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Non-surgical transfer of vitrified porcine embryos using a catheter designed for a proximal site of the uterus

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Abstract

This study aimed to compare the efficiency of non-surgical embryo transfer (ET) using a newly developed catheter, which enables transferring embryos into a proximal site of the uterus (mostly uterine body), and surgical ET of vitrified porcine embryos. In Experiment 1, the catheter was inserted into 12 gilts, with each half of the group allocated to skilled or novice operators. The time required for insertion into the uterus did not differ between skilled and novice operators (4 min 9 s and 4 min 6 s, respectively). In Experiment 2, 12 gilts were used as recipients for non-surgical and surgical ET with vitrified embryos ($n = 6$, each). There was no significant difference in the rate of piglet production based on the number of transferred embryos between surgical and non-surgical ET (25.8% vs. 15.4%, $p = .098$). The results suggest that non-surgical ET catheter allowed for easy insertion and transfer of embryos without special training. Although the catheter is effective for deposition of embryos into the proximal site of uterus, the efficiency of piglet production is not enhanced compared with surgical ET. The ET method using this catheter, being labor-saving and less-invasive, may contribute to the improvement of ET in pigs.

KEYWORDS

catheter, embryo transfer, non-surgical, pig, vitrification

1 | INTRODUCTION

Embryo transfer (ET) is a useful technique in the swine industry. Compared with the introduction of living pigs into the farms, ET has several advantages, such as low cost for transportation and a minimal risk of disease transmission, which is associated with transportation during introduction of live animals. ET in pigs has usually been performed surgically because of their inherently

complex reproductive tracts. However, non-surgical ET techniques that do not require any special apparatuses, facilities and surgical operation techniques are now paid more attention to, due to their practicality compared to surgical ET. Recent studies obtained piglets by non-surgical deep intrauterine ET (in other words, ET into the distal site of the uterine horn) using special instruments (Martinez et al., 2004; Nakazawa et al., 2008; Yoshioka, Noguchi, & Suzuki, 2012). However, it requires very delicate manipulations to avoid intrauterine infection. Additionally, the instruments developed in these previous studies were difficult to handle due to the long length of the inner tube or injector. These problems need to be resolved in order to implement non-surgical ET especially if performed on many farms.

The cryopreservation of porcine embryos improves the convenience of ET and enables long-term preservation of valuable genetic material. Thus, it can be expected that ET technology will dramatically improve by combining the cryopreservation of embryos and non-surgical ET technology. Some studies were able to achieve acceptable farrowing rates with in vivo derived vitrified and warmed porcine embryos by surgical ET (Beebe, Cameron, Blackshaw, & Keates, 2005; Berthelot, Martinat-Botte, Locatelli, Perreau, & Terqui, 2000; Cameron, Beebe, Blackshaw, & Keates, 2004; Fujino et al., 2008). In addition, vitrification methods have been developed with the "closed system" (Beebe, Bouwman, Mcllfatrick, & Nottle, 2011; Men et al., 2011; Misumi et al., 2013) to prevent microbial contamination from liquid nitrogen. Piglets were also successfully produced from the surgical ET of a few embryos (8 to 15 embryos) by the micro volume air cooling (MVAC) method (Misumi et al., 2013). More numbers (as many as 40 of embryos) are required for successful non-surgical ET (Martinez et al., 2015). If fewer than 15 embryos were collected from one donor and adapted for non-surgical ET resulting in a successful production of piglets, it would contribute enormously to the swine industry. For this purpose, a catheter that enables solid pregnancy and piglet production are being demanded.

Recently, Misumi, Egawa, Misawa, and Hirayama (2020) achieved a 60% farrowing rate in MVAC-vitrified embryos by non-surgical ET into the uterine body with a stainless ET catheter that was originally designed for cattle (mo-No.4; Misawa Medical Industry, Ibaraki, Japan). This result indicates that the deposition of the embryos in the uterine body may yield good results even with the vitrified and warmed embryos. However, using this modified catheter required a skilled technique to avoid perforating the genitals of the recipients when inserting the inelastic stainless catheter. Thus, a safe and effective catheter is required.

In this study, we developed a new flexible non-surgical ET catheter consisting of an injector attached to an outer guide to allow easy deposition of porcine embryos in the proximal site of the uterus (uterine body and uterine bifurcation area). The present study aimed was to evaluate the efficiency of non-surgical ET by MVAC-vitrified method and warmed porcine embryos using the developed non-surgical ET catheter, and to compare the efficiency of this newly developed catheter with that of commonly used surgical ET.

2 | MATERIALS AND METHODS

2.1 | Animals

All animals used in the present study and experimental procedures were approved by the Animal Care and Use Committee of the National Livestock Breeding Center (NLBC) (protocol no. 29-10) and the Animal Care and Use Committee of the Saga Prefectural Livestock Experiment Station.

All gilts were observed daily to find signs of estrus such as standing to be mounted, routing, congestion, swollen vulva, and clear mucus streaming from the vulva.

2.2 | Donors and embryo recovery

Sixteen Duroc (15 gilts and 1 sow) and 6 Large White (3 gilts and 3 sows) donor pigs (8 to 50 months old) were used for the collection of embryos. To adjust the schedule for the implementation of efficient embryo collection, synchronization of ovulation was induced in donors using one of the four methods (Figure 1a–d); those were used for producing vitrified embryos with high pregnancy rates in the previous studies (Misumi et al., 2020; Misumi et al., unpublished

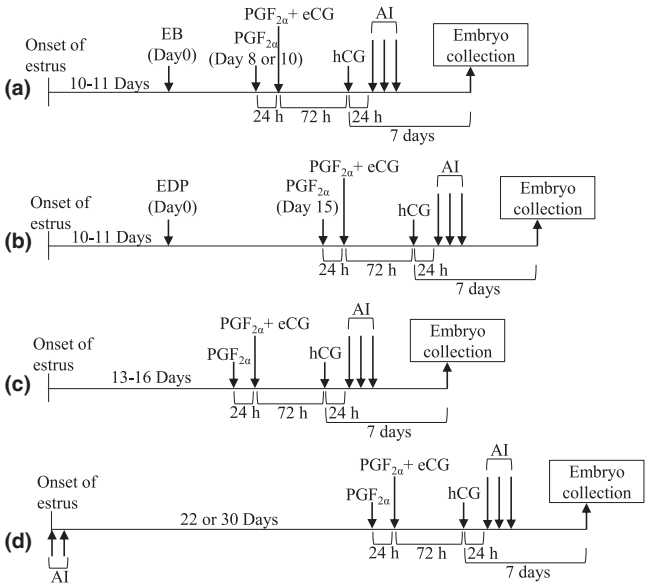

FIGURE 1 Summary of four methods of ovulation synchronization of the donors. AI, artificial insemination; EB, estradiol benzoate; eCG, equine chorionic gonadotropin; EDP, estradiol dipropionate; hCG, human chorionic gonadotropin; $PGF_{2\alpha}$, prostaglandin $F_{2\alpha}$. In methods (a) and (b), pigs were administered 20 mg EB or EDP, respectively. In method (c), donors were used their natural estrous. In method (d), pigs were artificially inseminated daily from onset of estrus to end. All pigs were administered $PGF_{2\alpha}$ (0.276 mg cloprostenol) twice, 1,000 or 1,500 IU eCG, and 500 or 750 IU hCG. They were artificially inseminated three times: at 24 hr, 41 hr, and 48 hr after the hCG treatment

data). All donors received prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) as 0.276 mg cloprostenol (Planate, MSD Animal Health, Tokyo, Japan) twice at 24 hr intervals, beginning at 11 days before the embryo collection. The gilts were given 1,000 or 1,500 IU of equine chorionic gonadotropin (eCG) (Serotropin; ASKA Animal Health, Tokyo, Japan) with the same dose of the second $PGF_{2\alpha}$ administration and 500 or 750 IU of human chorionic gonadotropin (hCG) (Gonatropin 3,000IU; ASKA Animal Health) at 72 hr after the eCG treatment. They were artificially inseminated (AI) 24, 41, and 48 hr after the hCG treatment with semen from boars of the same breed (5.6×10^9 spermatozoa in 80 ml) that had been diluted with HIROSWINE B (Hiroshima Cryopreservation Service Co, Hiroshima, Japan). Embryos were collected surgically ($n = 20$) or after slaughter ($n = 2$) 7 days after the hCG administration.

Donors for surgical collection of embryos underwent mid-ventral laparotomy under general anesthesia. They were sedated with 1 mg of medetomidine hydrochloride (Domitor; Nippon Zenyaku Kogyo Co., Ltd. Fukushima, Japan) and 10 mg of midazolam (Dormicum Injection 10mg; Astellas Pharma Inc, Tokyo, Japan) intramuscularly, followed by administration of 500 mg thiamylal sodium (Isozol for Injection 0.5 g; Nichi-Iko Pharmaceutical Co., Ltd. Toyama, Japan) intravenously for inducing anesthesia, and the condition was maintained by inhalation of 3%–4% isoflurane (Isoflurane Inhalation Solution; Pfizer, Tokyo, Japan). During surgical embryo collection, the female reproductive tracts and ovaries were exteriorized and both uterine horns flushed with M2 solution (Quinn, Barros, & Whittingham, 1982) for collecting the embryos. Two donors were slaughtered according to the regulations, their reproductive tracts removed immediately, and their uteri flushed in the same manner as that done for surgical collection.

Embryos were evaluated for quality and their developmental stage identified morphologically based on the criteria categorized by the International Embryo Technology Society (Wright, 2009). One hundred and eighty embryos (Duroc: $n = 126$, Large White: $n = 54$) with intact zona pellucida and developed until the early blastocyst, blastocyst or expanded blastocyst stage, were used for vitrification. Embryos at the morula stage were allowed to reach the blastocyst stage after incubation for a maximum of 6 hr at 38.5°C under 5% CO_2 and 5% O_2.

Blastocysts were vitrified and warmed using the MVAC method as described by Misumi et al. (2013). All media for the vitrification and warming process described below were from a porcine embryo vitrification kit (PEV-SK; Research Institute for the Functional Peptides, Yamagata, Japan). Two types of equilibration media were used: primary porcine equilibration solution (PES-1) and secondary porcine equilibration solution (PES-2). Porcine vitrification solution (PVS) was used as vitrification media. Porcine warming and dilution solution (PWDS) were used for warming and dilution. All media were kept at 45°C in a water bath until use. The manipulation during vitrification and warming procedure under a stereo microscope was performed on a 38°C warm plate. A vertically folded, thin, stainless steel stick was used as the device for vitrification (Embryo-stick; Misawa Medical Industry, Ibaraki, Japan). The 0.25 ml straw sealed with a cap was placed vertically in liquid nitrogen (LN_2) for precooling. Embryos were equilibrated with PES-1 for 5 min, followed by PES-2 for 5 min, and were then transferred into the PVS. Approximately 1 μl PVS containing the embryos was placed on the tip of the vitrification device and inserted into the precooled straw immediately. The duration from the transfer of embryos to PVS to insertion in the straw was within 1 min. The straw was immersed in LN_2 and preserved until warming for ET.

For the embryo warming procedure, the device-attached embryos were pulled out from the 0.25 ml straw and the device tip with embryos immediately immersed into a 35-mm dish plate containing approximately 4 ml PWDS to liberate the embryos from the device. Embryos were warmed for 3 min in the PWDS and then placed in the 20 mM HEPES-buffered PBM for ET (Mito et al., 2012).

2.3 | Distinctive features of the developed catheter for non-surgical ET

The disposable catheter we designed for non-surgical ET into a proximal site of the uterus is shown in Figure 2 ("Kurenai-3" Prototype; Misawa Medical Industry). The catheter consists of an outer catheter (defined as "guide" in the present study) and a slide-able inner tube (also as "injector") housed within the guide. It is flexible, made of plastic, silicone rubber, and metal springs; and consists of a soft inner injector. The guide is 61.5 cm long, and the outer diameter of the coiled region at the tip is 8 mm. The outer diameter of the injector is 2 mm, and the inner diameter is 1.2 mm.

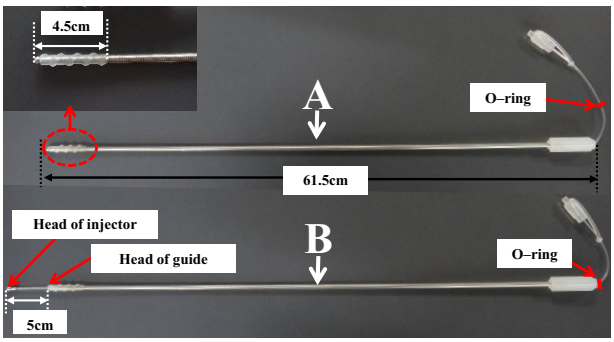

FIGURE 2 Catheter developed in the study for transferring into proximal site of uterus of female pigs ("Kurenai-3" Prototype; Misawa Medical Industry, Ibaraki, Japan). The catheter was flexible and consisted of a soft injector installed inside an outer catheter. The length of the catheter was 61.5 cm and the outer diameter of coiled tip was 8 mm. The outer diameter of the injector was 2 mm and the working canal was 1.2 mm. "A" in the figure indicates a catheter at the time of insertion through the cervix, and "B" indicates a catheter at the time of the transfer of embryos. At the time of the transfer, the injector is projected 5 cm into the uterus from the tip of the outer catheter. The protruding length is adjusted by a guide attached to the injector on the side of the handle

2.4 | Experimental design

2.4.1 | Experiment 1: insertion of the catheter

Nine Duroc and 3 Large White cyclic gilts (9 to 16 months of age) were used to investigate the insertion of the catheter. Synchronization of ovulation was induced by the same methods for donors as described earlier (Figure 1a and c). The gilts 7 days after hCG administration were moved into a small cage and carried to the room maintained at approximately 25°C. They were administered 10 mg midazolam intramuscularly for sedation in the standing position (Nakamura et al., unpublished data). The perineal area of the gilts was washed and cleaned thoroughly. When they showed signs of sedation, such as shutting eyes or moony behavior, catheter insertion was initiated through the vagina using a vaginal speculum and propelled into the cervix. The injector was then pushed into the proximal site of the uterus without resistance. The time from the insertion of the catheter into the vaginal speculum to insertion of the injector was recorded. To evaluate the simplicity of this catheter, insertion was done by a skilled operator and 6 novice operators, with each group using 6 gilts that were randomly allocated. In this study, the skilled operator was defined as a person who has experiences of more than 20 cases of inserting any type of commercially available non-surgical ET catheter, and the novice operators were defined as those with less than 2 cases of inserting experience, but were skilled in artificial insemination. When the injector was inserted completely to the position of the O-ring that was attached to limit the insertion depth of the injector, the catheter was fixed to the tail of the gilts with adhesive tape to prevent deviation of the catheter original position. The gilts were administered anesthetics in the same way as described for embryo collection, except for midazolam by mid-ventral laparotomy. Their reproductive tracts were exteriorized while sedated, then the position of the tip of the guide and injector in the uterus was determined visually and by palpation. Furthermore, after the catheter was removed, the distance from the tip of the guide to its exposed section outside the pubic area was measured to investigate the depth of insertion.

2.4.2 | Experiment 2: ET

Eight Duroc, three Large White, and one crossbred (Duroc × Large White) cyclic gilts (8 to 14 month of age) were used as recipients for comparison of non-surgical and surgical ET using vitrified and warmed embryos. Synchronization of ovulation was induced with the method (Figure 1d) after small modification; briefly, recipients were administrated the first $PGF_{2\alpha}$ between 21 and 34 days after AI, and 750 IU eCG with the second $PGF_{2\alpha}$ administration, and 500 IU hCG 72 hr after eCG administration. The AI services were not conducted after hCG administration. The two methods for ET were performed 6 days after hCG administration.

In non-surgical ET, recipient preparation and catheter insertion were carried out in the same manner as described in Experiment 1. The time from the start of the catheter insertion to the completion of the injector insertion was measured. After the injector was inserted completely, the warming of vitrified embryos was initiated. These procedures were conducted by the skilled operator. After warming, a group of 13 to 16 embryos was loaded into a 0.25-ml straw. The composition in the straw was as follows: 20 μl of transfer medium, an air bubble, 30 μl of transfer medium containing embryos, an air bubble, and finally 20 μl of transfer medium. A 5-ml syringe containing 2 ml transfer medium was attached to the injector through sterilizing filter, and 1 ml was used to fill the injector with the medium. The 0.25-ml straw containing the embryos was then connected to the injector, and the 5 ml syringe containing the remaining 1 ml medium was connected to the top of the straw. The medium containing the embryos were then introduced into the uterus through the injector. Finally, 1 ml of air in another 5 ml syringe attached to a sterilizing filter was pushed out into the injector to introduce all the contents that remained in the catheter into the uterus. The catheter was then removed from the recipient. The distance from the tip of the guide to its exposed section outside the pubic area was then measured.

In surgical ET, mid-ventral laparotomy was performed on recipients under general anesthesia the same as that done for the donors. The recipients' reproductive tracts and ovaries were exteriorized, and 12–16 embryos that were vitrified/warmed were loaded into a polypropylene catheter (PP catheter for cats with tip-hole type; Fujihira Industry Co., Ltd. Tokyo, Japan) with transfer medium. The embryos were deposited into the one uterine horn (approximately 5 cm to the uterotubal junction) through an incision that was made with ophthalmic scissors in the uterine wall.

Pregnancy was diagnosed by transcutaneous ultrasonography (HS-101V; Honda Electronics CO, Aichi, Japan) at 20 days after ET. All pregnant recipients were allowed to carry litters to term, and the farrowing rates and litter sizes were recorded.

2.5 | Statistical analysis

The percentage data were compared using Fisher's exact test. All other data were tested using Student's *t*-test. All statistical analyses were performed using Easy R (Saitama Medical Center, Jichi Medical University; http//www.jichi.ac.jp/saitama-sct/SaitamaHP.files/statmedEN.html, Saitama, Japan) (Kanda, 2013). A value of $p < .05$ was considered to be significant.

3 | RESULTS

3.1 | Experiment 1

The results of the insertion of the catheter for non-surgical ET into a proximal site of the uterus by skilled and novice operators are

TABLE 1 Results of the insertion of the catheter for non-surgical embryo transfer by skilled and novice operators

Operator	Pigs	Age (month)	Times required to insert (min:sec)	Depth of catheter (cm)	Successful insertion of head of guide in the correct position (uterine body)	Successful insertion of head of injector in the correct position (uterine body)
Skilled	A	14	5:58	44.5	o	o
	B	11	4:13	39.5	o	o
	C	12	3:45	40.0	o	(uterine horn bifurcation)
	D	10	2:08	37.0	o	o
	E	9	4:43	44.0	o	o
	F	10	15:45	48.0	cervix[b]	-
	Average	11 ± 0.7[a]	4:09 ± 0:38[a]	41.0 ± 1.4[a]	83.3%[c]	66.7%[c]
Novice	G	9	2:39	36.5	o	o
	H	12	9:25	52.0	(uterine horn)	(uterine horn)
	I	16	3:24	41.5	o	o
	J	14	2:40	39.0	o	(uterine horn bifurcation)
	K	12	2:55	42.5	o	o
	L	13	3:35	52.5	(uterine horn bifurcation)	(uterine horn)
	aveRage	12.6 ± 1.0[a]	4:06 ± 1:04[a]	44.0 ± 2.7[a]	66.7%[c]	50.0%[c]

[a]Mean ± *SEM*.

[b]The insertion of the catheter was impossible because the cervix was tightly closed.

[c]Proportion of head of guide or injector in expected position (uterine body).

shown in Table 1. The times required for insertion and the depths of insertion were not different between both operators. It was possible to insert the guide and injector through the cervix of almost all (11 of the 12) gilts, whereas one gilt had a tightly closed cervical canal. The tip of the outer catheter was observed in the uterine body (Figure 3)—the preferred location for access—in 83.3% and 66.7% in the skilled group and the novice group, respectively. However, there were two novice cases wherein the tip of the guide was not located in the uterine body, but instead reached the uterine horn of the position that was close to the uterine body. Furthermore, in the skilled group, there was only one out of the five cases wherein the catheter passed through the cervix, but insertion of the injector into the uterine body was unsuccessful, instead, the insertion was at the uterine horn bifurcation. Conversely, among the novices, three out of six cases were not inserted in the uterine body, two reached the uterine horn and one reached the uterine horn bifurcation.

3.2 | Experiment 2

As shown in Table 2, no significant differences were observed in the number of piglets and live born piglets between the surgical and non-surgical ET groups. In the non-surgical ET, pregnancy was achieved in five out of six recipients, and four of them farrowed a total of 14 live piglets (3.5 ± 1.3 per recipient), and there was one stillbirth. In the surgical ET, four of six gilts became pregnant, and all of them farrowed a total of 23 live piglets (5.8 ± 0.6 per recipient), with two stillbirths. All live-born piglets were healthy and had no morphological or physiological abnormalities. The efficiency of piglet production using surgical and non-surgical ET, as measured by the ratio of the number of live-born piglets to the number of embryos transferred to all recipients, was 25.8 and 15.4%, respectively ($p = .098$).

Table 3 presents the results of the transfer of vitrified/warmed embryos to six gilts using the catheter for non-surgical ET. The time taken to complete the insertion of the catheter ranged from 2:29 to 6:40 (4:17 ± 0.35 in average) (min:sec). The mean length of the outer catheter inserted into recipients was 45.3 ± 1.6 cm. One recipient that became pregnant but did not farrow resulting in an abortion at 34 days after ET. Another non-pregnant recipient exhibited a purulent discharge from the perineum several days after ET.

4 | DISCUSSION

In this study, we evaluated the efficiency of a newly developed non-surgical ET catheter for the transfer of embryos into the proximal site of the uterus in gilts. Non-surgical ET in pigs has yielded good reproductive performance by deep transfer into the uterus (Angel et al., 2014; Martinez et al., 2004, 2014, 2015). The catheter used in these methods consisted of an outer guide catheter and an inner, flexible, long (1.2–1.58 m) injector separated from the outer catheter. When ET was performed using this catheter, the outer guide catheter was first inserted into the genitals of female pigs, and then the injector was inserted deeply through the outer guide catheter. However, the insertion of the injector required very delicate manipulations to avoid possible intrauterine infection since the injector was separated from the outer catheter. The handling of the injector was also difficult because of its length. Thus, we developed a

catheter that integrates the outer catheter and injector to prevent intrauterine contamination and allows for easy transfer of the embryo into the proximal site of the uterus. We investigated the usability of the catheter in Experiment 1. Furthermore, the reproductive performance of non-surgical ET using this catheter with MVAC-vitrified and warmed embryos were investigated and compared to that of surgical ET commonly used in pigs, in Experiment 2.

In Experiment 1, the average time required for insertion was less than 5 min, and was not significantly different between skilled and novice operators. This suggests that no special training is required to insert this catheter through the cervical canal of recipient gilts to reach the uterus. On the other hand, novice operators appeared to insert catheters more deeply than experienced operators, although the difference was not statistically significant. Since the catheter was easy to insert, the feeling of its passage through the cervical canal was not easily sensed by the operator. This may be the reason why novice operators tended to insert the catheter so deeply. Since the coiled tip of the guide has a shape suitable for passing through the cervix, it exhibits a certain degree of hardness and thickness for its advancement into the uterus, unlike an injector. Therefore, there is a possibility to damage the uterus when the guide is inserted too deeply into the uterus. The described effects of operator experience and the resulting depth of insertion on transfer outcomes are issues for further study.

Non-surgical ET in pigs has evolved as a transfer to a uterine body since the first report by Polge and Day (1968). However, when Martinez et al. (2004) developed a method for non-surgical ET into the deep uterus and reported high farrowing rates, the standard for non-surgical ET in pigs shifted to ET into deep position of the uterus. In previous studies, non-surgical ET to the uterine body using fresh embryos had a farrowing rate of 9.0%–64.0% (Galvin, Killian, & Stewart, 1994; Hazeleger & Kemp, 1994; Li, Rieke, Day, & Prather, 1996; Reichenbach, Modl, & Brem, 1993; Yonemura, Fijino, Irie, & Miura, 1996), whereas those of deep-uterine ET were

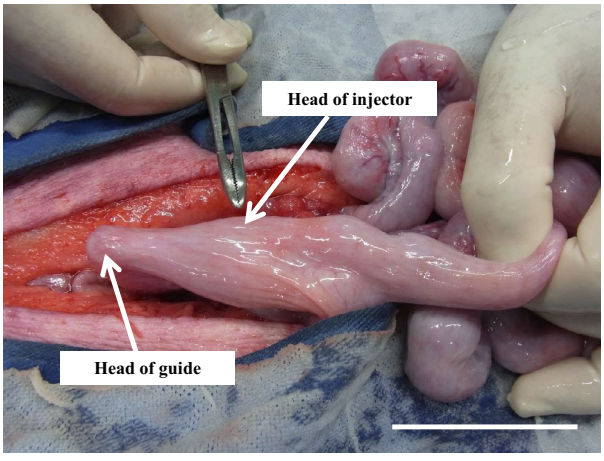

FIGURE 3 Position of the inserted catheter in the uterus of a gilt. The gilts in which the catheter was inserted was immediately anesthetized, and their reproductive tracts was exteriorized by mid-ventral laparotomy, and the insertion status of the catheter was confirmed. (Bar = 5 cm)

TABLE 2 Results of surgical embryo transfer and nonsurgical embryo transfer with MVAC-vitrified and warmed embryos

Experimental group	No. of recipients	No. of embryos transferred[a]	No. of pregnant	No. of farrowed	No. of live (stillbirth) piglets	No. of total piglets (per recipient)[a]	No. of live born piglets (per recipient)[a]	The efficiency of piglet production (%)[b]
Surgical	6	14.8 ± 0.7	4	4	23 (2)	6.3 ± 0.9	5.8 ± 0.6	25.8
Non-surgical	6	15.2 ± 0.5	5	4	14 (1)	3.8 ± 1.4	3.5 ± 1.3	15.4

Note: Non-surgical, nonsurgical embryo transfer; Surgical, surgical embryo transfer.
[a]Mean ± *SEM*.
[b]The ratio of the number of live born piglets to the number of embryos transferred to all recipients.

TABLE 3 Details of data of surgical and nonsurgical embryo transfer

Recipients	Experimental group	Total number of transferred embryos	Pregnancy	Number of live born piglets		Number of Stillbirth or miscarriage
				Male	Female	
M	Surgical	12	+	2	4	1
N		16	+	4	3	1
O		15	+	2	2	–
P		16	–	–	–	–
Q		16	+	3	3	–
R		14	–	–	–	–
Average		14.8 ± 0.7	66.7%	2.8 ± 0.5	3.0 ± 0.4	1.0
S	Nonsurgical	15	+	5	2	–
T		16	+	1	1	–
U		13	+[a]	–	–	–
V		16	+	2	2	1
W		15	–[b]	–	–	–
X		16	+	0	1	–
Average		15.2 ± 0.5	83.3%	2.0 ± 1.1	1.5 ± 0.3	

[a]The recipient that became pregnant but not farrowed had an abortion at 34 days after ET.
[b]The non-pregnant recipient exhibited a discharge from the perineum after ET.

70.8%–92.0% (Angel et al., 2014; Martinez et al., 2004, 2014), suggesting that the proper intrauterine position for transfer appeared to be the deep position of the uterus rather than the uterine body. In support of this, Wallenhorst and Holtz (1999) reported that the surgical transfer of embryos into the uterine body resulted in a significantly lower pregnancy rate than that of transfer into the uterine horn. Conversely, Misumi et al. (2020) reported that non-surgical transfer of vitrified/warmed embryos into the uterine body using a transferring device modified from the catheter for cattle, obtained a farrowing rate of 60% and piglet production rate of 17.9% equivalent to deep uterine transfer. This result was in contrast to previous studies reporting that deep uterus was more suitable for ET compared with the uterine body. In this study, the non-surgical transfer of vitrified/warmed embryos into the proximal site of the uterus resulted in a farrowing rate of 66.7% and a piglet production rate of 15.4%. In previous studies, non-surgical deep uterine transfer of vitrified/warmed embryos was reported with a farrowing rate of 42.9%–72.7% and a piglet production rate of 11.0%–17.3% (Cuello et al., 2005; Gomis et al., 2012; Martinez et al., 2015). Our results are similar to those by Misumi et al. (2020) which were equivalent to these non-surgical deep uterine ET. These results showed that non-surgical ET to the proximal site of the uterus may not necessarily be inferior to deep uterus ET in terms of piglet production. The possible explanation for these results may be due to the catheter being propelled by only a small distance into the uterus, and/or how the injector of the catheter was made of a very flexible (soft) material so that the uterus was less likely to be stimulated and/or damaged.

In addition, since the superiority of transfer into the deep uterus is that Wallenhorst and Holtz (1999) reported was the case of using a surgical method and that Misumi et al. (2020) also reported that no pregnant recipient was obtained by surgical ET into uterine body, it was considered that the suitable intrauterine sites for ET were not the same for surgical and non-surgical ET. The reason for this discrepancy may be the position of the recipients at the time of transfer, such as supine position or standing, or the presence or absence of general anesthesia.

In this study, we compared the results of surgical and non-surgical ET performed using the newly developed catheter. Although no significant difference was seen, piglet production efficiency was 25.8% in surgical ET, which tended to be higher than in non-surgical ET (15.4%, $p = .098$). Similar trends were seen in previous studies: in the case of surgical ET, about 20–35 vitrified/warmed embryos had been transferred per recipient in order to obtain a farrowing rate of 70.0%–83.0% and a piglet production efficiency of 17.2%–20.2% (Beebe et al., 2005; Cameron et al., 2004; Fujino et al., 2008; Misumi et al., 2013). In non-surgical ET, on the other hand, farrowing and piglet production rates are reported to be 42.9%–72.7% and 11.0%–17.3%, respectively (Cuello et al., 2005; Gomis et al., 2012; Martinez et al., 2015). Thus, surgical ET seemed to be superior to non-surgical ET.

Notably, studies comparing the reproductive performance of deep uterine, non-surgical ET of vitrified/warmed embryos showed that both farrowing and piglet production efficiency in the group that transferred 40 embryos were higher than the group that transferred only 30 embryos (38.9% vs. 72.7% and 7.1% vs. 17.3%, respectively) (Martinez et al., 2015). From these reports, in order to obtain good results when performing deep uterine non-surgical ET using vitrified/warmed embryos, many embryos are required per recipient. However, we obtained good reproductive performance with as few as 13–16 embryos per recipient. This result was similar to that from the study of Misumi et al. (2020) that non-surgically transferred 15–16 vitrified/warmed embryos per recipient into the uterine body or bifurcation and obtained a piglet production rate of 17.9%–20.5%. Therefore, in non-surgical ET, it may be

very important to avoid damaging the uterus rather than focusing on the position where the embryos are deposited in the uterus. In addition, the introduction of breeding pigs into a farm or the regeneration of genetic resources must comply with the registration system even for the piglets derived from the transferred embryos in Japan. Theoretically embryos from one single donor should be treated as single embryo transfer. However, since it is not always possible to collect many embryos from a single donor (Hirayama, Yoshioka, Noguchi, & Misumi, 2019; Ratky, Brussow, Solti, Torner, & Sarlos, 2000), embryos from multiple pigs may be mixed and used as a group of embryos attaining the required large number to be transferred into one recipient. In such a case, extra labor and costs becomes necessary for the genetic testing required to clarify the pedigree of the offspring. Since the method proposed in this study requires only a small number of embryos for transfer into one recipient, it may have an advantage in these situations. However, since the number of gilts used in this study was as small as 6 in each experimental group, it is necessary to increase the number of cases in the future for solid outputs.

In conclusion, our newly developed non-surgical ET catheter allowed for easy transfer of embryos without requiring special training of operators, and yielded a high reproductive outcome in transferring MVAC-vitrified embryos. The methods described in this report, combining long-term storage of embryos and labor-saving transfer methods, may contribute to the improvement of ET in the pig industry.

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CONFLICT OF INTEREST

All authors declare no conflict of interests.

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