Transcervical insemination in bitch

Stuart Mason, Nicole Rous, Aimee Lougher
Monash Veterinary Clinic, Oakleigh East, Victoria, Australia

Abstract

To perform a successful transcervical insemination, the operator must be able to accurately stage the estrous cycle, have a thorough understanding of the anatomy of the bitch and confidence to perform the procedure diligently. Estrous cycle timing is best undertaken using a combination of LH assay, vaginal cytology, progesterone assay and vaginoscopy. Transcervical insemination results in higher pregnancy rates with both fresh semen when compared to vaginal insemination and frozen semen when compared to surgical (laparotomy) insemination. Using this technique, a pregnancy rate of 86% was attained from 1850 inseminations using fresh semen with an average of 5.4 puppies per litter. In addition, utilising the same insemination technique, a pregnancy rate of 77% was attained from 940 inseminations when > 150 million frozen-thawed dog sperm were inseminated. In regards to frozen semen, there was a trend for greater pregnancy rate with increasing sperm numbers; however, in this study, there was no benefit to using > 150 - 200 million sperm.

Keywords: Frozen semen, bitch, canine, artificial insemination, transcervical insemination

Introduction

Transcervical insemination (TCI) in the bitch is becoming more common in practice, with increased availability of endoscopic equipment and increased awareness of the welfare of breeding bitches. In order to perform a successful TCI, the operator must be able to accurately stage the estrous cycle, have a thorough understanding of the anatomy of the bitch and confidence to perform the procedure diligently. Results are presented here related to 1850 fresh semen TCI's, 111 chilled semen TCI's and 940 frozen semen TCI's over a 14 year interval.

Timing of insemination

The most common cause of pregnancy failure in the bitch is poor timing of insemination or breeding.[1] Bitches spontaneously ovulate a primary oocyte arrested in Prophase I of the second stage of meiosis.[2, 3] The luteinizing hormone (LH) surge occurs ~ 2 days before ovulation.[4,5] The LH surge stimulates granulosa cells to luteinise and produce progesterone, which may aid in resumption of meiosis of the oocytes.[3] After ovulation, a corpus luteum forms due to luteinization of thecal and granulosa cells. The corpus luteum secretes progesterone, the hormone solely responsible for pregnancy maintenance in the bitch. Six days after ovulation, the bitch enters diestrus.[6]

Laboratory tests and procedures for staging bitch's cycle

LH assay

LH concentrations are undetectable until the point of the LH surge. Daily serum analysis of LH is required due to the short, varied, duration of the LH surge in the bitch (24 - 60 hours). There is a rising phase of up to 12 - 24 hours and then a decline over 12 - 36 hours.[7] The only readily available LH tests are Qualitative LH tests, which give a positive result at concentrations > 1 ng/ml. Even with daily LH testing, it is possible to not detect the LH surge, since LH concentrations vary at the peak of the LH surge (4 - 14 ng/ml[7,8]), and there is often a slow rise and fall. Therefore, LH assays on their own are not recommended, since the LH surge occurs 3 - 8 days before oocyte fertilisation,[8] coupled with the possibility of anovulatory cycles.

Progesterone assay

Progesterone concentrations rise from the LH surge and can be used as an indirect indicator of ovulation. Progesterone continues to rise through estrus and early diestrus, with the cervix closing under the influence of increasing progesterone. Progesterone is most commonly measured today by chemoluminescence, with serum being recommended rather than plasma.[9] Collection of blood for progesterone is best done on a fasted sample. Refrigeration of samples within the first 2 hours after collection will reduce measured progesterone concentrations.[9]

The LH surge occurs at ~6 nmol/l (2 ng/ml) and ovulation at 15 - 25 nmol/l (4 - 8 ng/ml), with ovulation being deemed complete with values > 30 nmol/l (10 ng/ml).[10] In the authors' opinion, there is no 'magical' value of progesterone at which fertility is maximal; changes in values are more important to help identify ovulation. Consistency of progesterone analysis is important, as different analytical machines will give different results.

Vaginal cytology

The cytology of the vagina changes as a result of changing estrogen concentrations from developing ovarian follicles causing hyperplasia of the vagina, represented by increasing cornification of vaginal cytology samples. As the vagina undergoes hypertrophy, surface cells suffer effects of reduced oxygenation which presents as increasing enlargement of the cytoplasm, nuclear pyknosis and cornification of surface cells.[11] Percentage of anuclear cells increases to 50% of the cornified cells by the start of estrus. Six days after ovulation, diestrus ensues, characterised by an influx of neutrophils and parabasal cells, reducing the percentage of cornified vaginal epithelial cells.[6] Vaginal cytology alone is a poor indicator of the ideal time to breed;[6] however, in conjunction with progesterone assays and vaginoscopy, it aids in determination of the phase of the cycle (proestrus, estrus, diestrus or anestrus) and the appropriateness of insemination.[12,13]

Vaginoscopy

Vaginoscopy is performed using a 25 cm long, 11 mm diameter Welch Allyn sigmoidoscope. Under the influence of rising progesterone and increasing estrogen concentrations after the LH surge, the deep oedematous folds of proestrus, begin to flatten out and reduce. As estrogen continues to decline and progesterone continues to rise through estrus, the hypertrophy and hyperplasia of the vagina reduces, resulting in a cobblestone appearance of the vagina (crenulation), indicating ovulation is complete and the bitch is in the fertile period. By the start of diestrus, the vagina returns to a flat state with no significant folds in the mucosa.[14]

To determine the appropriate time for breeding/insemination a combination of progesterone assay, LH assay, vaginal cytology, and vaginoscopy are used.[12]

Transcervical insemination procedure

Anatomy of the bitch

Understanding the anatomy of the bitch is important to ensure a successful TCI. The external genitalia of the female begins at the vulva, the entrance to the vestibule. The vestibule contains the clitoris and the urethral opening. The vestibule is quite short, with the lumen heading at approximately a 45° angle from horizontal towards the lumbar vertebrae of the bitch. The vestibule terminates at the cingulum which is important for ensuring a tied mating. From the cingulum, the vagina extends forwards approximately parallel to the lumbar spine. The vagina of the bitch is quite long and extends to the level of the cervix. Caudal to the cervix are 3 dorsomedian folds which consist of 3 tubercles (caudal, middle and cranial). The cranial vagina ends at the cervix, which protrudes caudally into the vagina, creating a cavity between the cervical os and the cranial vagina (fornix). The cervical lumen extends cranially from the cervical os, and in most cases, the cervical os faces down towards the floor of the vagina. The uterus of the bitch is bicornuate, extending cranially from the short body attached to the cervix in 2 distinct horns to the uterine tubes and ovaries.

Transcervical insemination procedures

There are 2 described methods of transcervical insemination: the Norwegian Catheter and endoscopic assisted transcervical insemination. Results of TCI presented in this paper were all via the endoscopic assisted method.

Norwegian catheter

Norwegian catheter (Gartnerservice, Haslum, Norway, jan@basbergdata.no) was the first developed nonsurgical intrauterine device in 1975.[15] It consists of an outer nylon sheath with an inner metal stylet with a blunted, rounded distal tip. The procedure is performed in any size of bitch without the need of any anesthesia. With the bitch standing, restrained on a table, the operator will

transabdominally palpate the cervix of the bitch while passing the catheter into the vagina of the bitch with the opposing hand. While holding the cervix steady with the thumb and index finger, the stylet is passed into the cervical os and through the cervix of the bitch. The semen is then inseminated through the stylet into the uterine lumen.[16,17]

The procedure is quick, simple and cheap to perform; however, the learning curve is steep and the procedure difficult on large breed bitches. Unlike surgical insemination, the procedure may be performed more than once if required, as there is no anesthesia or surgery.[17] Stimulation of the vestibule during this procedure is likely to stimulate uterine contractions resulting in an insemination process more like a natural mating than that obtained by surgical insemination.[18] The Norwegian catheter may be used for fresh, fresh chilled or frozen semen. The majority of published reports on the use of frozen semen in the bitch pertain to the use of the Norwegian catheter.[17,19-23]

Endoscopic assisted transcervical insemination

A procedure commonly known as TCI, was first described in 1993 with the use of a cystoscope.[24] Endoscopic assisted transcervical insemination (EIU) is simple to learn with persistence and care; however, it is a technique wherein all steps must be monitored closely to maximize results.[25, 26] Nowadays, most operators use a uterorenoscope, as this endoscope is longer and thinner (in diameter) than the cystoscope, allowing it to be used more adequately in all bitches from toys to giant breeds.[25,26] The cystoscope was often too large in diameter for toy bitches and too short to reach the cervix of large bitches. The procedure is performed with the bitch standing, restrained on a table. Using a uterorenoscope, cold xenon, halogen, or LCD light source, and camera with images projected onto a monitor, the operator is able to pass the endoscope to the level of the cervical os. The endoscope is initially passed into the vagina and at this point, air is instilled into the vagina via a rectal insufflation pump attached to the endoscope (30200 rectal insufflation bulb Welch Allyn, Skaneatele, NY). The use of a vaginal shunt (Minitube, Verona, WI) may aid in sealing the vagina to maintain vaginal distension. The endoscope is advanced cranially past the dorsomedian folds to the cervix. The cervical os is visualised on the ventral aspect of the cervix in most cases. A CH 5 (or CH 4) TCI catheter (Minitube) is advanced through the endoscope and through the cervical os. Once the catheter is within the cervix, the stylet is removed and the catheter only advanced into the body of the uterus. The TCI catheter has marks at increments of 1 cm to aid in positioning the opening to the catheter within the body of the uterus. The semen is then inseminated through the catheter, slowly, watching for leakage at the cervical os,[24-27] and massaging the vulva to promote uterine contractions. The semen is inseminated first, followed by prostate fluid (fresh semen; if prostate fluid is not diseased) or additional extender (fresh chilled, frozen semen) until the uterus is deemed full (appearance of fluid at the cervical os).[25,26] Slow insemination will allow for insemination of large volumes.[26]

EIU is suitable for fresh, fresh chilled and frozen semen. EIU is more successful than surgical insemination when performed appropriately,[25] and EIU is more successful than vaginal insemination with fresh semen.[28,29] Unlike with the Norwegian catheter, with EIU, the cervix is visualised so it is guaranteed the catheter is intrauterine 100% of the time.[24-27]

Why perform transcervical insemination?

Comparative studies of vaginal insemination of fresh semen with EIU have ~ 33 - 35% higher pregnancy rate with EIU.[28,29] This is likely a result of semen not actually being put at the cervical os in many vaginal inseminations, and additionally intravaginal insemination requires sperm to traverse the cervix into the uterus, in contrast to TCI wherein the sperm are placed directly intrauterine. It is most probable that with intravaginal insemination, a reduced number of sperm traverse the cervix into the uterus than the number placed in the vagina. This loss of sperm entering the uterus is counteracted by the recommendation of > 1 vaginal insemination; however, pregnancy results are still lower than TCI.[28,29]

Although transcervical insemination by EIU or Norwegian catheter can be repeated, there is no benefit to > 1 insemination, unless timing of insemination is poor.[19,20] With frozen semen use in bitches, many advocates of surgical insemination have discounted TCI as wasting more semen by doing the procedure twice (compared to 1 surgical insemination). However, pregnancy rate of 1 TCI is widely published,[23-27,30] and more recently, pregnancy rate of TCI compared to surgical

insemination.[25] The authors recommend performing 1 intrauterine insemination with optimal timing of insemination.[26] Publications describing 2 inseminations by Norwegian catheter or EIU were only recommended and performed in situations when timing was poor (to cover a larger possible window of fertilization)[19,20] or when quality of semen was poor.[25]

If timing of insemination is poor, and intrauterine insemination occurs in diestrus, there is both a reduced expected pregnancy rate[22,31,32] and an increased probability of inducing CEH and/or pyometra whether it be by laparotomy, EIU or Norwegian catheter.[33-36] Surgical insemination continues to be advocated by many, as sperm are placed closer to the oviduct than with TCI. However, studies on uterine motility concluded site of insemination within the uterus is irrelevant.[37,38]

The higher success rate of TCI with frozen semen than surgical insemination is most likely due to lack of stress of surgery and anaesthesia, the presence of uterine contractions during the procedure and lack of effects of wound healing and inflammation.[18,25,26] Aside from the reduced success rate, ethical issues of surgical insemination (anesthesia, surgery, healing, risk of death from anesthesia, production of anti-sperm antibodies via blood sperm contact) lead many to question why the procedure continues to be advocated and hence why TCI should be promoted.

Results of 1850 fresh semen, 111 chilled semen and 940 frozen semen TCI's (2005 - 2019) Using the aforementioned technique of timing and insemination, pregnancy rates were attained as detailed in Table 1 (fresh and chilled semen) and Table 2 (frozen semen).

Table 1. Results of TCI inseminations using fresh and chilled semen in the bitch.

	Fresh semen	Chilled semen
Total number performed	1850	111
Average motility (%)	80.4 (39 - 95)	70.5
Pregnancy rate (%)	86	67
Pregnancy rate < 150 x 10^6 sperm (%)	82	-
Pregnancy rate > 150 x 10^6 sperm (%)	88	67
Pregnancy rate < 70% motility (%)	68	75
Pregnancy rate > 70% motility (%)	87	64
Resorption numbers (%)	0.8	0
Average number sperm x 10^6	730 (25 - 3318)	513 (155 - 1290)
Average number of pups	5.42 (1 - 19)	6.67 (4 - 11)

These results are presented and analysed in relationship to the number and quality of sperm inseminated. However, it must be remembered that many other factors affect the outcome of any artificial insemination such as, but not limited to, genetic history, uterine health, health status of the male when frozen, age of frozen semen when used and health status of the bitch inseminated through estrus and early pregnancy. Most of this information was unavailable in data collection for this study and hence is not discussed.

Fresh semen results

To the authors' knowledge, there is no other study with such a large number of results of fresh semen inseminations using the endoscope assisted technique. Until now, most large published studies pertaining to the use of fresh semen in the bitch are related to vaginal and transcervical insemination using the Norwegian catheter.[16,21,22] Two papers comparing pregnancy rate of fresh semen via vaginal insemination to EUI reported a higher pregnancy rate with EUI, with pregnancy

Table 2. Results of TCI inseminations using frozen semen in the bitch.

	Frozen semen
Total number performed	940
Average motility (%)	50.87
Pregnancy rate (%)	69
Pregnancy rate < 100 million sperm (%)	62
Pregnancy rate 100 - 150 million sperm (%)	70
Pregnancy rate > 150 million sperm (%)	77
Pregnancy rate 150 - 200 million sperm (%)	80
Pregnancy rate > 200 million sperm (%)	70
Pregnancy rate < 40% PMS (%)	57
Pregnancy rate > 40% PMS (%)	76.60
Pregnancy rate < 30% PMS (%)	55
Resorption (%)	18
Average number sperm x 10^6	127 (10 - 781)
Average number of pups	5.04 (1 - 16)

rates similar to those attained in this study.[28,29] When analysing pregnancy, rate, there was no significant difference between the under and over 150 million progressively motile sperm ($p = 0.5189$, Graphpad quickcalc), However, there was a significant difference when samples were used with > 70% progressively motile sperm than < 70% progressively motile sperm ($p < 0.05$, Graphpad quickcalc). This concurs with current recommendations for sperm motility for optimal results. There were very few samples with < 150 million progressively motile sperm in this study, and this is likely to have impacted the analysis of inseminating over or under 150 million progressively motile sperm with fresh semen.

Frozen semen results

Pregnancy rates for frozen semen are consistent with those reported in other studies.[17,19-23,25,26,29,40] As per previous studies, there is a trend with greater pregnancy rate with more progressively motile sperm inseminated.[25,26] However in contrast to other studies,[39,40] there was no significant difference in pregnancy rate with > 200 million progressively motile sperm ($p = 1$, Graphpad quickcalc). This may be due to the small numbers of inseminations in the >200 million group, however as comparable results (pregnancy) were attained to other studies,[17,19-23,25,26] and higher results than those advocating the use of > 200 million sperm,[39,40] use of > 200 million sperm is possibly unwarranted. It is the authors' belief that adequate sperm should be used. However, conservation of sperm for future pregnancy attempts is equally important. Additionally, use of semen with < 40% progressive motility or < 30% progressive motility did not significantly reduce pregnancy rates ($p = 0.0564$ and $p = 0.2566$ respectively, Graphpad quickcalc); however, there was a trend to higher pregnancy rate with higher motility in thawed samples.

Conclusion

With good estrous cycle timing, semen handling and insemination technique, pregnancy rates in bitches using fresh, chilled or frozen semen can be maximised.

Conflict of interest

None to declare.

References

1. Johnson SD, Root-Kustritz MV, Olson PNS: Editors. Canine and Feline Theriogenology. Philadelphia: Saunders; 2001.
2. Tsutsui T: Gamete physiology and timing of ovulation and fertilization in dogs. J Reprod Fertil Suppl 1989;39:269-275.

3. Reynaud K, Fontbonne A, Marseloo N, et al.: In vivo meiotic resumption, fertilization and early embryonic development in the bitch. Reproduction 2005;130:193-201.
4. Phemister RD, Holst PA, Spano JS, et al.: Time of ovulation in the beagle bitch. Biol Reprod 1973;8:74-82.
5. Concannon PW, McCann JP, Temple M: Biology and endocrinology of ovulation, pregnancy and parturition in the dog. J Reprod Fertil Suppl 1989;39:3-25.
6. Root Kustritz MV: Managing the reproductive cycle in the bitch. Vet Clin North Am Small Anim Pract 2012;42:423-437.
7. Concannon PW: Endocrinologic control of normal canine ovarian function. Reprod Domest Anim 2009;44 Suppl 2:3-15.
8. Concannon PW, Hansel W, Visek WJ: The ovarian cycle of the bitch: plasma estrogen, LH and progesterone. Biol Reprod 1975;13:112-121.
9. Volkmann DH: The effects of storage time and temperature and anticoagulant on laboratory measurements of canine blood progesterone concentrations. Theriogenology 2006;66:1583-1586.
10. Jeffcoate IA, Lindsay FE: Ovulation detection and timing of insemination based on hormone concentrations, vaginal cytology and the endoscopic appearance of the vagina in domestic bitches. J Reprod Fertil Suppl 1989;39:277-287.
11. Olson PN TM, Wykes PM, Nett TM: Vaginal cytology. Part I. A useful tool for staging the canine oestrous cycle. Compend Contin Educ Pract Vet 1984;6:288-298.
12. Goodman MF: Canine ovulation timing. Probl Vet Med 1992;4:433-444.
13. Silva LD, Onclin K, Verstegen JP: Cervical opening in relation to progesterone and oestradiol during heat in beagle bitches. J Reprod Fertil 1995;104:85-90.
14. Lindsay FEF: The normal endoscopic appearance of the caudal reproductive-tract of the cyclic and non-cyclic bitch - Post-uterine endoscopy. Journal of Small Animal Practice 1983;24:1-15.
15. Andersen K: Insemination with frozen dog semen based on a new insemination technique. Zuchthygiene 1975;10:1-4.
16. Linde-Forsberg C: editor Artificial Insemination. ESAVS-EVSSAR-ENVN Reproduction in companion, exotic and laboratory animal; Nantes:2005.
17. Linde-Forsberg C: Achieving canine pregnancy by using frozen or chilled extended semen. Vet Clin North Am Small Anim Pract 1991;21:467-485.
18. England GC, Moxon R, Freeman SL: Stimulation of mating-induced uterine contractions in the bitch and their modification and enhancement of fertility by prostatic fluid. Reprod Domest Anim 2012;47 Suppl 6:1-5.
19. Thomassen R, Farstad W, Krogenaes A, et al.: Artificial insemination with frozen semen in dogs: a retrospective study. J Reprod Fertil Suppl 2001;57:341-346.
20. Thomassen R, Sanson G, Krogenaes A, et al.: Artificial insemination with frozen semen in dogs: a retrospective study of 10 years using a non-surgical approach. Theriogenology 2006;66:1645-1650.
21. Linde-Forsberg C, Forsberg M: Results of 527 controlled artificial inseminations in dogs. J Reprod Fertil Suppl 1993;47:313-323.
22. Linde-Forsberg C, Forsberg M: Fertility in dogs in relation to semen quality and the time and site of insemination with fresh and frozen semen. J Reprod Fertil Suppl 1989;39:299-310.
23. Linde-Forsberg C, Strom Holst B, Govette G: Comparison of fertility data from vaginal vs intrauterine insemination of frozen-thawed dog semen: a retrospective study. Theriogenology 1999;52:11-23.
24. Wilson MS: Non-surgical intrauterine artificial insemination in bitches using frozen semen. J Reprod Fertil Suppl 1993;47:307-311.
25. Mason SJ, Rous NR: Comparison of endoscopic-assisted transcervical and laparotomy insemination with frozen-thawed dog semen: a retrospective clinical study. Theriogenology 2014;82:844-850.
26. Mason SJ: A retrospective clinical study of endoscopic-assisted transcervical insemination in the bitch with frozen-thawed dog semen. Reprod Domest Anim 2017;52 Suppl 2:275-280.
27. Wilson MS: Transcervical insemination techniques in the bitch. Vet Clin North Am Small Anim Pract 2001;31:291-304.
28. Eilts B, editor Fertility in a canine breeding colony using vaginal artificial insemination or transcervical insemination of fresh semen. ANZCVS Science Week; 2013; Gold Coast, Queensland, Australia.
29. Daskin A, Tekin N, Akcay E: The effect of transcervical intrauterine and intravaginal insemination methods on fertility in dogs. Turkish Journal of Veterinary & Animal Sciences 2003;27:235-239.
30. Pretzer SD, Lillich RK, Althouse GC: Single, transcervical insemination using frozen-thawed semen in the Greyhound: a case series study. Theriogenology 2006;65:1029-1036.
31. Tsutsui T, Takahashi F, Hori T, et al.: Prolonged duration of fertility of dog ova. Reprod Domest Anim 2009;44 Suppl 2:230-233.
32. Verstegen JP, Silva LD, Onclin K: Determination of the role of cervical closure in fertility regulation after mating or artificial insemination in beagle bitches. J Reprod Fertil Suppl 2001;57:31-34.
33. Nomura K: Histological evaluation of canine deciduoma induced by silk suture. J Vet Med Sci 1995;57:9-16.
34. Nomura K: Induction of a deciduoma in the dog. J Vet Med Sci 1994;56:365-369.
35. Nomura K, Funahashi H: Histological characteristics of canine deciduoma induced by intrauterine inoculation of E. coli suspension. J Vet Med Sci 1999;61:433-438.
36. Nomura K, Nishida A: Histological variations of canine deciduoma induced in non pregnant horn at different stages of unilateral pregnancy. J Vet Med Sci 1998;60:623-626.
37. Fukushima FB, Malm C, Henry M, et al.: Site of intrauterine artificial insemination in the bitch does not affect sperm distribution within the uterus. Reprod Domest Anim 2010;45:1059-1064.

38. Tsutsui T, Shimizu T, Ohara N, et al.: Relationship between the number of sperms and the rate of implantation in bitches inseminated into unilateral uterine horn. Japanese Journal of Veterinary Science 1989;51:257-263.
39. Burgess DM, Mitchell KE, Thomas PG: Coeliotomy-assisted intrauterine insemination in dogs: a study of 238 inseminations. Aust Vet J 2012;90:283-290.
40. Hollinshead FK, Hanlon DW: Factors affecting the reproductive performance of bitches: A prospective cohort study involving 1203 inseminations with fresh and frozen semen. Theriogenology 2017;101:62-72.