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The effects of superovulation of donor sows on ovarian response and embryo development after nonsurgical deep-uterine embryo transfer

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ABSTRACT

This study aimed to evaluate the effectiveness of superovulation protocols in improving the efficiency of embryo donors for porcine nonsurgical deep-uterine (NsDU) embryo transfer (ET) programs. After weaning (24 hours), purebred Duroc sows (2–6 parity) were treated with 1000 IU (n = 27) or 1500 IU (n = 27) of eCG. Only sows with clear signs of estrus 4 to 72 hours after eCG administration were treated with 750 IU hCG at the onset of estrus. Nonhormonally treated postweaning estrus sows (n = 36) were used as a control. Sows were inseminated and subjected to laparotomy on Days 5 to 6 (Day 0 = onset of estrus). Three sows (11.1%) treated with the highest dosage of eCG presented with polycystic ovaries without signs of ovulation. The remaining sows from nonsuperovulated and superovulated groups were all pregnant, with no differences in fertilization rates among groups. The number of CLs and viable embryos was higher ($P < 0.05$) in the superovulated groups compared with the controls and increased ($P < 0.05$) with increasing doses of eCG. There were no differences among groups in the number of oocytes and/or degenerated embryos. The number of transferable embryos (morulae and unhatched blastocysts) obtained in pregnant sows was higher ($P < 0.05$) in the superovulated groups than in the control group. In all groups, there was a significant correlation between the number of CLs and the number of viable and transferable embryos, but the number of CLs and the number of oocytes and/or degenerated embryos were not correlated. A total of 46 NsDU ETs were performed in nonhormonally treated recipient sows, with embryos (30 embryos per transfer) recovered from the 1000-IU eCG, 1500-IU eCG, and control groups. In total, pregnancy and farrowing rates were 75.1% and 73.2%, respectively, with a litter size of 9.4 ± 0.6 piglets born, of which 8.8 ± 0.5 were born alive. There were no differences for any of the reproductive parameters evaluated among groups. In conclusion, our results demonstrated the efficiency of eCG superovulation treatments in decreasing the donor-to-recipient ratio. Compared with nonsuperovulated sows, the number of transferable embryos was increased in superovulated sows without affecting their quality and *in vivo* capacity to develop to term after transfer. The results from this study also demonstrate the effectiveness of the NsDU ET procedure used, making possible the commercial use of ET technology by the pig industry.

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1. Introduction

The use of embryo transfer (ET) has numerous important applications in pig production, including the movement of genetic resources with minimal risk of disease transmission, reduced transportation costs, and the absence of an effect on animal welfare during transport, compared with the transport of live animals. Despite the enormous interest shown by the pig industry in the development of this technology, its commercial use was very limited, mainly because of the requirement of surgical transfer procedures. Nonsurgical ET was considered an impossible technique for many years because of the complex anatomy of the swine genital tract (reviewed in [1–3]). The cervical folds and the length and coiled nature of the uterine horns were the main barriers to the insertion of a catheter in gilts or sows during metaestrus. However, in the last decade, new perspectives have arisen with the development of a successful procedure for nonsurgical deep-uterine (NsDU) ET (reviewed in [4,5]). The procedure is simple, safe, and well tolerated by the recipients. During the first attempt at NsDU transfer using fresh embryos, an acceptable reproductive performance (71.4% farrowing rate and 6.9 piglets born) was achieved [6], opening new possibilities for the commercial use of ET technology in the pig industry.

A high number of fresh, good-quality embryos per transfer are necessary to achieve optimal reproductive performance in the porcine recipients. Although no studies have compared surgical and nonsurgical ET procedures, it seems that the number of embryos that can be effectively transferred ranges between 15 and 23 for surgical transfers [7–9] and between 24 and 30 for NsDU transfers [6]. Although ovulation rates in pigs vary greatly among breeds and among animals of different ages (gilts and sows) within a breed [10,11], 15 to 25 oocytes can be proposed as typical in this species. These data indicate that the embryos collected from one donor would be enough to perform one ET with a donor-to-recipient ratio very close to 1:1. However, in practice, a percentage of donors do not become pregnant after insemination, some oocytes from pregnant donors are not fertilized, some embryos collected are nontransferable, and the embryo recovery rates are approximately 90%. All these factors together cause the actual donor-to-recipient ratio to be closer to 2:1, resulting in a high cost per transferable embryo. To reduce this ratio, a lower number of embryos per transfer could be used, although this possibility has not yet been evaluated. Another possibility is the superovulation of the donors through the use of eCG. When prepubertal gilts are used as donors, superovulation treatments not only produce a higher ovulation rate and number of embryos compared with nonstimulated mature gilts [12] but also a high percentage (~25%–50%) of unfertilized oocytes and/or degenerated embryos [12–15] and a high individual variability in the ovulatory response [12,16]. For these reasons, the use of prepubertal gilts as embryo donors should be considered carefully. Mature gilts and sow donors can also be stimulated to increase their ovulation rate through the use of gonadotropins after synchronization treatment or after weaning (reviewed in [17]). In addition to the high ovulatory response variability and the increased ovulation rate, it has been shown that the administration of eCG increases embryonic losses in gilts [18,19] and sows [20] at Days 24 to 40 of pregnancy. A limited number of studies have investigated the quality of 5- to 6-day-old embryos collected from superovulated gilts [21] and sows [22] and the subsequent reproductive performance of recipients transferred with this type of embryos. Because these studies, and others using superovulation of the donors for ET, did not include a control (nonsuperovulated) group [8,21–30] making comparisons is impossible, and the efficacy of superovulation has not yet been clearly established.

The objective of the present study was to determine (1) the effect of two doses of eCG to induce superovulation in sows on the number and quality of 5- to 6-day-old embryos and (2) the reproductive performance of recipients after NsDU transfer of embryos collected from superovulated and nonsuperovulated donors.

2. Materials and methods

All chemicals were purchased from Sigma-Aldrich Co. (Alcobendas, Madrid, Spain) unless otherwise stated. All experimental procedures used in the present study were carried out 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.

2.1. Animals

Experiments were conducted under field conditions at a commercial pig-breeding farm (Selección Batallé S.A., Girona, Spain). Purebred Duroc sows (2–6 parity) were used as donors and recipients. The sows were allocated individually to crates in a mechanically ventilated confinement facility and were fed a commercial ration twice a day. Water was provided ad libitum.

2.2. Detection of estrus

Estrus detection was performed by experienced personnel twice a day (7 AM and 5 PM) beginning 2 days after weaning by allowing nose-to-nose contact of females with a vasectomized mature boar and by applying back pressure. Sows exhibiting a standing heat reflex in the presence of the boar were considered to be in estrus. The first day of onset of estrus was designated as Day 0.

2.3. Estrous synchronization and superovulation treatments

Weaning was used to synchronize estrus between donors and recipients. To standardize the schedule of ETs, only sows with a weaning to estrus interval of 3 to 4 days were selected as donors or recipients.

Superovulation of the donors was induced by an intramuscular administration of different doses of eCG (Folligon; Intervet International B.V., Boxmeer, The Netherlands) 24 hours after weaning. Only sows with clear signs of estrus