAMBELLI & CUNTO 2005). Because AI time in relation to ovulation is less dependent on sperm viability when the semen is freshly collected and frozen-thawed semen yields less satisfactory results (SWANSON 2019, PLATZ et al. 1978); in the current study, each queen was inseminated with an average of 3×10^6 fresh spermatozoa following the BIUI or novel UBI method.

After pharmacological induction of estrus, behavioral estrous signs were observed in 11 out of 15 queens. Estrus was assessed by examination of vaginal cytology and observation of behavioral signs. Combining artificial insemination with estrus induction allows for greater control over the parameters that can affect insemination success, such as the precise timing of the procedure (PLATZ et al. 1978). Ovulation is considered to occur 25–27 h after hCG administration (SOJKA et al. 1970). HOWARD et al. (1992) reported ovulation 30 h after hCG treatment. According to HOWARD et al. (1992), performing anesthesia before ovulation can interfere with ovulation triggers and lead to a decreased fertility. In contrast to these findings, TSUTSUI et al. 2000 obtained a higher conception rate when insemination was performed before ovulation. The differences between the two studies could be explained by the fact that TSUTSUI et al. 2000 used queens in natural estrus and hCG at a higher dose than HOWARD et al. 1992. In our study, the total ovulation induction rate was 73.33% (11/15) by the time of exploratory surgery. Yet it is worth noting that the queen number 6 and 11 were diagnosed pregnant following AI, even though no evident ovulation was detected at the time of surgery. This phenomenon implicates that some of queens might have ovulated after surgery.

Considering the facts that high doses of gonadotropins could adversely affect the quality of oocytes and hCG at the dose of 100 IU brought about acceptable results (GRAHAM et al. 2000, SWANSON et al. 1996, GOODROWE et al. 1988), we chose the hCG dose of 100 IU for induction of ovulation in queens.

The first successful AI in domestic cats was reported more than 50 years ago following the intravaginal deposition of fresh semen into natural-estrous hCG-treated females (SOJKA et al. 1970). Although single insemination had a high pregnancy rate (50%), this method required high sperm counts (10–50 million spermatozoa) for consistent conception, as well as the need to inseminate females during their natural estrous period. TSUTSUI et al. 2003 reported a conception rate of 80% after surgical intrauterine AI (IUAI) with 8×10^6 fresh spermatozoa (SWANSON 2019). TSUTSUI et al. 2000 reported a conception rate of 57% after unilateral intrauterine insemination with 50×10^6 frozen-thawed spermatozoa.

DAŞKIN et al. 2022 reported pregnancy with the intravaginal insemination technique. ZAMBELLI et al. 2015a reported a 85.71% pregnancy rate with a new nonsurgical endoscopic transcervical insemination technique. ZAMBELLI et al. 2015b also reported a 100% pregnancy rate with the same endoscopic transcervical insemination technique. The last three reports are among the few studies on the efficacy of UC-collected sperm in terms of the pregnancy rate of queens, which indicates the need for further advancements. In this study, the urethral semen yielded a pregnancy rate of 75.00% and 42.85% after BIUI and UBI, respectively. So various procedures for domestic cat AI have been documented in recent years with varying results in conception rate, demonstrating the increased interest in developing new preservation strategies.

This is the first study to compare the fertility of fresh urethral sperm in the uterine body and uterine horns. Also, as far we know, this is the first study assessing fresh urethral sperm fertility injected directly into the uterine body. It is clear that the uterine horns are closer to the fertilization site when compared to the uterine body, so we chose these two insemination sites (uterine body and uterine horns) to investigate

whether the distance from the insemination site can affect the pregnancy rate. In our study, a 75.00% and 42.85% pregnancy rate were obtained following BIUI and UBI, respectively. The higher pregnancy rate in the BIUI group clearly showed that the distance between the insemination site and the fertilization site could contribute to a better pregnancy rate. However, the UBI technique only requires one injection, and by increasing the sperm dose, it may be possible to increase the pregnancy rate. Also, we observed that insemination in the uterine body is easier since only one injection is required, and post-injection bleeding was less than the uterine horns since the uterine horns were more vascular than the uterine body at AI. Another factor that facilitates insemination by the UBI technique is that the uterine body has a greater diameter when compared to the tip of the uterine horns. So, the inseminator must keep the diameter differences of these insemination sites (uterine body and uterine horn) in mind when inserting the needle, since felines produce low semen volumes (especially in UC technique), and semen loss due to inseminator mistakes at AI are not acceptable (JOHNSON 2018).

Spontaneous abortion occurred at approximately day 35 of pregnancy in queen number 11. There are some reports of spontaneous abortion after intravaginal and intrauterine deposition with fresh semen as well as by intrauterine deposition with frozen sperm and epididymal sperm (TSUTSUI et al. 2000, WATSON 2000, TSUTSUI et al 2001, TSUTSUI et al 2004. HOWARD & WILDT 2009 reported successful pregnancy and parturition following laparoscopic IUAI in felids. CHATDARONG et al. 2007 used a transvaginal catheter to perform noninvasive IUAI with frozen semen and reported conceptions, but no spontaneous abortions occurred. Hence, it is unclear whether the invasiveness of the surgical procedure played a role in the current study's abortion. Furthermore, it would be preferable to develop nonsurgical AI approaches that are easy to perform and applicable to all queens, particularly for non-domestic felid applications. Two mummified fetuses were diagnosed in queen number 1, and the reason is not clear, but it may be attributable to chromosomal and developmental abnormalities of the fetuses, infectious pathogens, and maternal endocrine abnormalities (PLANELLAS et al. 2012).

Despite observing fresh CLs and using the same male spermatozoa for insemination in both experimental groups, AI results in the BIUI group were considerably greater than those in the UBI group. This finding indicates that the BIUI could be a more efficient method of AI in cats as compared with UBI method.

It is commonly believed that a quiescent ovary is more likely to respond consistently to synchronization protocols (HOWARD & WILDT 2009). Therefore, pretreatment with a progesterone analog in synchronization protocols appears beneficial for achieving a more consistent ovarian response and improved oocyte quality (PELICAN et al. 2001, PELICAN et al. 2008). Absence of CLs after implant removal seems to improve the ovarian response to exogenous gonadotropins (PELICAN et al. 2006), so it is better to consider it in future studies.

CONCLUSION

The present study offers a novel approach to AI in felids by using laparotomy to deposit semen into the uterine bifurcation region. In this experiment, we obtained 75.00% and 42.85% pregnancy rates following BIUI and UBI, respectively. Our findings showed that intrauterine insemination in gonadotropin-induced cats could result in high pregnancy rates and that intrauterine insemination after eCG/hCG treatment generates normal and healthy offspring that can be carried on to term. Although the UBI method did not yield a high pregnancy rate, it can be improved by increasing the semen dose and quality and using a progesterone analog as a pretreatment. In conclusion, BIUI with fresh semen resulted in higher pregnancy rates than UBI.

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REFERENCES

ACKERMANN CL & LOPES MD. 2020. Training tom cats for semen collection using an artificial vagina: a retrospective study. Journal of feline medicine and surgery 22: 1155-1159.

AZUMANO A et al. 2022. Successful laparoscopic oviductal artificial insemination in the endangered Tsushima leopard cat (Prionailurus bengalensis euptilurus). Animals 12: 777.

CHATDARONG K et al. 2007. Pregnancy in the domestic cat after vaginal or transcervical insemination with fresh and frozen semen. Theriogenology 68: 1326-1333.

CONFORTI VA et al. 2013. Laparoscopic oviductal artificial insemination improves pregnancy success in exogenous gonadotropin-treated domestic cats as a model for endangered felids. Biol Reprod. 89: 4.

DAŞKIN A et al. 2022. Artificial insemination in a cat: Report of first successful performance resulted with parturition in Turkey. Ankara Üniversitesi Veteriner Fakültesi Dergisi 69: 225-227.

FARSTAD W. 2000. Current state in biotechnology in canine and feline reproduction. Animal Reproduction Science 60: 375-387.

FILLIERS M et al. 2010. In vitro evaluation of fresh sperm quality in tomcats: a comparison of two collection techniques. Theriogenology 74: 31-39.

GOODROWE K et al. 1988. Influence of gonadotropins on and developmental competence of in vitro fertilized feline follicular oocytes. Theriogenology 29: 250.

GRAHAM LH et al. 2000. Chorionic gonadotropin administration in domestic cats causes an abnormal endocrine environment that disrupts oviductal embryo transport. Theriogenology 54: 1117-1131.

HOWARD J & WILDT DE. 2009. Approaches and efficacy of artificial insemination in felids and mustelids. Theriogenology 71: 130-148

HOWARD JG et al. 1992. The effect of pre-ovulatory anaesthesia on ovulation in laparoscopically inseminated domestic cats. J Reprod Fertil 96: 175-186.

JOHNSON AK. 2018. Assisted reproduction in the female cat. Veterinary Clinics: Small Animal Practice 48: 523-531.

LERMEN D et al. 2009. Cryobanking of viable biomaterials: implementation of new strategies for conservation purposes. Molecular Ecology 18: 1030-1033.

LINDE-FORSBERG C. 2005. Recent advances in small animal reproduction. Ithaca: International Veterinary.

LUEDERS I et al. 2012. Improved semen collection method for wild felids: urethral catheterization yields high sperm quality in African lions (Panthera leo). Theriogenology 78: 696-701.

PELICAN KM et al. 2001. (Editors). Short-term ovarian suppression with levonorgestrel before gonadotropin stimulation enhances IVF embryo production in the domestic cat. In: MONROE ST & MADISON WI. Biology of Reproduction. USA: SOC STUDY REPRODUCTION.

PELICAN KM et al. 2006. Ovarian control for assisted reproduction in the domestic cat and wild felids. Theriogenology 66: 37-48.

PELICAN KM et al. 2008. Priming with progestin, but not GnRH antagonist, induces a consistent endocrine response to exogenous gonadotropins in induced and spontaneously ovulating cats. Domestic animal endocrinology 34: 160-175.

PLANELLAS M et al. 2012. Mummified fetus in the thoracic cavity of a domestic short-haired cat. Topics in companion animal medicine 27: 36-37.

PLATZ C et al. 1978. Pregnancy in the domestic cat after artificial insemination with previously frozen spermatozoa. Reproduction 52: 279-282.

PROCHOWSKA S et al. 2015. Characteristics of urethral and epididymal semen collected from domestic cats—A retrospective study of 214 cases. Theriogenology 84:1565-1571.

PUKAZHENTHI BS & WILDT DE. 2003. Which reproductive technologies are most relevant to studying, managing and conserving wildlife? Reproduction, Fertility and Development 16: 33-46.

RIJSSELAERE T & VAN SOOM A. 2010. Semen collection, assessment and artificial insemination in the cat. Vlaams Diergeneeskundig Tijdschrift 79: 467-470.

ROTH TL et al. 1997. Effects of equine chorionic gonadotropin, human chorionic gonadotropin, and laparoscopic artificial insemination on embryo, endocrine, and luteal characteristics in the domestic cat. Biology of reproduction 57: 165-171.

SOJKA N et al. 1970. Artificial insemination in the cat (Felis catus L.). Laboratory animal care 20: 198-204.

SWANSON W et al. 1996. Ancillary follicle and secondary corpora lutea formation following exogenous gonadotropin treatment in the domestic cat and effect of passive transfer of gonadotropin-neutralizing antisera. Theriogenology 45: 561-572.

SWANSON WF. 2012. Laparoscopic Oviductal Embryo Transfer and Artificial Insemination in Felids – Challenges, Strategies and Successes. Reproduction in Domestic Animals 47: 136-140.

SWANSON WF. 2006. Application of assisted reproduction for population management in felids: the potential and reality for conservation of small cats. Theriogenology 66: 49-58.

SWANSON WF. 2019. Practical application of laparoscopic oviductal artificial insemination for the propagation of domestic cats and wild felids. Reproduction, Fertility and Development 31: 27-39.

TANAKA A et al. 2000. Artificial intravaginal insemination using fresh semen in cats. J Vet Med Sci. 62: 1163-1167.

TSUTSUI T et al. 2000. Unilateral intrauterine horn insemination of fresh semen in cats. J Vet Med Sci. 62: 1241-1245.

TSUTSUI T et al. 2001. Intratubal insemination with fresh semen in cats. Journal of reproduction and fertility Supplement. 57: 347-351.

TSUTSUI T et al. 2003. Artificial insemination with frozen epididymal sperm in cats. Journal of veterinary medical science 65: 397-399.

TSUTSUI T et al. 2004. Relationship between the sperm count and the fertilization rate of ova ovulated from the contralateral ovary in intrauterine horn insemination in cats. Journal of veterinary medical science 66: 1143-1145.

WATSON PF. 2000. The causes of reduced fertility with cryopreserved semen. Animal reproduction science 60: 481-492.

WILDT DE et al. 1977. Laparoscopy for direct observation of internal organs of the domestic cat and dog. American Journal of Veterinary Research 38:1429-1432.

WILDT DE & SEAGER SW. 1978. Ovarian response in the estrual cat receiving varying dosages of HCG. Hormone Research in Paediatrics 9: 144-150.

WILDT DE. 1991. Fertilization in cats. In: DUNBAR BS & O'RAND MG. (Eds) A comparative overview of mammalian fertilization: Boston:Springer. p. 299-328.

ZAMBELLI D & CASTAGNETTI C. 2001. Transcervical insemination with fresh or frozen semen in the domestic cat: new technique and preliminary results. ESDAR Newsletter 6: 34.

ZAMBELLI D & CUNTO M. 2005. Transcervical artificial insemination in the cat. Theriogenology 64: 698-705.

ZAMBELLI D et al. 2004. Vaginal and cervical anatomic modifications during the oestrus cycle in relation to transcervical catheterization in the domestic cat. Reproduction in domestic animals 39: 76-80.

ZAMBELLI D et al. 2008. Quality and in vitro fertilizing ability of cryopreserved cat spermatozoa obtained by urethral catheterization after medetomidine administration. Theriogenology 69: 485-490.

ZAMBELLI D et al. 2015a. Endoscopic transcervical catheterization in the domestic cat. Reproduction in Domestic Animals 50:13-16.

ZAMBELLI D et al. 2015b. First deliveries after estrus induction using deslorelin and endoscopic transcervical insemination in the queen. Theriogenology 84: 773-778.