—Original Article—

An Earlier Uterine Environment Favors the *In Vivo* Development of Fresh Pig Morulae and Blastocysts Transferred by a Nonsurgical Deep-uterine Method

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Abstract. This study aimed to evaluate the effect of recipient-donor estrous cycle synchrony on recipient reproductive performance after nonsurgical deep-uterine (NsDU) embryo transfer (ET). The transfers (N=132) were conducted in recipients sows that started estrus 24 h before (–24 h; N=9) or 0 h (synchronous; N=31), 24 h (+24 h; N=74) or 48 h (+48 h; N=18) after the donors. A total of 30 day 5 morulae or day 6 blastocysts (day 0=onset of estrus) were transferred per recipient. The highest farrowing rates (FRs) were achieved when estrus appeared in recipients 24 h later than that in the donors (81.1%), regardless of the embryonic stage used for the transfers. The FR notably decreased (P<0.05) when recipients were –24 h asynchronous (0%), synchronous (61.3%) or +48 h asynchronous (50%) relative to the donors. No differences in litter size (LS) and piglet birth weights were observed among the synchronous and +24 h or +48 h asynchronous groups. While a +24 h asynchronous recipient was suitable for transfers performed with either morulae (FR, 74.3%; LS, 9.2 ± 0.6 piglets) or blastocysts (FR, 84.6%; LS, 9.8 ± 0.6 piglets), a + 48 h asynchronous recipient was adequate for blastocysts (FR, 87.5%; LS, 10.4 ± 0.7 piglets) but not for morulae (FR, 30.0%; LS, 7.3 ± 2.3 piglets). In conclusion, this study confirms the effectiveness of the NsDU-ET technology and shows that porcine embryos tolerate better a less advanced uterine environment if they are nonsurgically transferred deep into the uterine horn.
Key words: Blastocysts, Estrous asynchrony, Morulae, Nonsurgical embryo transfer, Porcine

The success of any embryo transfer (ET) program is significantly influenced by the quality of the embryos, the recipients and/or the interaction of both factors [1]. Therefore, the degree of synchrony of the estrous cycle (or endometrial status) between the recipients and donors is crucial. Despite this fact, previous studies have yielded variable results, including variation among the species. Pregnancy rates were not compromised when synchrony between the recipient and donor was within 48 h, 24 h and 12 h in the sheep [2–4], cow [1, 5, 6] and buffalo [7], respectively. In commercial equine ET programs, it is generally accepted that optimum pregnancy rates are achieved in recipients that have ovulated within a range of 24 h before to 72 h after the donors (reviewed by Stout [8]). Transfers performed outside these ranges invariably decrease the pregnancy rate and increase embryonic loss (ovine [2, 3], bovine [9] and equine [10]).

In pigs, high pregnancy rates (over 70%) were achieved when

surgical transfers were made to recipients in which the onset of estrus was either synchronous relative to that of the donors or 24 h or 48 h later; in contrast, pregnancy rates dramatically decreased in recipients that were ahead of the donors by the same intervals [11]. Similarly, other studies that also used surgical transfer indicated certain advantages when transfers were performed on recipients that were in estrus 24 h after the donors [12, 13]. In contrast, studies involving nonsurgical ET into the uterine body have shown that transfers into recipients ovulating 18 to 36 h after the donors resulted in reduced pregnancy rates compared with those performed on recipients that had ovulated within the range of 24 h before to 12 h after the donors [14]. In addition, using nonsurgical ET and blastocysts produced *in vitro*, Yoshioka *et al*. [15] found that the pregnancy and farrowing rates did not differ in recipients in which the estrous cycle was delayed 24 to 72 h compared with that of donors, although the efficiency of piglet production was greater when the asynchrony was 24 h. Differences in the transfer procedures used (surgical and nonsurgical), the source of embryos (*in vivo* and *in vitro*), the small sample sizes used and the scarce number of studies performed limit comparison of these studies with contemporary application of newer methods, such as nonsurgical deep uterine (NsDU) ET. This technique was developed from the successful deep-uterine insemination procedure in sows [16–18], which demonstrated that it was possible to nonsurgically

insert a catheter through the cervix deep into a uterine horn, a concept considered impossible at the time. The insemination technique was thereafter adapted for ET, leading to the creation of a novel and unique procedure for nonsurgical insertion of a catheter deep into a uterine horn of gilts and sows during metaestrus [19, 20]. The NsDU-ET procedure is simple, safe, rapid and well tolerated by the recipients (reviewed by Martinez *et al*. [21, 22]). The excellent reproductive performance of the recipients after NsDU transfer of fresh embryos [19, 23] and the promising results obtained using cryopreserved embryos [20, 21] represent a fundamental advance to widespread commercial use of ET by the pig industry. However, as with the development of any new technology, it is necessary to reevaluate specific factors that can affect the success rate of ET, since they were based on the use of surgical transfer or nonsurgical transfer into the uterine body.

Therefore, the objective of this study was to determine the effect of several specific degrees of recipient-donor asynchrony on the reproductive performance of recipients following NsDU-ETs of morulae or blastocysts.

Materials and Methods

All experimental procedures used in this study were performed in accordance with the 2010/63/EU EEC Directive for animal experiments and were reviewed and approved by the Ethical Committee for Experimentation with Animals of the University of Murcia, Spain.

Animals

This work was conducted in a pig genetics company (Selección Batallé S.A., Girona, Spain). Purebred Duroc sows (2–6 parities) were selected at weaning and used as donors and recipients. Females were allocated individually to crates in a mechanically ventilated confinement facility under field conditions. They were fed a commercial ration twice per day, and water was provided *ad libitum*.

Superovulation and detection of estrus

Weaning was used to synchronize estrus between donors and recipients. To standardize the ET–schedule, only sows with a weaning-to-estrus interval of 3 to 4 days were selected as donors and recipients. Superovulation of donors was induced by intramuscular administration of 1,000 IU equine chorionic gonadotropin (eCG; Folligon, Intervet, Boxmeer, The Netherlands) at 24 h after weaning. Only sows with clear signs of estrus at 48–72 h post eCG administration were further intramuscularly administered 750 IU of human chorionic gonadotropin (Veterin Corion, Divasa, Farmavic S.A., Barcelona, Spain) at the onset of estrus. Beginning at 2 days after the administration of eCG, estrous was checked twice per day (0700 h and 1700 h) by exposing sows to a mature boar (nose-to-nose contact) and applying manual back pressure. Females that exhibited a standing estrous reflex were considered to be in estrus.

Artificial insemination and embryo recovery and evaluation

The sperm–rich fractions of the ejaculates were manually collected from healthy sexually mature Duroc boars (2–3 years of age) that were fertile and undergoing regular semen collection for commercial AI using liquid semen. The donors were inseminated at 0 h, 24 h and 36 h after the onset of estrus with seminal doses (1.5×10^9 spermatozoa in 45 ml) prepared from semen diluted in Beltsville thawing solution (BTS) extender [24]. The seminal doses were stored for a maximum of 72 h at 18 C.

Embryo collection was performed in a surgical room located at the farm. The donors were subjected to a midventral laparotomy on days 5 and 6 of the estrous cycle (day 0: onset of estrus) to obtain morulae and unhatched blastocysts, respectively. The donors were sedated by administration of azaperone (2 mg/kg body weight, intramuscular). General anesthesia was induced using sodium thiopental (7 mg/kg body weight, intravenous) and maintained with isoflurane (3.5–5%). After exposure of the genital tract, the corpora lutea were counted in the ovaries. Embryos were collected by flushing the tip of each uterine horn with 30 ml of protein-free embryo recovery medium consisting of Tyrode's lactate (TL)-HEPES-polyvinyl alcohol (PVA) (TL-HEPES-PVA) [25] with some modifications. This medium was composed of 124.3 mM NaCl, 3.2 mM KCl, 2 mM NaHCO$_3$, 0.34 mM KH$_2$PO$_4$, 10 mM Na-lactate, 0.5 mM MgCl$_2$·6H$_2$O, 2 mM CaCl$_2$·2H$_2$O, 10 mM HEPES, 0.2 mM Na-pyruvate, 12 mM sorbitol, 0.1% (w/v) PVA, 75 μg/ml potassium penicillin G and 50 μg/ml streptomycin sulfate. Recovered embryos were evaluated under a stereomicroscope at a magnification of × 60 to grade their developmental stage and quality. One–cell eggs and poorly developed embryos were classified as unfertilized oocytes and degenerate embryos, respectively. The remaining embryos that exhibited appropriate morphology according to the criteria determined by the International Embryo Transfer Society [26] were considered viable. Only compacted morulae and/or unhatched blastocysts graded as excellent or good for morphological appearance were classified as transferable. The collected embryos were washed six times in embryo recovery medium and placed in Eppendorf tubes containing 1.5 ml of the same medium in a thermostatically controlled incubator (39 C) and maintained for up to 6 h prior to transfer.

Nonsurgical deep uterine embryo transfer

The NsDU–ETs were conducted on days 3 to 7 of the estrous cycle in nonhormonally treated synchronous or asynchronous recipients using the method previously described by Angel *et al*. [23]. Six hours prior to transfer, each recipient received a single intramuscular injection of a long–acting amoxicillin suspension (Clamoxyl LA®; Pfizer, Madrid, Spain) at a dosage of 15 mg/kg body weight. Recipients were housed in gestation crates in a small room (12 crates) exclusively used for that purpose. The perineal area of the recipients was thoroughly cleaned with soap and water using a different sponge for each sow. The tail of each recipient was covered with a latex glove to protect the vulva from possible contamination. The vulva was then washed and decontaminated (inside and outside) using sterile gauze soaked with chlorhexidine. Commercial nonsurgical ET catheters (DeepBlue® ET catheter, Minitüb, Tiefenbach, Germany), which were individually packaged and sterilized, were used for the transfers. Each ET catheter was composed of an AI spirette containing a flexible catheter (FC; 1.8 m length) inside and a protective sanitary sheath outside. Prior to insertion, the inner tubing of the FC was rinsed with 0.3 ml of TL-HEPES-PVA medium at 39 C, and the protective sheath was lubricated with silicone (Rüsch Silkospray®, Willy Rüsch, Kernen-Rommelshausen, Germany). Next, the spirette was inserted

through the vulva into the first 20 to 25 cm of the vagina. In this region, the spirette tip was pushed through the sheath and inserted into the cervix. The FC was then moved through the cervical canal and propelled forward along one uterine horn until the length of the FC outside of the recipient was approximately 30 to 40 cm. The FC was flushed with 0.3 ml of TL-HEPES-PVA medium at 39 C using a 1 ml disposable syringe when the tip of the FC reached the uterine body. When the FC was completely inserted into one uterine horn, a 1 ml syringe containing the embryos in 0.1 ml of TL-HEPES-PVA medium was connected to the FC, and the contents were introduced into the FC. Finally, an additional volume of 0.3 ml of TL-HEPES-PVA medium was used to force the embryos out of the FC into the uterus. Correct positioning of the FC was assumed if no bends or kinks in the catheter were present after its removal [19].

Experimental design

A total of 193 donors were selected based on their reproductive history (fertility, $96.1 \pm 0.9\%$; litter size, 11.0 ± 0.1; parity number, 4.9 ± 0.1; and lactation length, 21.3 ± 0.1 days). Transfers were conducted in recipients that started estrus 24 h before (–24 h; N=9) or 0 (synchronous; N=31), 24 (+24 h; N=74) or 48 (+48 h; N=18) h after the donors. The recipients (N=132) were selected based on their reproductive history and body condition. There were no differences in reproductive history of the recipients assigned to each group (fertility range, $93.7 \pm 3.3\%$ to $95.1 \pm 4.1\%$; litter size range, 9.9 ± 0.2 to 10.9 ± 0.4; parity number range, 2.4 ± 0.1 to 2.7 ± 0.2; lactation length range, 21.6 ± 0.4 to 22.2 ± 0.4 days). Thirty transferable embryos (morulae and unhatched blastocysts) were nonsurgically transferred into one uterine horn of each recipient. Each trial was conducted in separate sessions over a 2-year period and included 18 to 20 donors and 11 to 13 NsDU-ETs. The ovulatory response of the donors was determined by counting the number of corpora lutea in both ovaries. To evaluate the effectiveness of the superovulation treatment, the number of viable and transferable embryos and the number of oocytes and/or degenerated embryos were counted in each donor. The recovery rate was defined as the ratio of the number of embryos and oocytes and/or degenerated embryos recovered to the number of corpora lutea present. The fertilization rate was defined as the ratio of the number of viable embryos to the total number of embryos and oocytes and/or degenerated embryos collected. In addition, the presence of follicular cysts (ovarian structures filled with a transparent liquid, without ovulation signs, and with a diameter >2 cm at the time of laparotomy) and polycystic ovaries (ovaries with more than eight follicular cysts) was recorded for each donor. Starting at 12 days after NsDU-ET, the recipients were evaluated daily for signs of estrus. Pregnancy was diagnosed by ultrasonography on days 20 to 22 post transfer. All pregnant sows were allowed to carry litters to term, and the farrowing rates and litter sizes were recorded. The piglet production efficiency was calculated as the ratio of the number of live–born piglets to the number of embryos transferred to all recipients.

Statistical analysis

The data were analyzed using IBM SPSS Statistics for Windows, Version 19.0 (IBM, Armonk, NY, USA). The percentage data were compared using the Fisher's exact test. Continuous variables were evaluated using the Kolmogorov–Smirnov test to assess the assumption of normality, and groups were compared with analysis of variance or the Student's *t*-test. Post hoc analysis was performed using Bonferroni's test. The coefficient of variation (CV, standard deviation/mean) was used as a measure of variability of the ovulatory response. Differences were considered significant when P < 0.05. Differences among values with 0.05<P<0.10 were accepted as representing tendencies toward differences. The results are expressed as percentages and means ± standard error of the mean (SEM).

Results

Of 193 donors, 184 (95.3%) had embryos on days 5 to 6 post AI, 6 (3.1%) had only oocytes after flushing and 3 (1.5%) had polycystic ovaries with no corpora lutea in their ovaries. The proportion of donors with ovarian cysts was 39.1%, and sows with cysts had 2.4 ± 0.2 cysts. The mean ovulation rate was 24.9 ± 0.4 corpora lutea (range 11 to 51 corpora lutea, CV=25.4%). The recovery and fertilization rates were 94.8% and 92.8%, respectively, and the mean number of viable embryos and oocytes and/or degenerate embryos obtained in the pregnant sows was 21.9 ± 0.4 and 1.7 ± 0.4, respectively. The proportion of transferable embryos in relation to the number of viable embryos was 95.0%. The total number of transferable embryos collected from the inseminated donors (N=193) was 3,828, resulting in a donor to recipient ratio of 1.5:1.

Ten out of 132 ETs (7.6%) were removed from the study due to incorrect insertion of the NsDU-ET catheter. The reproductive performance of recipients after transfers is shown in Table 1. The highest pregnancy and farrowing rates were achieved when estrus in recipients was 24 h later that in the donors (85.1% and 81.1%, respectively), regardless of the embryonic stage (day 5 morulae or day 6 blastocysts) used for the transfers. The pregnancy and farrowing rates decreased (P<0.05) when recipients were synchronous or –24 h or +48 h asynchronous relative to the donors. Although no differences in litter sizes, piglet birth weights and sex ratio at birth were observed among the groups, the piglet production efficiency was higher (P<0.001) for the synchronous and +24 h groups.

The reproductive parameters of the recipients in estrus 0 h and 24 h later than the donors after NsDU transfers of day 5 morulae and day 6 blastocysts are shown in Tables 2 and 3, respectively. In both cases, no differences were observed between the groups for any of the parameters evaluated with the exception of the piglet production efficiency, which was higher (P<0.02) for blastocysts in the + 24 h group. Table 4 shows the reproductive performance after NsDU transfers of morulae and blastocysts in recipients with asynchrony of +48 h. The results indicate that such asynchrony was adequate for transfers performed with blastocysts but not for those with morulae. The farrowing rate (87.5%; P<0.05) and piglet production efficiency (27.9%; P<0.001) were increased in the blastocyst group compared with the morula group (30.0% and 7.0%, respectively). There were no differences between groups in the other parameters evaluated, although the litter size and number of piglets born alive tended (P=0.09) to be higher when the blastocyst stage was used.

Table 1. Effects of the different degrees of synchrony between recipients and donors on the farrowing rates and litter sizes after nonsurgical deep intrauterine transfers of 30 fresh embryos at the morula and/or unhatched blastocyst stages

	Synchrony recipients–donors (h)*			
	−24	0	+ 24	+ 48
Recipients, N	9	31	74	18
Pregnancy rate, N (%)	1 (11.1)[a]	19 (61.3)[b]	63 (85.1)[c]	9 (50.0)[ab]
Farrowing rate, N (%)	–	19 (61.3)[a]	60 (81.1)[b]	9 (50.0)[a]
Total born (mean ± SEM)	–	10.5 ± 0.8	9.6 ± 0.4	9.2 ± 1.0
Born alive (mean ± SEM)	–	10.1 ± 0.8	8.9 ± 0.4	8.6 ± 0.9
Stillborn (mean ± SEM)	–	0.4 ± 0.2	0.7 ± 0.1	0.6 ± 0.3
Piglet birth weight (kg; mean ± SEM)	–	1.2 ± 0.1	1.7 ± 0.1	1.6 ± 0.1
Sex ratio at birth (%; male/female)	–	47.7/52.3	49.0/51.0	46.4/53.6
Piglet production efficiency (%)	–	20.6[d]	24.0[d]	14.3[e]

*Recipients in estrus before (–) or after (+) donors. [a,b,c,d,e] Different superscripts within the same row indicate differences: [a,b,c] P<0.05; [d,e] P<0.001.

Table 2. Reproductive parameters of the synchronous recipients after nonsurgical deep-uterine transfer of 30 fresh porcine embryos

	Embryonic stage*	
	Morula	Blastocyst
Recipients, N	17	14
Pregnancy rate, N (%)	10 (58.8)	9 (64.3)
Farrowing rate, N (%)	10 (58.8)	9 (64.3)
Total born (mean ± SEM)	10.3 ± 1.1	10.8 ± 1.0
Born alive (mean ± SEM)	9.9 ± 1.0	10.2 ± 0.9
Stillborn (mean ± SEM)	0.4 ± 0.3	0.6 ± 0.3
Piglet birth weight (kg; mean ± SEM)	1.2 ± 0.2	1.2 ± 0.2
Sex ratio at birth (%; male/female)	47.0/53.0	48.4/51.6
Piglet production efficiency (%)	19.4	21.9

*Embryos at the morula and unhatched blastocyst stages were collected from donors at days 5 and 6 (day 0=onset of estrus), respectively, and were transferred into the recipients on days 5 and 6 of the cycle, respectively.

Table 3. Reproductive parameters of the recipients in estrus 24 h later than the donors after nonsurgical deep-uterine transfer of 30 fresh porcine embryos

	Embryonic stage*	
	Morula	Blastocyst
Recipients, N	35	39
Pregnancy rate, N (%)	29 (82.8)	34 (87.2)
Farrowing rate, N (%)	26 (74.3)	33 (84.6)
Total born (mean ± SEM)	9.2 ± 0.6	9.8 ± 0.6
Born alive (mean ± SEM)	8.6 ± 0.6	9.1 ± 0.5
Stillborn (mean ± SEM)	0.6 ± 0.2	0.7 ± 0.2
Piglet birth weight (kg; mean ± SEM)	1.9 ± 0.1	1.5 ± 0.1
Sex ratio at birth (%; male/female)	48.1/51.9	49.5/50.5
Piglet production efficiency (%)	21.3[a]	25.6[b]

*Embryos at the morula and unhatched blastocyst stages were collected from donors at days 5 and 6 (day 0=onset of estrus), respectively, and were transferred into the recipients on days 4 and 5 of the cycle, respectively. [a,b] Different superscripts within the same row indicate differences (P<0.02).

Table 4. Reproductive parameters of the recipients in estrus 48 h later than the donors after nonsurgical deep-uterine transfer of 30 fresh porcine embryos

	Embryonic stage*	
	Morula	Blastocyst
Recipients, N	10	8
Pregnancy rate, N (%)	3 (30.0)[a]	7 (87.5)[b]
Farrowing rate, N (%)	3 (30.0)[a]	7 (87.5)[b]
Total born (mean ± SEM)	7.3 ± 2.3[e]	10.4 ± 0.7[f]
Born alive (mean ± SEM)	7.0 ± 2.0[e]	9.6 ± 0.7[f]
Stillborn (mean ± SEM)	0.3 ± 0.3	0.8 ± 0.5
Piglet birth weight (kg; mean ± SEM)	1.7 ± 0.2	1.5 ± 0.2
Sex ratio at birth (%; male/female)	52.4/47.6	43.7/56.3
Piglet production efficiency (%)	7.0[c]	27.9[d]

*Embryos at the morula and unhatched blastocyst stages were collected from donors at days 5 and 6 (day 0=onset of estrus), respectively, and were transferred into the recipients on days 3 and 4 of the cycle, respectively. [a,b,c,d] Different superscripts within the same row indicate differences: [a,b] P<0.05; [c,d] P<0.001. [e,f] Different superscripts within the same row indicate tendencies for differences (P=0.09).

Discussion

This study confirmed previous reports on the effectiveness of the NsDU-ET technology and provides evidence for the first time that the degree of estrous synchrony between recipients and donors can markedly influence the success of NsDU-ET.

As previously reported in surgical ET studies in the pig [11], the pregnancy and farrowing rates dramatically decreased when the recipient started estrus 24 h (–24 h) ahead of the donor. Studies in other species have demonstrated that transferring embryos to a more advanced uterus can result in accelerated growth by increasing their rate of cell division [27, 28], which could fatally modify their subsequent development [4, 29] and adversely affect the pregnancy outcomes.

On the other hand, our results clearly demonstrate that increased pregnancy and farrowing rates are achieved when NsDU-ETs are

performed on recipients that started estrus 24 h (+24 h) after the donors compared with those obtained using synchronous or +48 h asynchronous recipients. These results partially confirm those achieved in surgical experiments, where increased pregnancy rates and embryonic survival were obtained with transfers performed on recipients in which the onset of estrus was 0, 1 or 2 days later than in donors [11]. The hypothesis that porcine embryos are tolerant of a less advanced uterine environment has also been supported by other studies using surgical transfers [12, 13, 30]. In contrast, Hazeleger *et al*. [14] reported that transfers to recipients ovulating 24 h before donors might be optimal for nonsurgical transfers into the uterine body, whereas transfers to recipients ovulating 24 h or more after the donors appear to result in significantly reduced pregnancy rates. Several factors might be accountable for such discrepancies. It has previously been speculated that after surgical transfer, embryos might benefit from younger uterine environments due to anesthesia or surgical trauma [14]. However, our results using NsDU-ETs mirror those achieved in the surgical experiments but not those obtained with the nonsurgical uterine body ETs, clearly indicating that the surgery is not associated with the tolerance of asynchrony of the embryos. The most plausible explanation for these discrepancies might be the location of embryo deposition, which is related to the ET technique used. In surgical ET and NsDU-ET procedures, the embryos are deposited into the tip or anterior quarter of a uterine horn, respectively [11, 19, 21, 22]; in contrast, in the nonsurgical ET technique used by Hazeleger's group, the embryos are placed within the uterine body [14]. Under natural conditions, morulae and blastocysts remain near the tip of the uterine horn until day 6 to 7 of the cycle, progressing subsequently toward the uterine body [31]. Thus, as it has been demonstrated in studies using surgical ETs [32], transfer of these embryos to anterior portions of the uterine horns of recipients confers some advantages compared with transfer within the uterine body, likely because the uterine environment in this region is unfavorable for embryos during these stages of development. Regional differences in the uterine environment might also explain the synchrony discrepancies among the abovementioned studies. More research is needed to corroborate this hypothesis.

The reasons underlying the enhanced tolerance that transferred embryos exhibit for less advanced uterine environments remain to be elucidated. Consistent with Blum-Reckow and Holtz [13], collection and handling of the embryos and/or short–term storage *in vitro* prior to transfers might cause a transitory delay of embryonic development, as occurs under *in vitro* culture conditions [33, 34], which might increase the chances for embryo survival in a less advanced uterus.

In our study, a recipient asynchrony of +24 h was satisfactory for NsDU-ETs performed with both morulae and blastocysts. However, interestingly, a recipient asynchrony of + 48 h was adequate for blastocysts but not for morulae. The most likely explanation for this observation is that transfers of day 5 morulae into +48 h asynchronous recipients involve the use of recipients on the third day of the cycle, which means that some of the recipients are still in estrus or shortly after estrus. At that point in the cycle, the hormonal events associated with corpora lutea formation and the endometrial secretory products in the uterine lumen might be inadequate for survival of the embryos at the morula stage. In this context, the concentration of serum progesterone in the recipient at the moment of transfer plays an important role in the success of porcine somatic cell nuclear transfer ET programs [35]. The importance of progesterone in promoting embryo survival after ET has also been demonstrated in other species [36, 37].

Consistent with our previous studies [19, 23], NsDU-ET catheter insertion did not cause uterine infections in the form of vaginal discharge, reflecting the efficacy of the aseptic measures taken. Furthermore, it did not disturb the animal behavior or the reproductive tract of the recipients (data not shown). Together with the high number of transfers performed, these data indicate that the procedure is safe and well tolerated by the recipients.

A high proportion of donors (39.1%) had cysts in the ovaries with an average of 2.4 cysts per sow. The high incidence of cysts might be attributed to the superovulation treatment used in this experiment. However, in a previous study performed in the same farm using the same breed, we observed a similar frequency of ovarian cysts in superovulated and non-superovulated donors [23], indicating that the superovulation treatment was not associated with this problem. In the present study, these cysts were likely nonfunctional and did not interfere with the reproductive cycle as previously reported for single cysts [38]. The excellent reproductive history and quality of the embryos collected support this hypothesis. Although the origin of these cysts is unclear, they might be attributable to innate characteristics of the Duroc breed.

The high pregnancy and fertilization rates, high numbers of viable and transferable embryos and low numbers of oocytes and/or degenerated embryos obtained in this study indicate the effectiveness of the superovulation treatment used and confirm the results obtained in our previous study [23]. The ovulatory response variability found in the present study was high (CV=25.4%). This finding was not abnormal because such variability has been widely reported [39–42]. However, the variation observed in this study and our previous study [23] was low compared with those reported for cyclic gilts and sows, wherein the CVs were approximately 40% [39–42]. The different superovulation protocols and the different lines and breeds used among these studies, which can exhibit widely variable superovulatory responses [21], likely contributed to this discrepancy.

In conclusion, our results indicate that using NsDU transfers of day 5 morulae and day 6 blastocysts, the ideal recipient should start estrus 24 h after the donors. This asynchrony can be increased to +48 h for transfers performed with day 6 blastocysts. In contrast, the use of synchronous recipients or recipients with heat ahead (–24 h) of the donors does not result in adequate pregnancy and farrowing rates. The excellent reproductive performance of the recipients following NsDU-ETs reported in this study represents an important advance for the widespread commercial use of ET by the pig industry.

Acknowledgments

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