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Research Article

Field Non-Surgical Embryo Transfer of *In Vitro*-Produced Porcine Embryos

Transferência Não Cirúrgica em Campo de Embriões Suínos Produzidos In Vitro

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ABSTRACT

Non-surgical embryo transfer (NSET) offers several advantages over surgical embryo transfer (SET), including improved animal welfare, simplified procedures, reduced costs, and the potential for repeated use. In pigs, the combination of *in vitro* embryo production (IVP) and NSET supports advanced biotechnologies, potentially enabling rapid genetic improvement without the need for surgery or live animal transport. This pilot study evaluated the feasibility of IVP for field NSET. Porcine IVP were transferred to synchronized recipient females one day prior to the developmental stage of IVP. Three protocols were tested: (I) Field NSET – two procedures were conducted on multiparous, fertility-proven sows under field conditions, with estrus synchronization achieved through natural cycling, with presumptive pregnancy assessed by the absence of estrus return. (II) Closed conditions NSET – four procedures were performed under controlled indoor conditions on multiparous, fertility-proven sows under field conditions, with estrus synchronization achieved through natural cycling, with pregnancy assessed by ultrasonography on day 25. (III) Closed conditions SET – six procedures were conducted indoors on hormonally synchronized gilts, also assessed by ultrasonography on day 25. No live offspring were obtained; early pregnancies initially detected did not continue. Results were: (I) Field NSET (2/2 recipients did not return to estrus), (II) Closed NSET (1/4 pregnancies confirmed), and (III) Closed SET (1/6 pregnancies confirmed). All apparent pregnancies were lost at early stages, suggesting possible causes such as early embryonic loss or handling-related infections. These preliminary findings underscore both the challenges and the potential of IVP–NSET in pigs, highlighting the necessity for large-scale validation studies and the mandatory inclusion of field ultrasonography to properly assess the feasibility and refine the practical implementation of this technology.
KEYWORDS: Swine. IVF. Pregnancy. Production. Reproduction. Ultrasonography.

RESUMO

A transferência embrionária não cirúrgica (NSET) oferece diversas vantagens em relação à transferência embrionária cirúrgica (SET), incluindo melhoria do bem-estar animal, procedimentos simplificados, redução de custos e a possibilidade de uso repetido. Em suínos, a combinação da produção in vitro de embriões (IVP) com a NSET apoia biotecnologias avançadas, potencialmente permitindo um rápido melhoramento genético sem a necessidade de cirurgia ou transporte de animais vivos. Este estudo piloto avaliou a viabilidade da IVP para NSET em condições de campo. Embriões porcinos produzidos in vitro foram transferidos para fêmeas receptoras sincronizadas um dia antes do estágio de desenvolvimento correspondente à IVP. Três protocolos foram testados: (I) NSET em campo – dois procedimentos foram realizados em porcas multíparas com fertilidade comprovada, em condições de campo, com sincronização do estro obtida por ciclagem natural e presunção de prenhez avaliada pela ausência de retorno ao estro. (II) NSET em condições controladas – quatro procedimentos foram conduzidos em ambientes internos controlados com porcas multíparas de fertilidade comprovada, com sincronização do estro por ciclagem natural e diagnóstico de prenhez por ultrassonografia no dia 25. (III) SET em condições controladas – seis

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procedimentos foram realizados em instalações internas com leitoas sincronizadas hormonalmente, também avaliadas por ultrassonografia no dia 25. Nenhum filhote vivo foi obtido; as prenhezes iniciais detectadas não tiveram continuidade. Os resultados foram: (I) NSET em campo (2/2 receptoras não retornaram ao estro), (II) NSET em condições controladas (1/4 prenhezes confirmadas) e (III) SET em condições controladas (1/6 prenhezes confirmadas). Todas as prenhezes aparentes foram perdidas em estágios iniciais, sugerindo possíveis causas como perda embrionária precoce ou infecções relacionadas ao manejo. Esses achados preliminares ressaltam tanto os desafios quanto o potencial da abordagem IVP–NSET em suínos, evidenciando a necessidade de estudos de validação em larga escala e da inclusão obrigatória de ultrassonografia em campo para avaliar adequadamente a viabilidade e aperfeiçoar a implementação prática dessa tecnologia.
PALAVRAS-CHAVE: Suínos. FIV. Prenhez. Produção. Reprodução. Ultrassonografia.

Embryo transfer (ET) in pigs has gained increasing interest over recent decades due to its potential to accelerate genetic progress and support advanced reproductive technologies such as cloning and gene editing. Although ET is routinely implemented in other livestock species, particularly cattle, its widespread adoption in commercial pig production remains limited. This limitation largely stems from anatomical and physiological characteristics and technical challenges specific to swine.

The complex structure of the porcine reproductive tract, featuring a highly convoluted cervix and elongated uterine horns, presents substantial barriers to non-surgical embryo deposition. As a result, traditional ET in pigs has relied predominantly on surgical procedures, which are labour-intensive, costly, and impractical for large-scale application (MARTINEZ et al. 2015).

Additionally, porcine embryos exhibit marked sensitivity to temperature fluctuations and possess high lipid content, rendering them especially vulnerable to damage during cryopreservation (NAGASHIMA et al. 1994). These limitations have impeded the development of robust protocols for embryo storage and transport.

Recent technological advances, however, have improved the feasibility of ET in swine. Refinement of non-surgical ET (NSET) and progress in cryopreservation techniques have expanded the potential for routine ET implementation. NSET has emerged as a safer, less invasive, and more welfare-conscious alternative to surgical ET (SET). Compared with SET, NSET offers reduced animal stress, eliminates the need for anesthesia, shortens procedure duration, and allows repeated attempts across successive cycles without compromising reproductive efficiency, often achieving performance comparable to SET.

The cost of non-surgical techniques is generally lower, making it more accessible to smaller pig producers. Furthermore, if NSET could be performed in the field, there would be no need for specialised facilities. NSET also could have the potential to reach accessibility levels similar to artificial insemination (AI), lowering economic and technical barriers for its adoption. However, NSET has some limitations. The number of embryos that can be transferred is typically lower than in SET, potentially reducing litter size. The timing of ET is less precise, as it relies on the natural oestrus cycle of the recipient sow.

NSET requires additional training and expertise to ensure the proper deposition of embryos into the reproductive tract. Its broader use in pig remains constrained by technical challenges: the sow’s tortuous cervix requires specialized training to minimize intrauterine infections and ensure accurate embryo deposition (MARTINEZ et al. 2004, YOSHIOKA et al. 2012, BIN ALI et al. 2014, HASLER 2014).

NSET has been evaluated with *in vivo*-derived embryos (MARTINEZ et al. 2004, 2015, CUELLO et al. 2005, BIN ALI et al. 2014) and also with *in vitro*-produced (IVP) embryos (SUZUKI et al. 2004, YOSHIOKA et al. 2012) with encouraging results. However, further research assessing the efficacy of NSET using IVP is essential to determine the reliability of this approach in swine reproduction. This combination represents a major advance in porcine reproductive biotechnology, facilitating the application of techniques such as IVF, cloning, and gene editing while enabling repeated embryo transfer attempts within short intervals (CHEN et al. 2022, MARTINEZ-SERRANO & MARTINEZ 2025).

When surgically collecting *in vivo*-derived embryos, the number of embryos available for transfer is limited by donor yield, which restricts transfer potential. Moreover, pregnancy establishment in pigs requires the presence of at least two embryos per uterine horn (DZIUK 1968). In contrast, IVP systems can generate larger numbers of embryos, enabling transfers of greater magnitude; for instance, transferring 40 embryos yields better reproductive outcomes than transferring 30 (MARTINEZ et al. 2015). Nonetheless, the survival of IVP embryos often remains lower than that of *in vivo*-derived embryos (HASLER 2014, MAHMOOD et al. 2024).

Regarding the potential application of NSET using IVP embryos in porcine farms, it would be very useful the possibility of applying it at the field. To date there are no reports describing field NSET using IVP embryos. The present study aimed to evaluate the feasibility of combining field NSET with IVP-derived embryos in sows in a small-scale trial.

With this objective, field NSET with IVP-derived embryos, and also NSET and SET in an animal facility with controlled conditions (that will be called “closed conditions”) were performed according to the experimental design described in Figure 1.

In vitro embryo production: *cumulus*-oocytes complex were collected from slaughterhouse ovaries, matured in IVM media for 44 h at 39 °C in 5% CO_2 (Medium 199 + 10% porcine follicular fluid, 2-mercaptoethanol, cysteine, pyruvate, antibiotics, ITS, 22 h with hMG and cAMP and 22 h without hMG and cAMP). Denuded mature oocytes were fertilized with boar semen (1×10^6 sperm/mL) under mineral oil at 39 °C for 4 h and cultured in SOF at 39 °C (5% CO_2, 7% O_2; LUCHETTI et al. 2023, LORENZO et al. 2024) to day 5 (morula) or 6 (blastocyst).

To reach the minimum number required for NSET (≥40 embryos per transfer), day 5 and 6 embryos from two consecutive IVF were selected and transferred together and PA blastocysts were included, and comprised 10% of the embryos transferred, as previously validated in pilot studies to not impair pregnancy outcomes. Parthenogenetic activation (10% of embryos) was used to achieve the transfer threshold of ≥ 40 embryos per non-surgical transfer. For this, denuded mature oocytes received two 60 V DC pulses, then incubated in activation medium and cultured in SOF at 39 °C (5% CO_2, 5% O_2; LUCHETTI et al. 2016).

All laboratory procedures were performed under sterile conditions inside Class II laminar flow hoods. Incubators were continuously monitored for temperature (39°C), gas composition (5% CO_2, 5–7% O_2), and humidity (>95%).

Recipients and synchronization: animal procedures were approved by the Institutional Ethics Committee (CICUAL, Universidad de Buenos Aires; protocol 2020/15).

Recipients (n=10 Landrace) were synchronized with a 1-day delay to that of IVP embryos (YOSHIOKA et al. 2012): 6 cycling gilts (< 100 kg) for SET, and 4 multiparous sows (~250 kg) for NSET. Gilts received 600 IU eCG followed by porcine LH 72 h later (HAZELEGER & KEMP 2001); in the sows, physiological oestrus was detected via AM–PM method (LLOVERAS et al. 2002). Animals were clinically examined, vaccinated according to farm protocols, and housed in clean, temperature-controlled facilities (18–24 °C) with free access to water.

SET and NSET procedures: SET was performed on Day 5 of the cycle. Oestrus was confirmed by the standing reflex. Gilts were fasted for 24 h but had access to water. Anaesthesia was applied with continuous cardiorespiratory monitoring. A midline laparotomy was performed, and the uterine horn ipsilateral to the ovary bearing *corpora lutea* was exposed. A Tomcat catheter was used to deposit ≥40 IVP embryos suspended in Handling Medium (PBS + streptomycin + BSA). Incisions were sutured in layers, and animals were monitored until full recovery.

NSET: 6 NSET procedures were carried out, 4 under controlled laboratory conditions and 2 under field conditions. NSET under controlled conditions was repeated in each recipient, resulting in 2 NSET per recipient. Animals were restrained in insemination crates. A DeepBlue® ET catheter (Minitube) lubricated with sterile Vaseline was inserted through the cervix and advanced into the upper third of the uterine horn (MARTINEZ et al. 2015). Embryos (≥40 per transfer) were deposited in 1–2 mL Handling Medium.

Regarding environmental and sanitary conditions, controlled NSET were performed indoors at 20–22 °C using sterile disposable equipment. Field NSET were performed in a commercial farm with standard biosecurity (clean crates, disinfected equipment, gloved handling) and embryo manipulation was made indoors on a work disinfected table using a portable stereomicroscope. Embryos were transported ~100 km in vitrification straws filled with Handling Medium placed inside thermally insulated styrofoam containers with heating devices maintaining 39 °C. Total transport time was 2 h. Before transfer, embryos were flushed from straws into a 10 mL syringe containing warmed Handling Medium before flushing into the uterine horn.

Pregnancy assessment: pregnancy was assessed in SET and lab-NSET by non-return to oestrus (day 21) and by ultrasound on day 25. In the field NSET, only non-return to oestrus was available as an indirect measure.

Data analysis: data were described in terms of cleavage and blastocyst rates and pregnancy outcomes (pregnant / non-pregnant) reported per recipient. Due to the small sample size and exploratory nature of the study, results were analysed descriptively. No inferential statistics were applied.

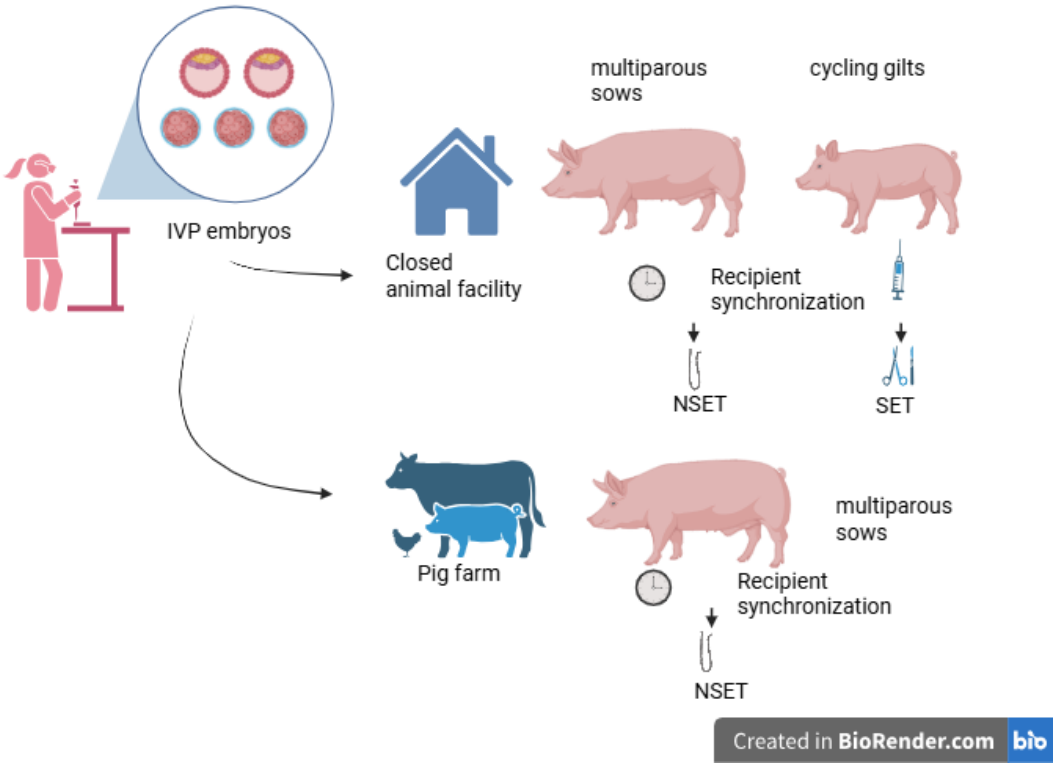


Figure 1. Experimental design consisted of IVP embryos obtained in the lab, recipient female synchronization, and ET as follows: NSET and SET in closed animal facility, and NSET at the field.

To facilitate clarity, results are organized by transfer technique (SET or NSET) and condition (field or closed conditions) and summarized in Table 1. Embryo development was consistent with previous results from our group. IVF embryos: cleavage 54%, blastocyst 16%; PA embryos: cleavage 70%, blastocyst 22% (LUCHETTI et al. 2016, LUCHETTI et al. 2023).

Table 1. Summary of embryo transfers performed under closed and field conditions.

Technique	Number of ET	Presumptive Pregnancies detected	Confirmation method	Embryonic Structures observed	Pregnancy rate	Early pregnancy loss rate
SET	6	1	Ultrasound, Day 25	≥4 sacs	1/6 (17)	1/1 (100)
NSET closed	4	1	Ultrasound, Day 25	≥4 sacs	1/4 (25)	1/1 (100)
NSET field	2	2	No	-	2/2 (100)	1/1 (100)

A total of 10 ET were performed under closed conditions (6 SET and 4 NSET). One pregnancy was detected following SET (1/6) and one following NSET (1/4). Pregnancies were confirmed by transabdominal ultrasonography on Day 25. In both cases, ultrasound examination revealed four or more structures compatible with early embryonic sacs, which were used as the criterion for pregnancy confirmation (Figure 2). Both pregnancies were subsequently lost around day 30, and the embryonic structures were completely resorbed.

A total of 2 NSET were performed under field conditions. Both sows did not return to oestrus, which suggests possible pregnancy, but given the inability to confirm via ultrasound and the known risks of contamination or infection, the pregnancies could not be conclusively verified and might represent either early embryonic losses or adverse effects related to handling procedures.

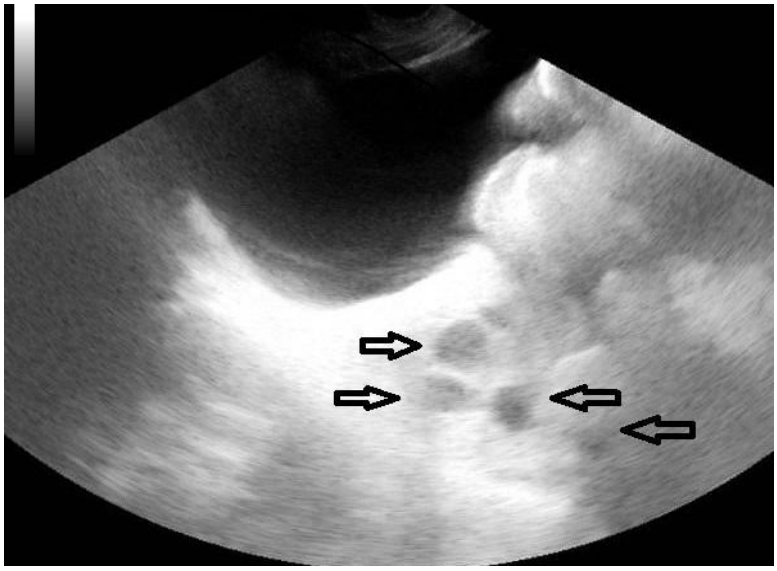


Figure 2. Representative image of the ultrasound performed on one gilt on day 25 of pregnancy. Four presumptive embryonic sacs can be seen (arrows).

This study explores the feasibility of applying field NSET with IVP embryos in sows in a small-scale trial. The novelty of this work is that the field condition was not previously assayed. In parallel, NSET and SET were performed an animal facility with controlled conditions. Concerning NSET in closed conditions, the pregnancy rate observed in this work (25%) is similar to previous reports: 17% (SUZUKI et al. 2004) and 33.3% (YOSHIOKA et al. 2012).

Regarding SET, the pregnancy rate obtained here (17%) is importantly lower than in previous reports: 100% (YOSHIOKA et al. 2011). But in these reports, live piglets were born, whereas in the present work gestations were early lost. Some possible causes for this could be early embryonic loss or handling-related infections. Several factors may have contributed to these outcomes. The developmental competence of IVP embryos remains inferior to that of *in vivo*–derived counterparts (BAUER et al. 2010), particularly when oocytes are sourced from abattoir ovaries, which are known for variable quality.

Current blastocyst formation rates by Day 6 typically reach only 35–40%, and IVP-derived blastocysts contain fewer cells and a lower inner cell mass-to-trophectoderm ratio than their *in vivo*-derived counterparts (CHEN et al. 2022). Other possible influences include the transfer medium volume, the deposition site (YOSHIOKA et al. 2012), the limited operator experience despite veterinary training (MARTINEZ et al. 2004) and the absence of confirmed fertility status in SET recipients, in contrast to the multiparous, proven-fertility females used for NSET.

The use of cycling gilts weighing less than 100 kg for the SET procedures was based on surgical management considerations, since multiparous sows with proven

fertility exhibited substantially greater body mass, which rendered them challenging to restrain in the operating room and to manage appropriately during anaesthesia administration. These observations align with previous reports highlighting the challenges of implementing ART in swine.

Establishment of pregnancy requires at least two embryos per uterine horn (DZIUK 1968), necessitating the transfer of large numbers of embryos—typically 150 one-cell stage embryos or 30 blastocyst-stage embryos per surrogate. High polyspermy rates, poor cryopreservation outcomes, and the absence of standardized non-surgical procedures continue to limit widespread adoption of IVP embryos in commercial settings.

Regarding field NSET, presumptive pregnancy rate observed is very high (100%) but this result is based on a very low number of animals (2) and there is no ultrasound confirmation. The reliance on non-return to oestrus as an indicator of pregnancy was informative in the field but ultimately insufficient, as early pregnancy loss prevented confirmation of gestation. The fact that pregnancies confirmed by ultrasound under controlled conditions were also lost suggests that similar embryonic resorption likely occurred in the field. The high incidence of loss between days 25 and 45 is consistent with well-documented patterns of embryonic mortality in pigs (LAI & PRATHER 2003, ALTHOUSE et al. 2019).

In Argentina, recently it has been made the first SET using *in vivo*-derived embryos (RAMOS et al. 2024). This is the first study of NSET with IVP embryos. The early losses highlight the necessity for further refinement of these techniques. Improvement of IVP culture conditions and oocyte selection strategies to enhance developmental competence.

Larger-scale studies with optimized and standardized protocols are essential to determine whether this technology can be reliably employed in pig reproduction. Importantly, the adaptation of NSET for porcine IVP could broaden access to genetic improvement technologies, particularly benefiting small-scale producers, while also aligning with animal welfare considerations. Besides, IVP-derived embryos are essential for generating genetically modified pigs for agricultural and biomedical research, owing to the physiological similarities between pigs and humans. Porcine models have already contributed substantially to the study of human diseases and to xenotransplantation research (KOPPES et al. 2020, HENDRICKS-WENGER et al. 2021).

Cryopreservation remains a critical bottleneck for IVP systems. The high lipid content of porcine embryos contributes to low survival after vitrification. The most consistent results have been achieved by applying centrifugation and micromanipulation at early developmental stages to remove lipids before blastocyst culture and cryopreservation; solid-surface vitrification has also been proposed to improve cryosurvival, yet further refinement of vitrification protocols is still required (NAGASHIMA et al. 1994, CUELLO et al. 2021, SOMFAI & KIKUCHI 2021).

These preliminary findings, although based on a limited sample size which precludes rigorous statistical analysis, suggest its technical feasibility under experimental conditions. Notably, there are no existing publications reporting pregnancy rates specifically for field NSET of IVP, limiting direct comparison with the

broader literature. This investigation represents a pioneering effort to evaluate field NSET with IVP, highlighting the need to improve IVP and NSET protocols highlighting the necessity for large-scale validation studies and the mandatory inclusion of field ultrasonography to properly assess the feasibility and refine the practical implementation of this technology, before establishing it in swine reproduction. At a local level, it represents also a first approach to NSET with IVP embryos practice.

Future progress will depend on overcoming the current limitations in embryo production and transfer protocols, ultimately enabling broader and more reliable application of this technology in the swine industry.

NOTES

AUTHOR CONTRIBUTIONS

All authors have contributed to this work. In particular:
Conceptualization, methodology, and formal analysis: Luchetti, Renoulin, Lorenzo and Lombardo. Software and validation: Luchetti and Lorenzo. Investigation: Luchetti and Renoulin.Resources and data curation: Luchetti, Renoulin, Lorenzo and Lombardo. Writing-original draft preparation: Luchetti. Writing-review and editing: Lorenzo and Lombardo. Visualization: Luchetti. Supervision: Lombardo.Project administration: Lombardo. Funding acquisition: Lombardo. All authors have read and agreed to the published version of the manuscript.

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INSTITUTIONAL REVIEW BOARD STATEMENT

Animal procedures were approved by the Institutional Ethics Committee (CICUAL, Universidad de Buenos Aires; protocol 2020/15).

DATA AVAILABILITY STATEMENT

The data that supports this study will be shared upon reasonable request to the corresponding author.

INFORMED CONSENT STATEMENT

Not applicable because this study did not involve humans.

CONFLICTS OF INTEREST

There are no conficts of interest.

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