

A New Device for Intrauterine Artificial Insemination in the Dog**

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ABSTRACT : The intrauterine inseminator (IUI) was developed to provide the method of depositing dog semen into the uterine body instead of the vagina. The IUI consists of a vaginal endoscope, a balloon sheath, and injection catheter. When the endoscope is inserted into the vagina and the balloon expanded with air, the cervical os becomes visible so a injection catheter can be inserted through the cervix for deposition of the frozen-thawed semen. The efficacy of the IUI device was compared to intra-vaginal artificial insemination using semen that had been collected and frozen from pooled sperm-rich fraction of ejaculates collected from two Jindo dog donors. Aliquots of semen were extended with a Tris-egg yolk diluent, centrifuged, the seminal plasma removed, the pellet resuspended with the same diluent, and cooled to 5°C over a 2 h period. A Tris-egg yolk-glycerol extender was added at 5°C; after 1 h, semen was loaded into 0.5 ml straws, and straws were frozen in LN vapor for 5 min, and immersed in LN for storage. The final sperm concentration for freezing was approximately 100×10^6 cells/ml. The straws were thawed at 70°C for precisely 6 sec, 1.5 ml Tris-egg yolk buffer at 38°C added, and the 2 ml of thawed semen was used for a single insemination using the IUI device. Each bitch was inseminated at optimal insemination point, which was estimated by vaginal epithelial cells staining and progesterone concentration analysis. Use of the IUI device resulted in 21 of 26 females giving birth to 89 pups (4.2 ± 1.6 pups per litter), while intra-vaginal AI resulted in 6 of 15 females whelping a total of 17 pups (2.8 ± 1.2 pups per litter). We believe the IUI device is easier to use than previously described devices used for intrauterine insemination. In our experience the expansion of the balloon has a calming effect on the bitch that aids the inseminator. These results indicate that the IUI device was able to provide high fertility with 50 million frozen sperm per insemination and two inseminations. (*Asian-Aust. J. Anim. Sci.* 2003, Vol 16, No. 2 : 180-184)

Key Words : Dog, Frozen Semen, Artificial Insemination, Intrauterine Inseminator

INTRODUCTION

Several factors are critical to obtain a high fertility rate with artificial insemination (AI) of frozen canine spermatozoa, e.g., high initial semen quality, proper timing of the insemination (Farstad and Andersen Berg, 1989; Linde-Forsberg and Forsberg, 1989; Linde-Forsberg and Forsberg, 1993) and intrauterine semen deposition (Fontbonne and Badinand, 1993; Govette et al., 1996). Semen quality after freezing and thawing and the number of spermatozoa required per insemination will, therefore, greatly depend on the quality of ejaculated semen, how well it survives the freezing and thawing process, and techniques used for the freezing-thawing process. The recommended number of dog spermatozoa to be used for each insemination with frozen-thawed semen is 150 to 200×10^6 spermatozoa (Anderson, 1972, 1975). A number of bitches that can be inseminated with one ejaculate ranges between 1 and 5. Timing of insemination is of great importance,

particularly when frozen-thawed semen is used, because cryopreservation generally reduces spermatozoal longevity. Although natural mating may be successful when performed several days before the fertilization period, the shorter duration of sperm survival of frozen-thawed semen requires that AI be made when the oocytes are ready to be fertilized. In the dog, primary oocytes are ovulated 24 to 78 h after the LH surge, and the oocytes require approximately 2 to 3 more days to mature to metaphase II (Mahi-Brown, 1991; Phemister et al., 1973; Wildt et al., 1978). Therefore, AI using frozen-thawed semen should be performed 3 to 7 d after the LH peak. Peripheral progesterone concentrations can be used to time the events related to the LH surge and ovulation in this species (Concannon et al., 1989; Linde-Forsberg, 1991; Tsutsui, 1989).

Although vaginal insemination is easy to perform, lower pregnancy rates are obtained than when intrauterine insemination is used (Fontbonne and Badinand, 1993; Govette et al., 1996; Linde-Forsberg, 2000, 2001). Deposition of semen in the anterior vagina can be performed using a disposable bovine uterine catheter (Linde-Forsberg, 1991), the Norwegian catheter (Farstad, 1984), or the Osiris catheter with a balloon on its tip, which can be inflated to prevent the reflux of semen (Mailot et al., 1985). Intrauterine insemination can be performed

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transcervically using the Norwegian catheter (Anderson, 1975; Farstad, 1984) or with the aid of a fiberoptic endoscope and urethral catheter (Wilson, 1993). Laparotomy or laparoscopy can also be used, although this entails increased levels of stress for the bitch and higher costs for the owner (Anderson, 1972; Silva and Versteegen, 1995). A shortened life span of frozen-thawed spermatozoa is considered to be the main reason for the lower fertility after vaginal deposition. In addition 10 times as many spermatozoa are needed to obtain the same results by vaginal AI as by IU AI (Linde-Forsberg et al., 1999; Tsutsui et al., 1988). This is supported by the need for optimal timing of insemination when using frozen spermatozoa (Linde-Forsberg and Forsberg, 1989).

The aim of this study was to determine if the IUI device would result in a high pregnancy rate when used for AI with 2×50 million frozen sperm.

MATERIALS AND METHODS

All chemicals except those otherwise indicated were purchased from Sigma Chemical Company (St. Louis, MO, USA).

Animals care

This study was conducted in accordance with the Korea legal requirements for animal welfare and experimentation, and the National Institute of Health guide for animal care and experimentation.

Two Jindo dog (2 to 4 years of age, about 25 kg) were used as semen donors. Forty one females of same breed (2 to 6 years of age, about 20-25 kg) in which the previous cycles had been characterized were used for the insemination. The bitches were located at the Department of Animal Science and Technology, Sunchon National University, Korea, and were kept in groups of 2 to 3 in 1.2×1.2 m kennels. The bitches had access to outdoor runs for at least 2 h per day, and were fed WooSung canine maintenance science plan diet in the amount suggested by the manufacturer; water was available *ad libitum*.

Diluents and extenders

Two semen extenders were prepared and frozen as described by Rota et al. (1997) with minor modifications. In brief, extender I (Ext I) contained 2.4 g Tris, 1.4 g citric acid, 0.8 g glucose, 0.06 g Na-benzylpenicillin, 0.1 g streptomycin sulphate, and 20 ml egg yolk diluted to 100 ml with milli-Q water. Extender II (Ext II) contained the same ingredients as Ext I plus 8 ml glycerol and 1 ml Equex STM paste diluted to 100 ml with Milli-Q water.

Semen collection, dilution, freezing and thawing

The sperm-rich fraction of the ejaculate was collected

by digital manipulation into a sterile plastic tube (Linde-Forsberg, 1991). The two semen samples were pooled together to avoid an effect of dog on the outcome of insemination. Pooled semen concentration was measured by a hemocytometer (Thoma, Tokyo, Japan), and the pooled volume, sperm abnormality and motility were assessed. Semen was only used for freezing if it had an initial sperm motility $>70\%$, a sperm concentration of 2 to 4×10^8 sperm/ml, and not more than 15% abnormal and dead spermatozoa.

Aliquots of semen containing 4×10^8 sperm were extended 1:3 with Ext I, centrifuged at $400 \times g$ for 5 min, the seminal plasma removed, the sperm pellet resuspended with 2 ml Ext I, and cooled to 5°C over a 2 h period. Two ml of Ext II was added at 5°C and equilibrated for 1 h before the semen was loaded into 0.5 ml straws, frozen in LN vapor for 5 min, and immersed in LN for storage. Each straw was thawed in a water bath at 70°C for 6 sec, emptied into a tube, and diluted 1:3 with Ext I at 38°C for AI.

Estrus detection

Decisions during estrus to perform AI were based on the reproductive history of the bitches, vaginal cytology and progesterone concentration in peripheral plasma with ICG Status-Pro (Synbiotics, San Diego, CA, USA). All bitches were examined once a day for the presence of vulva swelling and serosanguineous discharge, indicating the onset of proestrus. The estrous cycle was monitored daily by vaginal smears from onset of proestrus until the first day of cytological diestrus as shown Figure 1 (Schutte, 1967). The aim was to perform inseminations within the period during which the bitches were assumed to be most fertile, approximately 2 to 5 d after ovulation, with progesterone concentrations between 30 and 75 nmol/l (Concannon et al.,

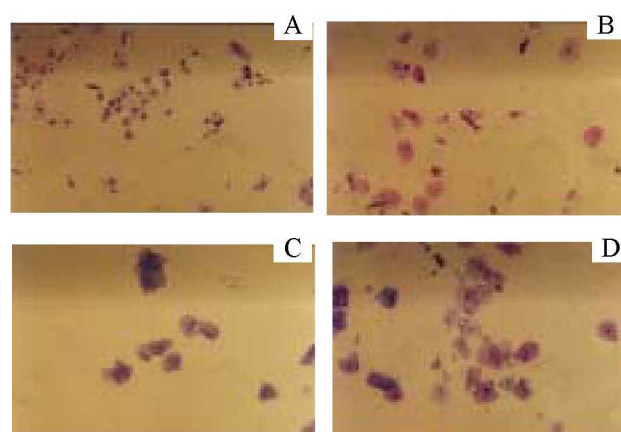


Figure 1. Monitoring of vaginal cytology following the presence of vulva swelling and serodanguineous discharge as Day 1 ($200 \times$). A) Day 1, B) Day 5, C) Day 10 and D) Day 12

1989; Linde-Forsberg, 1991, 1994; Tsutsui, 1989). The optimal insemination periods was decided at showing over 60% cornified cells and analysis of ICG kit.

Development of intrauterine inseminator

The IUI device shown in Figure 3 consists of a vaginal endoscope, a light source, a balloon sheath, an air pump, and an injection catheter. The endoscope is 20.5 cm total length with a 8 mm outside diameter and 6 mm inside diameter. The balloon sheath is 3 cm length before inflation and totally encircles the barrel of the endoscope. The metal injection catheter is 30 cm total length, and it is held in the center of the endoscope barrel by the guard. The endoscope can be used to examine the condition of the vagina and cervix in the days preceding insemination. To facilitate that examination air can be pumped into the vagina to expand the vaginal wall.

For insemination the endoscope is inserted into the vagina and the balloon sheath expanded with air using a 10 ml syringe attached to the air supply connector (Figure 3). The expanded balloon presses against the vaginal wall providing space for the cervix to move caudally against the endoscope so the injection catheter can be easily inserted into the cervical os. At this time, Artificial inseminator using the IUI needs to control of cervix by abdominal palpation. The expansion of the balloon sheath within the vagina aids in pacifying the estrous bitch during insemination.

Insemination

A total of 41 bitches received AI with frozen-thawed dog semen. During the insemination process each estrus bitch was maintained in a retaining cradle with the rear paws elevated 20 to 30 cm above the front paws as shown in Figure 2. For each insemination one straw of 0.5 ml frozen semen was thawed, mixed with 1.5 ml Ext I, and loaded into a 2.5 ml syringe. Thawed semen was deposited in the anterior vagina of 15 bitches using the IUI device without the balloon sheath and penetration of the cervix. Intrauterine inseminations were performed in 26 bitches using the same IUI device. The IUI was inserted into the vagina and the balloon sheath expanded with 6 to 10 ml of air depending on the size of the dog. The caudal part of the dog's abdomen was pushed upward with one hand forcing the cervix to move dorsally to align the cervical os with the endoscope for direct visualization of the cervical os. The injection catheter was passed through the cervix and into the uterine body 2 to 3 cm, the syringe was attached to the catheter and the frozen-thawed semen was deposited into the uterus. The injection catheter was withdrawn after semen injection. All females were maintained with the rump elevated for at least 5 min after insemination. Whelping rate data were analyzed using Chi-square analysis ($p < 0.05$).



Figure 2. A retaining cradle for intrauterine artificial insemination in dog.

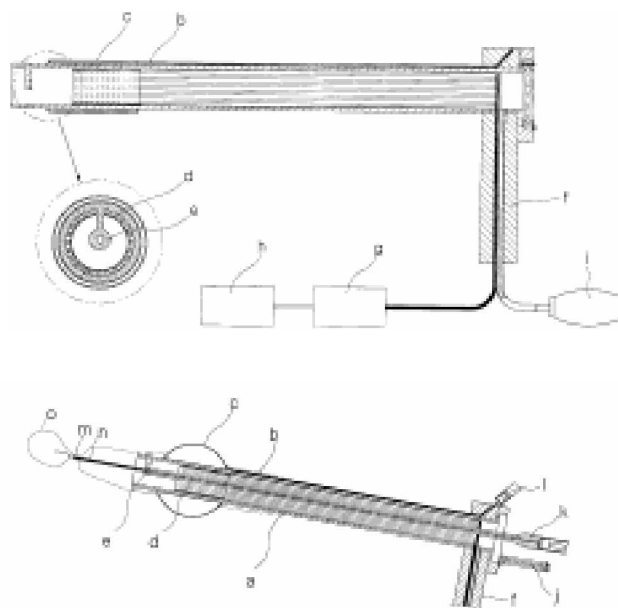


Figure 3. Scheme of canine intrauterine inseminator: a) vagina endoscope, b) balloon sheath, c) balloon, d) light cable, e) guard of injection catheter, f) handle, g) halogen lamp, h) electric cord, i) air pump, j) eye scope, k) injection catheter, l) air supply connector, m) cervix, n) end of injection catheter, and o) uterus.

RESULTS AND DISCUSSION

Data related to production of pups by intra-uterine AI with frozen-thawed semen using the IUI and by intravaginal deposition are shown in Table 1. Each bitch was inseminated twice 48 h apart per estrus with the optimal

Table 1. Whelping rate and litter size for frozen-thawed dog semen inseminated intravaginally or same equipment used

Items	Intravaginal	Intrauterine
No. of bitches inseminated ^a	15	26
No. of bitches whelping (%) [*]	6 (40.0)	21 (86.6)
No. of pups born	17	89
No. of pups per litter (Mean±S.E.)	2.8±1.2	4.2±1.6

^a Total of 50×10^6 spermatozoa used per in intra-uterine and 3 times more concentration (150×10^6 spermatozoa) in intra-vaginal insemination. Bitches were inseminated twice 48 h apart.

^{*} Whelping rate differed significantly ($p < 0.05$).

time for insemination decided by cytology staining and progesterone concentration in plasma as shown Figure 1. The total number of frozen-thawed spermatozoa used per insemination was 50×10^6 for intrauterine insemination and 150×10^6 for intravaginal insemination, respectively. Whelping rates for IUI and vaginal insemination differed significantly ($p < 0.05$). When fifteen bitches were inseminated intravaginally as shown Table 1, six became pregnant and delivered 17 pups (2.8±1.2 pups per litter). In contrast, intrauterine AI using the IUI device resulted in pregnancies in 21 out of 26 bitches and 89 pups were born (4.2±1.6 pups per litter).

The efficacy of the IUI for dog AI was tested using only 50×10^6 frozen-thawed sperm per insemination. Although a limited number of bitches were available for this study, the results would provide the basis for more extensive use by other investigators. In the present study 80.7% of the bitches became pregnant in intrauterine AI using the IUI compared to only 40.0% when frozen-thawed semen was deposited in the anterior vagina using the same device, although 3 times many spermatozoa of frozen semen was used for intravaginal insemination. We believe these results provide strong evidence that the IUI deserves consideration for more extensive use in the field. Previous studies have shown that to obtain similar results by vaginal AI as by IU AI around 10 times as many spermatozoa are needed (Tsutsui et al., 1988; Linde-Forsberg et al., 1999).

The balloon sheath provides the IUI with several attributes in comparison to previously used methods of insemination. Firstly, when the balloon is expanded it enlarges the vagina just caudal to the cervix providing space so that the cervical os can readily be moved caudally and dorsally into alignment with the endoscope. Secondly, the pressure on the vaginal wall provided by the inflated balloon is similar to what the bitch normally experiences during copulation. In our experience the pressure from the balloon has a calming affect on the bitch that assists the person in performing the insemination.

Each of the insemination techniques that have thus far been used in the dog appears to have shortcomings that limit their use in the field. Although the vaginal deposition of semen is simple to perform, when frozen semen was

deposited in vagina both whelping rate and litter size were lower compared with intrauterine insemination (Fontbonne and Badinand, 1993; Linde-Forsberg et al., 1999).

The Norwegian catheter, which consists of an outer nylon sheath and an inner steel catheter and metal injection catheter, has been used for intrauterine insemination of the blue fox (Fougner et al., 1973) and the dog (Anderson, 1972; Fontbonne and Badinand, 1993; Linde-Forsberg et al., 1999). While the Norwegian catheter has been used effectively for many years, it requires considerable training and practice before one becomes skilled in use of the procedure. The metal catheter poses a risk for a injury to the cervix if the dog suddenly moves during the procedure. Wilson (1993) reported use of a fiberoptic endoscope to visualize the cervix during insertion of an urethral catheter into the cervical os. While the urethral catheter eliminates the risks posed by use of the Norwegian catheter, passage of a flexible catheter through the cervix involves manipulation that requires a great deal of practice. Wilson (1993) found that some bitches were easy to catheterize while others were extremely difficult, depending on the length of the vagina, width of the paracervical area, and position of the cervical os. Linde-Forsberg et al. (1999) reported that a fiberoptic endoscope was used to inseminate 19 bitches in which use of the Norwegian catheter had failed.

The major difficulty in performing nonsurgical intrauterine AI is the necessity for the inseminator to use abdominal palpation with one hand to force the cervix caudally and dorsally while the catheter is being manipulated through the cervical os with the other hand (Anderson, 1975; Jedruch et al., 1989; Linde-Forsberg, 1991; Linde-Forsberg, 2001). A significant proportion of bitches resent the palpation, especially if a prolonged period of time is required before the catheter is passed through the cervical os. These obstacles are encountered with the IUI, however, the features provided by the inflated balloon sheath and the injection catheter guard are effective in overcoming these difficulties, although the IUI needed the abdominal palpation to control of cervix.

Intrauterine insemination during laparotomy has been described and is now used in several countries (Tsutsui et al., 1989). This technique is relatively expensive, time consuming, and requires deep surgical anesthesia, which may potentially interfere with uterine mobility and oocyte migration (Jedruch et al., 1989). Laparoscopy has also been used for intrauterine insemination of dogs (Silva and Verstegen, 1995; Silva et al., 1995). The laparoscopy is less invasive in compared with laparotomy, has a lower risk for abdominal infection, and post-surgical recovery is faster.

The optimal insemination time was determined by progesterone concentration analysis with ICG kit following showing over 60% cornified cells the vaginal cytology monitoring as shown Figure 1. In general, the optimal

insemination time accepted 10 to 13 days after the presence of vulva swelling and serosanguineous discharge, indicating the onset of proestrus.

In conclusion, the new IUI provides a technique that is effective and relatively easy to use for nonsurgical intrauterine insemination of the dog, and a relatively high rate of fertility can be achieved with 2×50 million frozen-thawed sperm per AI using the IUI. Use of this device has the potential to provide the means for expanded use of frozen dog semen.

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