The effect of **superovulation** with eCG on ovarian response and embryonic development on day 6 of gestation in **multiparous Duroc** sows

Embryo transfer (ET) is a technology with important applications in pig production because it allows the exchange of genetic material with minimal risk of disease transmission and at a reduced cost, thus avoiding the welfare problems associated with transporting animals.

Cuello C, Gil MA, Parrilla I, Martínez CA, Roca J, and Martínez EA

Department of Animal Medicine and Surgery, Faculty of Veterinary Medicine. University of Murcia. 30100 Campus de Espinardo, Murcia.

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Introduction

Despite enormous interest in the development of ET on the part of the swine industry, its commercial use is currently very limited, mainly because it requires surgical procedures. Non-surgical ET was considered impossible for many years because of the complex anatomy of the female porcine genital tract. However, new perspectives have opened over the last decade with the development of a non-surgical deep intrauterine transfer system (reviewed by Martinez *et al.*, 2019). This transfer procedure is simple, safe, and well tolerated by recipients, thus allowing embryos to be deposited deep within the uterine horn. In the first experiments carried out with this ET system with fresh embryos, acceptable reproductive parameters were obtained with a 71.4% farrowing rate and an average of 6.9 piglets born (Martinez *et al.*, 2004), thereby boosting the development of ET in this species.

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To achieve optimal reproductive parameters in recipient sows after ET, a high number of fresh, good-quality embryos must be used.

According to the reference ovulation rate for the species (15–25 oocytes), the embryos collected from a single donor may be sufficient to perform ET, which implies a donor-to-recipient ratio of 1:1.

Number of embryos required

No comparative studies on the number of embryos needed to perform surgical and non-surgical transfers are available, although it is thought that 15–23 embryos are required to guarantee good results in surgical procedures (Berthelot *et al.*, 2007; Cameron *et al.*, 1989; Polge, 1982) while 24–30 embryos are needed for acceptable results with non-surgical systems (Martinez *et al.*, 2004).

However, in practice this is not the case. It must be assumed that a percentage of donors will not become pregnant after artificial insemination; some oocytes from pregnant sows will not be fertilized; some of the embryos recovered from donor females will not be transferable; and the embryo recovery rate will not be 100%.

All these factors mean that the real donor-to-recipient ratio is around 2:1, which results in a significant increase in the cost per transferable porcine embryo.

To reduce this ratio, the following options can be considered:

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1. Consider using fewer embryos per transfer

This possibility has not yet been studied and its impact on reproductive parameters should be evaluated.

2. Achieve superovulation of donor sows with equine chorionic gonadotropin (eCG)

When gilts are used as embryo donors and undergo superovulation treatment, the ovulation rate and number of embryos retrieved is increased compared to untreated gilts (Ziecik *et al.*, 2005). Nonetheless, more unfertilized oocytes and/or degraded embryos are obtained (~25–50%) and there is a high level of individual variability in ovulatory responses (Holtz and Schlieper, 1991; Niemann *et al.*, 1989; Wallenhorst and Holtz, 2002; Ziecik *et al.*, 2005). Thus, the use of gilts as embryo donors should be considered with caution.

3. Stimulate gilts and multiparous sows with gonadotropins

Gilt and multiparous sows can be stimulated hormonally with gonadotropins to increase their ovulation rate after synchronization treatment or after weaning (Brüssow *et al.*, 2009). Very few studies are available on the quality of gestation day 5–6 embryos collected from nulliparous (Rátky *et al.*, 2001) or multiparous (Hazeleger *et al.*, 2000) superovulated donor gilts and/or the reproductive parameters obtained after the transfer of these embryo types to recipient sows. In addition, the little previous research performed in this field is limited by the fact that none of this work included a group of control sows that were not superovulated, thus making it impossible to compare these studies and clearly establish the impact of superovulation treatment.

Although porcine ovulation rates vary greatly between breeds and depend on whether the sow is nulliparous or multiparous, an ovulation rate of 15 to 25 oocytes is typical in swine.

Implementation

Our group set out to perform a series of studies with two objectives:

1. To determine the effect of two doses of eCG during the induction of ovulation in multiparous sows on the quality of embryos at day 5–6 of gestation and subsequently, to investigate the effect of superovulation treatment with gonadotropins in sows from different genetic lines.
2. To determine the reproductive parameters after the deep intrauterine transfer of embryos collected from superovulated and non-superovulated donors.

The first study on the superovulation of donor sows with different doses of eCG (Angel *et al.*, 2014) was carried out under field conditions at a commercial farm (Selección Batallé SA, Girona, Spain).

Material and methods

Donor synchronization and superovulation

90 purebred Duroc sows who had had 2–6 farrowings were used as embryo donors. Estrus synchronization was carried out at weaning and only sows with a weaning–estrus interval of 3 to 4 days were used as embryo donors. Donor superovulation was induced by intramuscular administration of different doses of eCG (Folligon®; Intervet International BV, Boxmeer, The Netherlands) 24 hours after weaning. The eCG doses used were 1,000 IU and 1,500 IU. Only sows showing signs of heat 48 to 72 hours after eCG administration were treated with 750 IU hCG (Veterin Corion, Divasa Farmavic SA, Barcelona, Spain). hCG was intramuscularly administered at the time of the onset of estrus.

Artificial insemination, collection, and embryo evaluation

The donors were inseminated intracervically 0, 24, and 36 hours after the onset of estrus. Subsequently, the embryos were collected by laparotomy from the donor sows on cycle days 5 or 6 (day 0 = onset of estrus), following the protocol previously described by Martínez *et al.* (Martinez *et al.*, 2014).

The recovered embryos were evaluated using a stereomicroscope and were classified according to their stage of development and quality, according to the criteria set out by the International Society for Embryo Transfer (IETS; Wright, 1998):

- Morulae and blastocysts classified as grade 1 or 2 (excellent and good quality, respectively) were considered viable.
- The remaining collected structures, including oocytes, single-cell embryos, and underdeveloped or poor-quality embryos, were discarded and considered unfertilized oocytes and/or degraded embryos.

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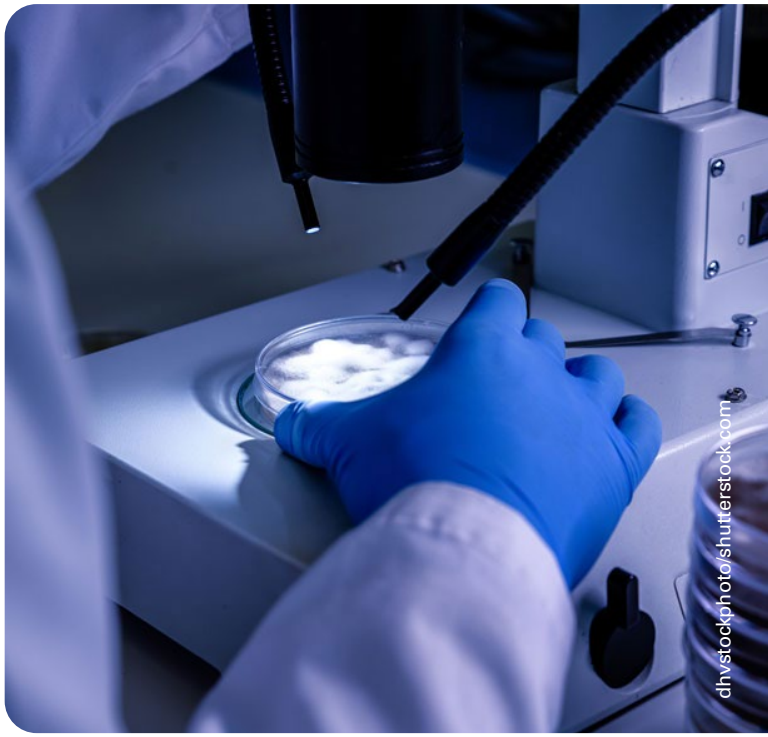
Evaluation of ovulation rates, recovery, fertilization, and the presence of cysts

The ovulation rate was determined by counting the number of corpora lutea (CL) present in the ovaries during the laparotomy procedure. To evaluate the effectiveness of the superovulation treatment, the total number of viable embryos and unfertilized oocytes and/or degraded embryos in each of the donors was quantified.

The recovery rate was defined as the ratio between the number of embryos and oocytes recovered with respect to the total number of CL.

The fertilization rate was defined as the ratio of viable embryos with respect to the total number of embryos and oocytes recovered.

In addition, the presence of follicular cysts (ovarian structures filled with transparent fluid without signs of ovulation and with a diameter exceeding 2 cm at the time of the laparotomy) in the ovaries or the presence of polycystic ovaries (ovaries with more than 8 follicular cysts) was recorded.



In this study we evaluated parameters related to the production and quality of the embryos.

Superovulation treatment results

The effect of superovulation treatment on gestation rates and the number of cysts is shown in the *table*. The potential pregnancy rate (percentage of donors with more than 4 viable embryos) tended (p = 0.07) to be lower in donors superovulated with the highest dose of eCG compared to the control sows. This result was because polycystic ovaries were present in 3 sows (11%) from the 1,500 IU eCG group. None of these sows presented CL in their ovaries, and the size of most of the cysts was > 3 cm. Polycystic ovaries were not observed in the control group or the 1,000 IU eCG

Parameter:	Treatment (IU of eCG)		
	0 (Control)	1,000	1,500
Sows, *n*	36	27	27
Pregnancy rate, *n* (%)	36 (100)	27 (100)	24 (88.9)[a]
Sows with cysts, *n* (%)	14 (38.9)	9 (33.3)	12 (44.4)
Number of cysts in sows with cysts (mean ± SEM)	2.4 ± 0.4	2.9 ± 0.6	3.6 ± 1.0
Sows with polycystic ovaries, *n* (%)	0 (0.0)	0 (0.0)	3 (11.1)[a]

[a]Trend with respect to the control group (p = 0.07); SEM: Standard error of the mean.

Effect of superovulation treatment on gestation rates and incidence of ovarian cysts in donor sows on days 5 and 6 of gestation.

Potential pregnancy rate

The potential gestation rate tended to be lower in the sows superovulated with the highest dose of eCG. This finding can be attributed to the presence of three sows (11.1%) with numerous follicular cysts and without additional CL in the ovaries, a condition associated with infertility (Heinonen *et al.*, 1998). The remaining sows, both in the superovulation group and the control group, were pregnant on days 5 and 6 after insemination, with no significant differences in gestation rates between the groups. Therefore, it seems clear that the superovulation treatment used had no adverse effects on maturation, gamete transport, fertilization, or early embryonic development.

group; the number of cysts ranged between 2.4 ± 0.4 and 3.6 ± 1.0 per sow, with no differences between the groups. There were also no differences in the recovery rates or fertilization rates.

The reproductive parameters we obtained regarding the donor sow ovulation rates and quality of the embryos on day 6 are shown in the *figure*.

The mean number of CL and total number of viable embryos was higher in the superovulation groups and increased in line with the gonadotropin dose ($p < 0.05$). There were no differences between the groups in terms of the number of degraded oocytes and/or embryos. The number of transferable embryos obtained was higher ($p < 0.05$) in superovulated sows than in the control sows. When all the treated sows (pregnant and non-pregnant) were considered, the mean number of transferable embryos was 20.6 ± 1.0 and 22.4 ± 2.1 for the 1,000 IU and 1,500 IU groups, respectively.

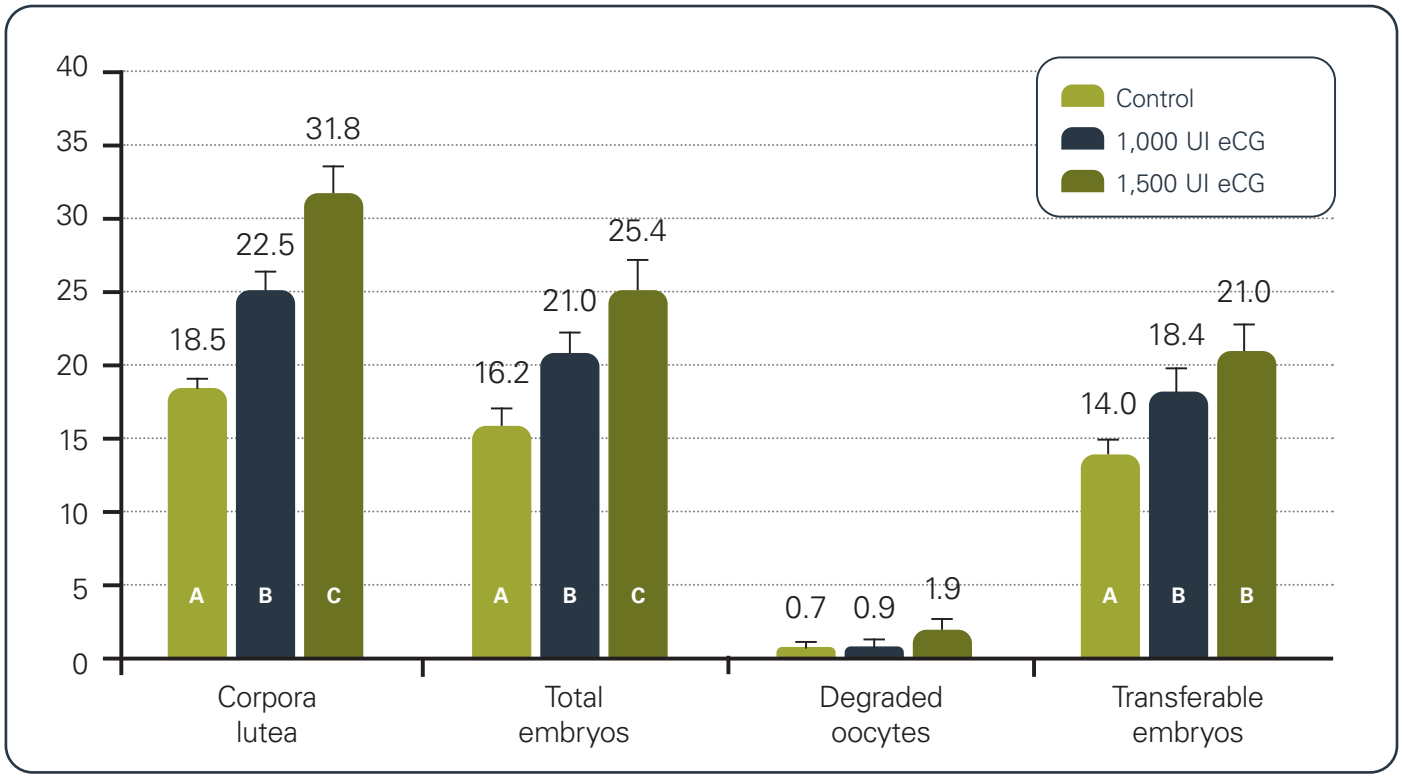
On day 5 of gestation, the percentage of non-transferable embryos was different ($p < 0.01$) between groups (3.5%, 0.0%, and 23.1% for the 1,000 IU, 1,500 IU, and control groups, respectively) only because of the presence of precompacted morulae. On day 6 of gestation, a higher ($p < 0.02$) percentage of non-transferable embryos (8.1%) was obtained in the 1,500 IU eCG group compared to the control (2.4%) and 1,000 IU eCG (2.9%) groups because of the presence of hatched blastocysts.

Presence of ovarian cysts

Regardless of the superovulation treatment applied (1,000 or 1,500 IU of eCG), we found a high proportion of donors (33–44%) had ovarian cysts, with an average of 3 to 3.5 cysts per sow. These figures were like those found in non-superovulated control sows, indicating that the superovulation treatment used did not affect the incidence of cysts.

Although cysts have been associated with a higher rate of return to estrus, a lower farrowing rate, and an increase in the number of sows in anestrus (Castagna *et al.* , 2004), in this study the cysts did not interfere with cyclicity, as shown by the excellent reproductive history and high-quality of the embryos collected. These cysts were most likely non-functional and therefore, did not interfere with the cycles of sows, as previously described for single cysts (Ryan, 1991).

Although the reason for the high percentage of sows with cysts observed in this study is unclear, we cannot rule out the hypothesis that this high incidence is an innate characteristic of purebred Duroc sows.



Influence of superovulation treatment on reproductive parameters in pregnant sows on day 6. Each different letter indicates the presence of a significant difference at $p < 0.05$.

Discussion

The results of this study clearly demonstrate that, at days 5 and 6 of gestation, the embryos obtained from donors superovulated with eCG presented a morphology and developmental capacity like those obtained from non-superovulated donors. Regardless of the superovulation treatment applied (1,000 or 1,500 IU of eCG), we found a high proportion of donors (33–44%) had ovarian cysts, with the number of cysts ranging from 3 to 3.5 per sow. These figures were like those found in non-superovulated control sows, indicating that the superovulation treatment used did not affect the incidence of cysts. Although cysts have been associated with a higher rate of return to estrus, a lower farrowing rate, and an increase in the number of sows in anestrus (Castagna *et al.*, 2004), in this study the cysts did not interfere with cyclicity, as shown by the excellent reproductive history and high-quality of the embryos collected. These cysts were most likely non-functional and therefore, did not interfere with the cycles of sows, as previously described for single cysts (Ryan, 1991). Although the reason for the high percentage of sows with cysts observed in this study is unclear, we cannot rule out the hypothesis that this high incidence is an innate characteristic of purebred Duroc sows.

The potential gestation rate tended to be lower in the sows superovulated with the highest dose of eCG. This finding can be attributed to the presence of three sows (11.1%) with numerous follicular cysts and without additional corpora lutea in the ovaries, a condition associated with infertility (Heinonen *et al.*, 1998). The remaining sows, both in the superovulation group and the control

group, were pregnant on days 5 and 6 after insemination, with no significant differences in gestation rates between the groups. Therefore, it seems clear that the superovulation treatment used had no adverse effects on maturation, gamete transport, fertilization, or early embryonic development.

Superovulation treatments have been associated with a high percentage of unfertilized oocytes and/or degraded embryos. In our study, we not only obtained a greater number of viable and transferable embryos in superovulated donors, but the number of unfertilized oocytes and/or degraded embryos (around 7% of all the collected structures) was also similar in all the groups and was not affected by superovulation. Furthermore, only a small percentage (< 6%) of viable embryos obtained from superovulated donors were classified as non-transferable. This percentage was not because of reduced embryo quality but rather, was because their stage of development (precompacted morula or hatched blastocysts) was not appropriate for ET. Our results contradict previous studies which showed that between 16% and 53% of the embryos recovered from superovulated donors were classified as poor-quality oocytes or embryos (Brussow *et al.*, 2000; Holtz and Schlieper, 1991; Niemann *et al.*, 1989; Ziecik *et al.*, 2005). However, in all these previous reports, superovulation had been carried out in prepubertal sows which produce embryos of poorer quality and which have an inherent lower capacity to produce embryos. It is important to note that in this current work, no correlation was found between the number of corpora lutea and the number of unfertilized oocytes and/or degraded embryos, which therefore contradicts the widely held belief that a high ovulation rate is associated with lower viability and embryonic quality. Considering all the superovulated sows we studied, the administration of 1,000 IU of eCG resulted in a number of transferable embryos like that of the treatment with 1,500 IU (20.6 ± 1.0 versus 22.4 ± 2.1, respectively). Therefore, a dose of 1,000 IU of eCG was considered adequate to induce superovulation in multiparous donor sows.

Conclusion

As a result of this pilot study, we reached the conclusion that the superovulation treatment used increased the efficiency of embryo collection in weaned sows without affecting cyst formation or the number of unfertilized oocytes and/or degraded embryos. A dose of 1,000 IU of eCG was sufficient to obtain an acceptable number of transferable embryos and reduced the donor-to-recipient ratio to 1.4:1 for Duroc sows, thereby helping to facilitate the application of ET in swine.

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