Theriogenology 87 (2017) 316–320

Contents lists available at ScienceDirect

Theriogenology

journal homepage: www.theriojournal.com

Surgical embryo collection but not nonsurgical embryo transfer compromises postintervention prolificacy in sows

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ARTICLE INFO

Article history:
Received 25 July 2016
Received in revised form 28 August 2016
Accepted 6 September 2016

Keywords:
Pigs
Embryos
Nonsurgical embryo transfer
Surgical embryo collection
Future reproductive performance

ABSTRACT

Recent advances in nonsurgical deep uterine (NsDU) embryo transfer (ET) technology allow the noninvasive transfer of porcine embryos into recipients, overcoming the most important impediment for commercial ET in this species. Although many factors in the porcine ET-field have been recently evaluated, many others remain to be explored. We investigated here the future reproductive performance of donors and recipients after artificial insemination subsequent to the default surgical embryo recovery approach and to the NsDU-ET procedure, respectively. Although surgical embryo collection did not influence subsequent farrowing rates (90.5%), litter size decreased severely (8.9 ± 0.8 piglets) compared to presurgery (10.8 ± 0.3 piglets) and control group (10.7 ± 0.3 piglets). In contrast, NsDU-ETs did neither affect fertility nor prolificacy of recipients in the cycle subsequent to ET, regardless of whether they were pregnant after NsDU-ET or not. These results indicate that while the surgical embryo collection procedure compromises the reproductive future of donor sows, the NsDU-ET approach does not affect the reproductive potential of the recipients after reintroduction to the breeding stock of the farm. Further research is thus needed to improve surgical embryo collection.

1. Introduction

Embryo transfer (ET) technology has been a demand of the pig industry for more than 60 years because of its many applications, particularly for the safe exchange of high-value genetic material. With the recent development of nonsurgical deep uterine (NsDU)-ET technology, the commercial use of ET in pigs is now possible. The NsDU-ET technique is a reliable procedure that yields good fertility levels when coupled to fresh embryos [1–3] or stored embryos, either short-term [4] or long-term [5].

Several factors affecting the success of the NsDU-ET procedure, including superovulation of the donors, synchrony between donors and recipients, use of fresh, short-term or long-term stored embryos, number of embryos transferred per recipient, and the recipient's parity, have all been recently assessed [2–6]. However, other factors not directly related to the success of ET have to be quantified to properly estimate the final costs of porcine ET, a most relevant factor for the successful establishment of commercial ET programs. An essential component is the impact that embryo collection and/or ET would have on the future reproductive performance of donors and recipients.

In pigs, unlike other species, the transcervical flushing of the uterus for embryo collection is not effective because of the length and coiled anatomy of the uterine horns [7]. Currently, the collection of embryos from donor sows is only efficient after slaughter or by surgical intervention. Since a common question from genetic companies is the reintroduction of genetically valuable donors into the

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0093-691X/$ – see front matter © 2016 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.theriogenology.2016.09.009

breeding stock of the farm after surgical embryo collection, the impact of this procedure on their future reproductive performance must be evaluated.

In contrast, the NsDU-ET procedure involves the insertion of a flexible and thin device into one uterine horn through a conventional insemination catheter previously inserted into the cervical folds as a guide (reviewed in [8]). The NsDU-ET device is able to progress along the uterine lumen, allowing the deposition of the embryos in the middle or anterior quarter of the uterine horn (reviewed in [9]). To the best of our knowledge, there is no information about the effects of NsDU-ET on the reproductive performance of the recipient sows in subsequent cycles, which is also a matter of concern for recipient farms.

The present study aimed to evaluate the effects of surgical embryo collection and NsDU-ET on the subsequent fertility of donors and recipients, respectively, after artificial insemination (AI).

2. Materials and methods

All experimental procedures 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 (research code: 638/2012).

2.1. Animals and farm management

This work was conducted in field conditions at a commercial farm located in southeastern Spain (Agropor SA, Murcia, Spain). Crossbred sows (Landrace × Large-White) from the same genetic line (1–5 parity), with a lactation period of 21 to 24 days, were randomly selected at weaning and used as donors or recipients. The females were allocated into individual crates in a mechanically ventilated confinement facility. The semen donors were sexually mature boars (2–3 years of age) of proven fertility, housed in climate-controlled individual pens (20 °C–25 °C) at a commercial breeding boar station for AI-dose semen production in Murcia (Spain). The animals had access to water *ad libitum* and were fed commercial diets according to their nutritional requirements.

2.2. Estrus detection in donors and recipients

Weaning was used to synchronize the estrus between the donors and recipients. Only sows with a weaning-to-estrus interval of 4 to 5 days were selected as donors or recipients. Estrus detection was performed by experienced personnel once a day beginning 2 days after weaning by allowing snout-to-snout contact of females with a mature boar and by the application of backpressure by experienced personnel. Animals that showed a standing estrous reflex were considered to be in estrus and used in the experiments.

2.3. Artificial insemination

The sows were postcervically inseminated at 0, 24, and 36 hours after the onset of estrus. The insemination doses (1.5×10^9 spermatozoa in 45 mL) were prepared from the sperm-rich fractions of the ejaculates, extended in Beltsville thawing solution extender [10] and stored for a maximum of 72 hours at 18 °C.

2.4. Surgical interventions

Surgeries were performed in a surgical room located on-farm. Donors were subjected to mid-ventral laparotomy on Day 6 of the estrous cycle (Day 0: onset of estrus). Animals were sedated by the administration of azaperone (2 mg/kg body weight, intramuscular) and narcosis induced using sodium thiopental (7 mg/kg body weight, intravenous) and maintained with 3 to 5% isoflurane. After exposure of the genital tract, corpora lutea were counted on the ovaries, and the embryos were collected as follows; a small incision in the uterine wall 30 to 40 cm below the uterotubal junction was performed with a blunt Adson forcep, and then, a glass cannula was inserted through the incision. A volume of 30 mL of protein-free embryo recovery medium, consisting of Tyrode's lactate (TL)-HEPES-polyvinyl alcohol (PVA; TL-PVA; [11]) with small modifications [4], was introduced into the uterine horn from the tip of the uterine horn using a 60-mL syringe connected to a blunt needle. The flushing medium was forced through the glass cannula into a 50-mL sterile tube by manual massage of the uterus. The incision in the uterus was closed with continuous 2-0 polyglactin 910 sutures. Then, the uterine horns were placed back inside the abdominal cavity, and the mid-ventral incision was closed with continuous one polyglactin 910 sutures in three layers: the peritoneum and linea alba, the adipose layer and the skin. Finally, the incision area was treated with chlorhexidine, and a single intramuscular injection of a long-acting amoxicillin suspension (Clamoxyl LA; Pfizer, Madrid, Spain) at a dose of 15 mg/kg was administered.

2.5. Nonsurgical deep uterine embryo transfer

All NsDU-ETs were performed in weaned recipients that started estrus 0 or 24 hours after the donors using a previously described method [2–4]. Briefly, 6 hours before ET, each recipient received a single intramuscular injection of a long-acting amoxicillin suspension (Clamoxyl LA) at a dosage of 15 mg/kg body weight. The recipients were housed in gestation crates in a small room exclusively used for that purpose. The perineal area was thoroughly cleaned with soap and water using a different sponge for each sow. The tail was covered with a latex glove to protect the vulva from possible contamination. The vulva/vestibulum were then washed using sterile gauze soaked with chlorhexidine. Commercial nonsurgical ET catheters (Deep Blue ET catheter, Minitüb, Tiefenbach, Germany) were used. When the catheter was completely inserted into one uterine horn, a 1-mL syringe containing 30 embryos in 0.1 mL of TL-PVA medium was connected to the catheter, and the contents introduced into the catheter. An additional volume of 0.3 mL of TL-PVA medium was used to force the embryos into the uterus.

2.6. Experimental design

The influence of surgical embryo collection and NsDU-ET procedures on the future reproductive potential of