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Deep intrauterine insemination and embryo transfer in pigs

E. A. Martinez[1], J. M. Vazquez[1], J. Roca[1], X. Lucas[1], M. A. Gil[1] and J. L. Vazquez[2]

[1]*Department of Animal Pathology, University of Murcia, 30071 Murcia, Spain; and* [2]*Department of Surgery, University Miguel Hernandez, Elche, Spain*

A new method for non-surgical deep intrauterine catheterization of pigs, without sedation of the sow, is described. Insemination results obtained with this method using fresh spermatozoa demonstrate that, in comparison to conventional artificial insemination (AI) (3×10^9 spermatozoa in 80–100 ml), a 20–60-fold reduction in the number of spermatozoa inseminated and at least a 8–10-fold reduction in the dose volume can be used without affecting fertility if spermatozoa are deposited deep (middle or upper) into one of the uterine horns. Results from deep intrauterine insemination with frozen–thawed spermatozoa and flow-sorted spermatozoa are also presented and the effect of deep intrauterine insemination on sperm transport is discussed. In addition, a brief description of the advances made in non-surgical embryo transfer technology is reported.

Introduction

Artificial insemination (AI) is gaining importance in the pig industry. Over the past 15 years, there has been an increase in the development of on-farm AI services in many countries, and the percentage of sows artificially inseminated can be more than 80% (Weitze, 2000). The demand for higher quality pork together with the optimal fertility results that can be achieved by using improvements to the AI technique have contributed to the growth in the use of AI. As a consequence of this increase, a more efficient use of semen samples of high quality and high genetic value would be of great importance for the pig industry because a larger number of females could then be inseminated with semen from boars that are genetically superior.

Numerous investigations have been carried out on the development of several semen extenders for short- or long-term storage of spermatozoa. Different tests for evaluating the quality and functionality of semen, and predicting boar fertility *in vivo* have been proposed. Other investigations have focused on the effect of timing of insemination relative to ovulation, the role of seminal plasma in sperm transport and the addition of different compounds to the insemination doses to enhance reproductive performance. Nevertheless, few investigations have been performed regarding three important aspects of the AI technique: site of deposition of the insemination dose, number of spermatozoa per dose and dose volume. A large volume of liquid (50–200 ml) and a large number of spermatozoa ($5–10 \times 10^9$ per insemination dose) deposited intracervically during AI were recommended 40 years ago to achieve maximum

Email: emilio@um.es

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fertility in pigs (Polge, 1956; Stratman and Self, 1960; Baker *et al.*, 1968). Few modifications of these recommendations have been introduced since these studies. Thus, current AI procedures in pigs use two inseminations during oestrus with a concentration of 2.5–4.0 × 10^9 spermatozoa in a large volume of liquid (80–100 ml) deposited intracervically at each insemination, which limits the number of doses that can be prepared from one semen sample to approximately 20.

However, it is now known that the number of spermatozoa per insemination can be reduced if the sperm dose is deposited into the uterine horn. Successful non-surgical deep intrauterine inseminations using very small numbers of spermatozoa have been reported in cattle (Seidel *et al.*, 1997) and horses (Morris *et al.*, 2000). In pigs, a 100-fold reduction of the standard AI dose (3 × 10^9 spermatozoa in 80 ml) can be made when spermatozoa are surgically deposited close to the uterotubal junction (Krueger *et al.*, 1999; Krueger and Rath, 2000).

This article describes a new procedure for non-surgical deep intrauterine insemination in pigs and subsequently focuses on the effectiveness of this procedure in sows using a small number of fresh, frozen or flow-sorted spermatozoa. Implications about transport of spermatozoa into the genital tract will also be discussed. Finally, a brief description of the advancements achieved in non-surgical embryo transfer in pigs is presented.

Procedure for non-surgical deep uterine catheterization

Non-surgical transcervical catheterization of the uterus has been successfully performed in cows, horses and dogs (Devine and Lindsay, 1984; Bracher *et al.*, 1992; Watts and Wright, 1995). However, there are no reports of transcervical catheterization of the uterus in sows. The main obstacle to this procedure is the complex anatomy of the cervix and uterus of the pig. The cervical folds and the length and coiled nature of the uterine horns have discouraged attempts at non-surgical transcervical introduction of a catheter into the uterine horn for AI.

Vazquez *et al.* (1999) reported a procedure to gain access to the uterine horn through the cervix by using a flexible fibreoptic endoscope (length 1.35 m, outer diameter 3.3 mm) and the difficulties of this technique when applied in oestrous sows. In their study, deep uterine catheterizations were performed in weaned sows in their own crates at 30–40 h after hCG treatment, without sedation. With this technique, it was possible to pass the cervical canal and to reach the depth of one uterine horn in 96.7% of sows and the procedure was completed within 3–7 min in about 90% of sows. The behaviour of the sows during the procedure was similar to the reaction of the sows during standard AI, indicating that this procedure is a relatively stress-free method that is well tolerated by sows, as is also the case for cows (Devine and Lindsay, 1984). After uterine catheterization, no symptoms of uterine infection were observed during the days after the hysteroscopy, and sows returned to oestrus after a normal period of time. Endoscopic images obtained during insertion of the fibrescope are shown (Fig. 1a–c). Upon entering the uterine body, the endometrial folds were apparent and occluded visualization of the bifurcation between the uterine horns. However, the fibrescope progressed without difficulty along one uterine horn until its total length was inserted. The tip of the fibrescope reached approximately the middle or the beginning of the anterior third of the uterine horn, which adapted to the fibrescope and formed a spiral shape, as determined by laparoscopy (Fig. 1d).

Although endoscopic deep intrauterine insemination is a successful technique for inseminating sows with a small number of spermatozoa, the fibrescope is expensive and fragile, and unsuitable for use under field conditions. As it is not necessary to have an optic system to pass through the cervix or to gain entry into the uterine horn, a new flexible catheter (1.8 m in length, 4.0 mm in outer diameter) was made on the basis of the propulsion force and

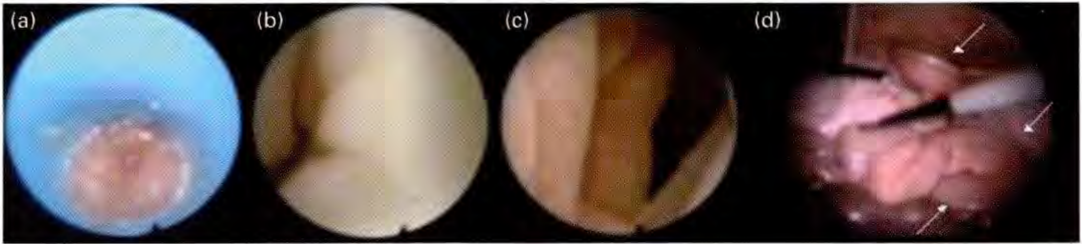

Fig. 1. Endoscopic (a,b,c) and laparoscopic (d) photographs of the sow genital tract during oestrus. The insertion of the endoscope was performed in sows housed in gestation crates. Sows were not sedated. (a) Artificial insemination spirette (blue) inserted into the cervix. (b) Cervical canal with cervical folds. (c) Lumen of a uterine horn. (d) Image illustrating the silhouette (arrows) of the endoscope in one uterine horn.

flexibility of the fibrescope used in the previous studies (Martinez *et al.*, 2001). With this new device, similar results in the passage of the catheter through the cervix into one uterine horn to those obtained with the fibrescope have been achieved, but the time required to complete the procedure was reduced (3–4 min). The tip of the flexible catheter was located in the anterior third of one uterine horn, as determined by laparotomy of sows of 2–3 parities.

Deep intrauterine insemination with a small number of spermatozoa in sows

Several experiments to determine the effectiveness of deep intrauterine insemination using a small number of spermatozoa have been, or are in the process of being, performed, including the use of fresh, frozen or flow-sorted spermatozoa.

Deep intrauterine insemination using fresh semen

Successful surgical intrauterine insemination with small numbers of spermatozoa has been reported for hormonally stimulated prepubertal gilts (Krueger *et al.*, 1999) and for sows (Krueger and Rath, 2000). These investigations demonstrated that the number of spermatozoa used for surgical intrauterine insemination (next to the uterotubal junction) can be reduced to 1×10^7 spermatozoa per uterine horn, without compromising fertility. Preliminary results (Martinez *et al.*, 2000) indicate that when spermatozoa are deposited non-surgically using the fibrescope deep into one of the uterine horns of hormonally treated post-weaning sows, normal farrowing rates and litter sizes are obtained by inseminating a concentration of 5×10^7 spermatozoa per sow (Fig. 2). Further experiments have been conducted in our laboratory to determine the minimum number of spermatozoa required to maintain optimal fertility using the flexible catheter for non-surgical deep intrauterine insemination in weaned sows undergoing induced oestrus (Martinez *et al.*, 2001). Farrowing rates and litter sizes after deep intrauterine insemination with 1.5×10^8 or 5.0×10^7 spermatozoa at 36 h after hCG treatment did not differ from those obtained after standard AI with 3×10^9 spermatozoa; however, a significant decrease in the farrowing rates was observed in sows inseminated with 2.5×10^7 or 1.0×10^7 spermatozoa (Fig. 3). An additional study is being conducted to evaluate the pregnancy rates, farrowing rates and litter sizes when deep intrauterine insemination is performed in sows undergoing natural oestrus after weaning. The possibility of carrying out deep intrauterine inseminations in natural oestrous sows after weaning would allow the application of this new technique under the same conditions as the conventional procedures of AI used in commercial pig units.

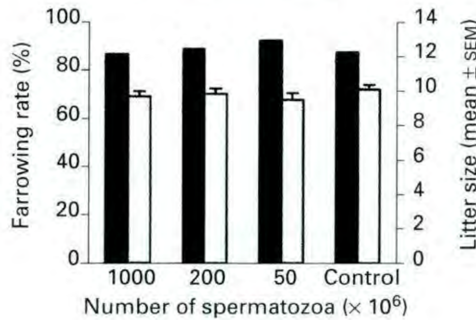

Fig. 2. Farrowing (■) rates and litter size (□) in oestrous-induced weaned sows that were inseminated once into one uterine horn with a concentration of 1000 ($n = 15$), 200 ($n = 18$) or 50 ($n = 13$) $\times 10^6$ spermatozoa in 10 ml of Beltsville thawing solution diluent using an endoscope. Control sows ($n = 48$) were inseminated twice by the standard artificial insemination method.

Deep intrauterine insemination with frozen semen

The first pregnancies using frozen semen in pigs were obtained 30 years ago by surgical insemination into the oviducts (Polge *et al.*, 1970) or by intracervical insemination of thawed spermatozoa (Crabo and Einarsson, 1971; Graham *et al.*, 1971). The fertility achieved with frozen semen from 1970 to 1999 was about 20–30% and two to three piglets fewer than that obtained using fresh semen or semen stored for a short time. Recently, increased fertility rates have been reported using spermatozoa frozen in a new flat plastic package of 5 ml (Eriksson and Rodriguez-Martinez, 2000) or in 0.5 ml straws (Bussiere *et al.*, 2000). Nevertheless, fertility and prolificacy are still lower than that expected with fresh semen and standard AI. It is established that boar spermatozoa are more susceptible to cold shock than spermatozoa of other species and that a high proportion of spermatozoa die during the freezing procedure.

After thawing, a large proportion of motile spermatozoa has decreased or suppressed fertilizing ability because the freezing–thawing causes destabilization of the sperm membrane (Watson, 1996). This process resembles capacitation, although it is not identical (Watson and Green, 2000). Consequently, a greater number of thawed spermatozoa (5–6 $\times 10^9$) extended in 80–100 ml of diluent are usually inseminated intracervically close to the time of ovulation to increase the fertility results.

Results of numerous studies on freezing procedures, diluents, cryoprotectants and other aspects of frozen boar semen have been published (for a review, see Johnson *et al.*, 2000a). However, the effect of frozen–thawed semen deposition in different parts of the uterine horn on fertility has not been reported. A preliminary field trial was performed to determine the effectiveness of deep intrauterine insemination with frozen–thawed semen (J. Roca, G. Carvajal, C. Cuello, I. Parrilla, X. Lucas, J. M. Vazquez and E. A. Martinez, unpublished). Sows ($n = 49$) were hormonally treated after weaning and subjected to one deep intrauterine insemination at 40 h after hCG treatment with 1×10^9 thawed spermatozoa extended in BTS diluent (Beltsville thawing solution; Pursel and Johnson, 1975) in a total volume of 7.5 ml,

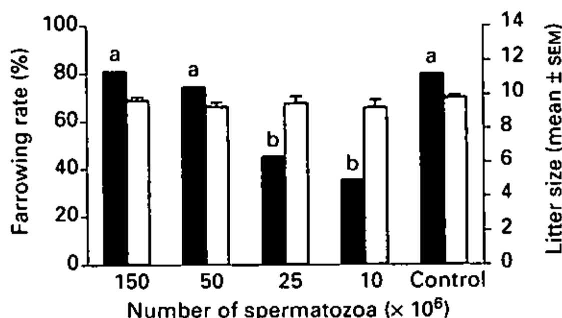

Fig. 3. Farrowing (■) rates and litter size (□) in oestrous-induced weaned sows inseminated once with a flexible catheter deep into one uterine horn with 150 (n = 84), 50 (n = 82), 25 (n = 31) or 10 (n = 34) × 10^6 spermatozoa in 10 ml of Beltsville thawing solution diluent. Control sows (n = 99) were inseminated twice by the standard artificial insemination method. [ab]Significantly different at $P < 0.01$.

using the flexible catheter for deep intrauterine insemination. Surprisingly, farrowing rates and litter sizes were approximately 80.0% and 9.5%, respectively, and did not differ from those obtained with traditional AI (sows (n = 52) inseminated twice with 3 × 10^9 fresh spermatozoa by the standard AI method, two inseminations per oestrus). Unfortunately, standard intracervical inseminations with a concentration of 5–6 × 10^9 frozen spermatozoa extended in 80–100 ml of medium were not performed in this experiment. Therefore, it was not possible to evaluate whether the results achieved after deep intrauterine insemination using frozen semen are attributable to the insemination method or other factors, such as the quality of thawed spermatozoa used. As the quality of spermatozoa inseminated was considered as normal for frozen–thawed semen (40–50% motility and 35–45% of normal apical ridge) it is possible that when thawed semen is deposited in the depth of a uterine horn, an improvement in fertility can be achieved by using a small number of thawed spermatozoa and a small dose volume.

Deep intrauterine insemination using flow-sorted semen

Currently, the only means of farrowing pre-sexed offspring in pigs is by using flow cytometric sorting of spermatozoa bearing X and Y chromosomes (for a review, see Johnson *et al.*, 2000b). The effectiveness of this method has been demonstrated using surgical intratubal inseminations (Johnson, 1991) and *in vitro* fertilization (Rath *et al.*, 1997; Abeydeera *et al.*, 1998). The flow-sorting method produces populations of weak spermatozoa and changes in spermatozoa similar to those that occur during capacitation. The current technique of high speed and high pressure sorting (Johnson and Welch, 1999) also produces a small number of sexed spermatozoa, which is limited to a concentration of 5–6 × 10^6 X or Y spermatozoa per h. Therefore, the practical application of the current technology for gender preselection in pigs depends partially on efficient techniques for non-surgical inseminations with a very small number of spermatozoa. Farrowing rates of 26.7 and 50.0%, and litter sizes of 7.8 and 9.5 were obtained in our laboratory when weaned oestrous sows were deeply inseminated once with 2–5 × 10^7 (n = 15) or 6–15 × 10^7 (n = 12) flow-sorted spermatozoa, respectively, using non-surgical deep intrauterine methods (J. M. Vazquez, I. Parrilla,

N. Garcia, C. Cuello, X. Lucas, J. Roca and E. A. Martinez, unpublished). Although the litter size could be considered as excellent after insemination with flow-sorted spermatozoa, the farrowing rate using a concentration of 2–5 $\times$ 10^7 flow-sorted spermatozoa is still too low to have economic advantages using both techniques together. Further investigations are being conducted on this matter to improve the fertility of sows undergoing deep intrauterine insemination with sex-sorted spermatozoa.

Possible mechanisms implicated in the effectiveness of deep intrauterine insemination

Our results from deep intrauterine insemination with fresh semen indicate that if spermatozoa are non-surgically deposited deep into one uterine horn, normal farrowing rates and litter sizes can be obtained when a dose of 5 $\times$ 10^7 spermatozoa per sow is used.

The mechanisms by which the number of spermatozoa per insemination can be reduced during insemination in the depth of the uterine horn are not clear. Billions of spermatozoa in a large volume of liquid are deposited intracervically during natural mating or artificial insemination. However, only about 1000 spermatozoa reach the sperm reservoir (Mburu *et al.*, 1996) that is located in the caudal 1–2 cm of the isthmus (Hunter, 1981, 1984), where the cells maintain their fertilizing ability and are released just before ovulation (Hunter, 1984). Most of the spermatozoa are eliminated rapidly from the uterus by back flow of semen at insemination or during 2 h after insemination (Steverink *et al.*, 1998) and by local phagocytosis (Rozeboom *et al.*, 1998), which occurs 2 h after insemination (Pursel *et al.*, 1978). Some spermatozoa are found in the oviducts within 15 min after insemination (First *et al.*, 1968) and sufficient spermatozoa to ensure subsequent fertilization are present in the isthmus reservoir within 1–2 h of mating (Hunter, 1981, 1984).

During natural mating, different stimuli from the boar, such as tactile, ejaculate volume and seminal plasma components can facilitate transport of spermatozoa in the female genital tract by increasing oxytocin and $PGF_{2\alpha}$ concentrations, which should stimulate myometrial contractions (for a review, see Soede, 1993). AI has a positive affect on uterine motility, probably as a result of a cervical stimulus. Hunter (1982) suggested that although uterine contractions should assist transport and redistribution of semen between the two uterine horns, an initial distribution of semen into the uterus might be achieved during natural mating due to the force of ejaculation and the volume of fluid involved. Thus, the large volume of semen deposited during natural mating ensures that the uterotubal junction is bathed in a sperm suspension by the completion of mating (Hunter, 1982). Therefore, spermatozoa from this suspension could enter the oviducts and establish the sperm reservoir soon after mating. A similar situation could occur when animals are artificially inseminated with a dose of 80–100 ml. In fact, when 100 ml is used, fluid can be collected from uterine cannulae inserted near the uterotubal junction as early as 1.5 min after the onset of insemination (Baker and Degen, 1972). The requirement for a large volume when semen is deposited intracervically is supported by the observation that neither spermatozoa in the oviducts nor accessory spermatozoa were found 12 h after insemination in gilts inseminated intracervically with only 20 ml of semen containing 10 $\times$ 10^9 spermatozoa (Baker *et al.*, 1968). Stratman and Self (1960) also found a positive effect of the insemination volume (50 versus 10 or 20 ml) on embryo survival and percentage of conception regardless of the number of spermatozoa used (2.5–10.0 $\times$ 10^9). The requirement for a large volume of semen for intracervical deposition also supports the finding that high fertilization rates (92%) and numbers of accessory spermatozoa (approximately 100) have been found in sows inseminated intracervically with only 5 $\times$ 10^8 spermatozoa in a total volume of 100 ml (Waberski *et al.*, 1996). In contrast, it

has been demonstrated that the dose volume is not important when the inseminations are carried out close to the uterotubal junction. Optimal fertility has been obtained after surgical inseminations next to the uterotubal junction with an insemination volume of only 0.5 ml containing 1×10^7 spermatozoa (Krueger and Rath, 2000) or after non-surgical deep intrauterine inseminations with 5×10^7 spermatozoa in a dose volume of only 10 ml (Martinez *et al.*, 2000, 2001). Thus, the deposition of a small number of spermatozoa in a small volume of liquid close to the uterotubal junction may be similar to an aliquot of the ejaculate or of a standard AI dose arriving at the uterotubal junction during natural mating or AI, as has been suggested in horses (Morris *et al.*, 2000). This finding is not surprising, as it was hypothesized by Hunter (1982) that for the future development of AI, a technique that permits deposition of a few milliliters of semen at the top of each uterine horn against the uterotubal junction should be beneficial.

Our results with endoscopic inseminations of fresh semen (Martinez *et al.*, 2000) indicate that it is not necessary to deposit spermatozoa at the uterotubal junction to obtain adequate farrowing rates and litter sizes by inseminating a small number of spermatozoa in a small volume of medium. Semen was deposited once near the middle of one uterine horn. These conditions were sufficient to enable formation of a sperm reservoir in the caudal isthmus of at least one oviduct, because no significant differences were observed in farrowing rates from those obtained after standard AI. When deep intrauterine insemination is performed closer to the uterotubal junction by using the flexible catheter (length 1.80 m), the number of spermatozoa cannot be lower than 5×10^7 without a reduction in fertility (Martinez *et al.*, 2001).

Other alternatives must be considered. Comparable fertility results to those obtained using deep intrauterine insemination might be obtained after insemination of a reduced number of spermatozoa in a small dose volume into the uterine body, as has been shown in horses (Woods *et al.*, 2000). In a study by Johnson *et al.* (2000c), two pregnancies in five sows and five pregnancies in six sows resulted after insemination once or twice, respectively, into the uterine body with $1\text{–}3 \times 10^8$ fresh spermatozoa in a total volume of 25 ml. Moreover, litter sizes obtained after one insemination into the depth of one uterine horn with fresh or frozen semen were not significantly different from the standard inseminated control groups (Martinez *et al.*, 2000, 2001; J. Roca, G. Carvajal, C. Cuello, I. Parrilla, X. Lucas, J. M. Vazquez and E. A. Martinez, unpublished). Although it was not evaluated in these studies, it is possible that the hormonal treatment of sows inseminated in the depth of one uterine horn probably increased the number of ovulations in each ovary and, as a result, a greater number of oocytes may be fertilized in the oviduct ipsilateral to the uterine horn inseminated. This might explain the large number of piglets born under our experimental conditions if fertilization had occurred in only one oviduct. However, embryos at four- to eight-cell stages could be collected from the tip of both uterine horns 2 days after deep uterine insemination with a concentration of 1.5×10^8 fresh spermatozoa in five of six sows (E. A. Martinez, J. M. Vazquez, X. Lucas, X. Roca, J. L. Vazquez and B. N. Day, unpublished). No significant difference was observed in the yield of embryos in each the uterine horns. This finding demonstrated that when spermatozoa are deposited close to the uterotubal junction in only one uterine horn, spermatozoa reach the contralateral oviduct and fertilize the oocytes. Whether spermatozoa reach the contralateral oviduct by a transperitoneal or intrauterine pathway is being investigated. Therefore, in addition to dose volume and site of deposition of the spermatozoa, other factors could be implicated in the differences in requirements between intracervical and deep intrauterine insemination procedures. There are two basic differences between the procedures. Firstly, during intracervical insemination the spermatozoa contact the cervical canal and its secretions, which does not occur during deep intrauterine insemination. As the

traditional deposition of semen in pigs has generally been considered 'intrauterine', little attention has been given to the sperm–cervical mucus interaction in this species. Secondly, deep intrauterine insemination produces a large distension of the cervix and uterine horn, which might induce a greater release of hormones implicated in uterine contractility and sperm transport compared with the traditional insemination method. Investigations are currently in progress to clarify some of these issues.

Non-surgical embryo transfer

Practical application of embryo transfer in pigs is limited because of the necessity to use surgical procedures for the collection and transfer of the embryos. Although Polge and Day (1968) demonstrated that pregnancy could be established in pigs by non-surgical embryo transfer, this procedure was considered impossible for many years. The complex anatomy of the cervix and uterus in pigs were the principal obstacles to the insertion of a catheter during metoestrus. In the 1990s, new procedures were developed to transfer embryos non-surgically (for a review, see Hazeleger and Kemp, 1999), although most of them have not been successful. Only one research group has performed non-surgical embryo transfer directly into the uterine body without sedation of the recipient sows (Hazeleger and Kemp, 1994, 1999). Hazeleger and Kemp (1994) showed that deposition of embryos in 0.1 ml of medium into the uterine body is possible in non-sedated sows using a specially designed flexible instrument. Use of this device resulted in a relatively high pregnancy rate of 59% and an average of eleven normal fetuses at day 37 after embryo transfer when 28–30 expanded blastocysts per embryo transfer were used. The farrowing rate was lower when the same embryo transfer procedure was used under field conditions (41% farrowing rate and 7.4 piglets born) (Ducro-Steverink *et al.*, 2001). Although the results achieved by this research group are similar to those obtained surgically, in which a pregnancy rate of 60–80% and an embryonic survival rate of 50–70% at 30 days have been reported (Polge, 1982; Wallenhorst and Holtz, 1999), further improvements are required to increase fertility results after non-surgical embryo transfer.

Results from surgical embryo transfer indicate that the uterine body is an inadequate site for embryo transfer in pigs. The pregnancy rate of the recipients is low when expanded blastocysts are deposited surgically in the uterine body (12%), compared with blastocysts deposited in the middle (88%) or the caudal quarter of the uterine horn (81%) (Wallenhorst and Holtz, 1999). Although these results are poor and not in agreement with those found after non-surgical embryo transfers into the uterine body (Hazeleger and Kemp, 1994; Galvin *et al.*, 1994; Li *et al.*, 1996; Hazeleger *et al.*, 1999), the possibility that the uterine body is not an optimum site to deposit embryos should be considered. However, with the current procedures for non-surgical embryo transfer, the embryos cannot be deposited into a uterine horn and, therefore, it has not been possible to determine whether embryo transfer results could be increased by depositing the embryos in the middle or even further up the uterine horn. In addition, another limitation of the current non-surgical embryo transfer systems is that only sows can be used as recipients because the cervix of gilts is too restrictive to allow penetration.

On the basis of the flexible catheter used for deep intrauterine insemination, a new device for non-surgical embryo transfer in the depth of one uterine horn of gilts and sows at days 4–6 of the oestrous cycle (day 0 = onset of oestrus) is being developed. The embryo transfer catheter (1.20 m in length, 4 mm outer diameter and 0.7 mm of working canal) can be inserted quickly and appropriately through the cervix into the uterus in about 90% of gilts (determined by laparotomy) and 95% of sows (determined by the flow of the catheter through the cervix

and uterus, and by the shape of the catheter after removal, whether it was straight or bent). The overall correct prediction of the location of the catheter in the uterus was 96.1% (25 correct predictions of 26 insertions performed) in gilts and 97.1% (34 correct predictions of 35 insertions performed) in sows, and no perforations of the cervix or uterine wall were observed. This procedure offers new possibilities to transfer embryos non-surgically in pigs and other experiments are being conducted to determine the overall effectiveness of this procedure. It is possible that a simple, effective and practical procedure for non-surgical embryo transfer in pigs will be available in the next few years and important advances in the commercial applications of this technique can be achieved.

Conclusion

A new technique for non-surgical deep intrauterine insemination in pigs is being developed. With this technique, it is possible to pass the cervical canal and to reach the depth of one uterine horn in about 95% of sows, and the time required is similar to that required to perform traditional AI. In comparison with standard AI (3×10^9 spermatozoa in 80–100 ml of liquid), a 20–60-fold reduction in the number of spermatozoa inseminated and at least an 8–10-fold reduction in the dose volume can be achieved without a decrease in fertility. Deep intrauterine insemination might have a high impact on the fresh semen AI industry by decreasing the number of boars that are used. Selection of the boars could be more rigorous and only boars that have high genetic value would be included in the AI centres. A decrease in space, food, management and replacement of boars and a decrease in the time required for semen collection, sperm evaluation and sperm dose preparation would be secondary benefits using this insemination procedure. Preliminary results indicate that the non-surgical deep intrauterine technique may also be a practical method for obtaining satisfactory fertility in sows inseminated with a low number of cryopreserved spermatozoa. The number of frozen spermatozoa per ejaculate could be increased at least five to six times (about 60 doses per ejaculate). In addition, deep intrauterine insemination could provide a technique for practical application of biotechnologies, such as flow-sorted spermatozoa or non-surgical embryo transfer and could be used for new investigations on sperm transport in the female reproductive tract. However, this technology is in the preliminary stages and many investigations will be necessary to elucidate numerous questions that cannot be answered now.

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