The effect of eCG and hCG-induced superovulation on the ovarian response and pre-implantation embryonic development in **multiparous DanBred, Duroc, and Pietrain sows**

The second part of this article, published in issue 9 of the digital ReproPig magazine, evaluates the effect of superovulation treatment and the efficacy of this protocol in the different genetic lines used.

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Introduction

Embryo transfer (ET) is a technology that offers huge productive and economic advantages to the swine sector. ET allows for the exchange of genetic material with a minimal risk of transmitting diseases and reduces the cost of commercializing animals.

In addition, using ET avoids the animal welfare issues associated with transport (Martinez *et al.*, 2019). Despite its important applications, the commercial use of ET in pigs pales in comparison to its use in cows, where it is routinely applied in production and in genetic improvement systems (Rodriguez-Martinez, 2012). More than 65 years ago, the Russians Kvasnicki and Novoje obtained the first litters using ET with pig embryos (Kavasnicki, 2001). Since then, the development of ET in pigs has been limited by the need to use surgical procedures, both to obtain embryos and for their transfer, because of the special anatomy of the genital tracts of sows. Another big obstacle in the application of ET in swine is the difficulty of using embryo cryopreservation in this species.

Despite all these problems, significant advances have been made over the past decade in porcine ET systems (Martinez *et al.*, 2001) and the vitrification of pig embryos (Cuello *et al.*, 2007, 2008), which could make the commercial application of ET in pigs a reality in the near future. A couple of decades ago, our research group started to develop a non-surgical ET technique that would allow embryos to be deposited inside the uterine

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horn of nulliparous gilts and multiparous sows (Martinez *et al.*, 2001). Since then, we have studied numerous aspects related to the development, safety and efficacy of the procedure (Martinez *et al.*, 2016; Martinez *et al.*, 2013; Martinez *et al.*, 2016). Thanks to the development and improvement of this system, very good results are currently being obtained after the non-surgical transfer of morulas and blastocysts, when transferred either fresh or after storage in liquid nitrogen for 24 h (a 80–90% farrowing rate and a litter size of 9.0–9.5 piglets), as well as for vitrified embryos (a 50–75% farrowing rate and a litter size of 9.0–10 piglets) (Cuello *et al.*, 2016; Martinez *et al.*, 2015; Martinez *et al.*, 2014).

Regardless, there is continued research on superovulation protocols for donor sows, with the aim of developing a safe, practical and efficient ET system for use in the pig industry. The acceptable number of fresh embryos needed to use the non-surgical transfer system is between 24 and 30 embryos (Martinez *et al.*, 2,004), with this figure increasing to 30–40 when vitrified embryos are used (Martinez *et al.*, 2,015). Although sows usually ovulate around 15–25 embryos, various factors mean that the donor–recipient ratio is around 2:1 when working with fresh embryos. Decreasing this ratio would significantly reduce the cost per transferrable embryo, thereby facilitating the commercial application of this technology.


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Thus, our group researched the efficiency of different superovulation protocols in multiparous Duroc sows (Angel *et al.*, 2014). This study showed that 1,000 IU of eCG administered 24 hours after weaning followed by 750 IU of hCG at the beginning of estrus increased the number of transferrable embryos collected and, unlike previous studies (Holtz and Schlieper, 1991), the incidence of follicular cysts, embryo quality and number of unfertilized oocytes were not affected by this treatment.


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What happens in other pig breeds or genetic lines?

An interesting question in superovulation treatment is whether it is equally effective in other breeds or genetic lines, and whether or not it affects the quality of the embryos obtained or the incidence of cysts. For this reason, this same group designed a study to evaluate the efficiency of the superovulation protocol using 1,000 IU of eCG and 750 IU of hCG with the aim of improving the reproductive efficiency of hybrid DanBred, pure Duroc and pure Pietrain pigs as donors.

The donor–recipient ratio was reduced to 1.4:1 in Duroc sows that received this superovulation treatment.

Detecting estrus and superovulation in donor sows

In this study, weaned multiparous sows that had farrowed 2–6 litters from different DanBred (Landrace × Large-White), Duroc and Pietrain genetic lines were used as donors. The donors were housed in individual crates in facilities with mechanical ventilation, fed with a commercial feed twice a day and had water available *ad libitum*. Estrus synchronization was performed at weaning and only the sows who showed a wean-to-estrus interval of 3–4 days were included in the study. Superovulation in the donors was achieved using a protocol previously tested by the same research group (Angel *et al.*, 2014) in which 1,000 IU of eCG (Folligon; Intervet International B.V., Boxmeer, The Netherlands) is administered 24 hours after weaning and 750 IU of hCG (Veterin Corion, Divasa Farmavic SA, Barcelona, Spain) is given when estrus begins. The hormones were administered intramuscularly by injection into the inner thigh (gracilis muscle).

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Estrus detection was carried out starting on day one post-weaning, allowing snout-to-snout contact between the sows and a mature vasectomized boar. Sows that showed the standing reflex in the presence of the boar when pressure was exerted upon their backs were considered to be in estrus. The embryo donors were post-cervically inseminated 0, 24, and 36 hours after the start of estrus with semen from mature boars (aged 2–3 years) from the same genetic line as the donors and with proven fertility. The sperm-rich fraction of the ejaculate was diluted with Beltsville Thawing Solution (Pursel and Johnson, 1975).

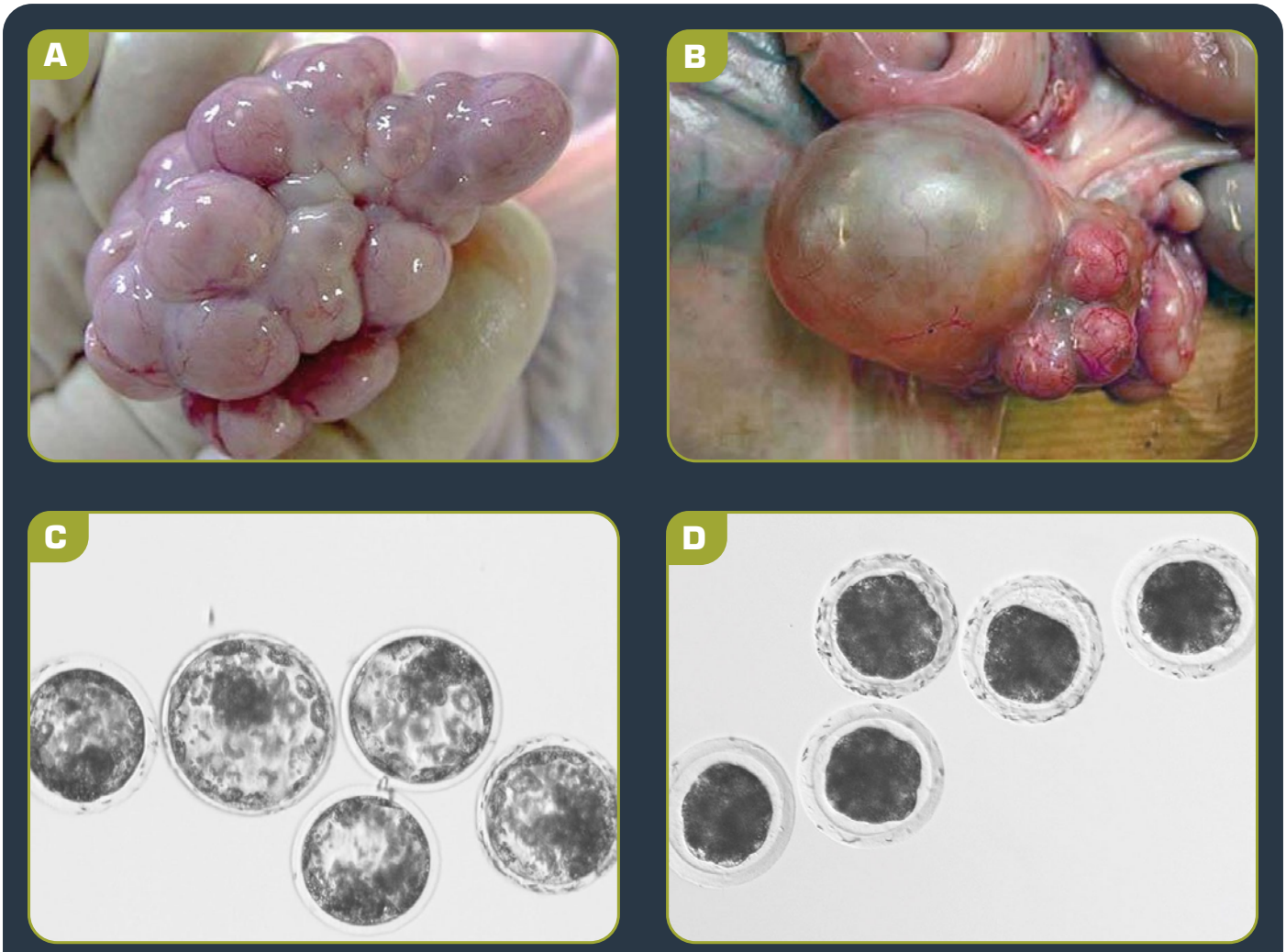
The doses used for artificial insemination contained 1.5×10^9 sperm cells in a volume of 45 mL stored at 18 °C for a maximum of 48 hours.

Evaluation of reproductive parameters and preimplantation embryonic development

On day 6 of gestation (where day 0 = start of estrus), the embryos were surgically collected from the donor, following the protocol described by Martinez *et al.* (2014). The corpora lutea present in the ovaries were counted during the surgeries. In addition, the presence of ovarian follicular cysts (ovarian structures full of transparent liquid without signs of ovulation and a diameter exceeding 2 cm at the time of the laparotomy) was evaluated (as shown in the *figure*). The embryos collected by washing both uterine horns were evaluated with the aid of a stereo microscope and were classified in accordance with International Embryo Transfer Society (IETS) guidelines, based on their developmental stage and quality (Wright, 1998).

A total of 20 sows from each of the genetic lines evaluated (DanBred, Duroc and Pietrain) were used in this study. For each line, 10 donors were superovulated and 10 donors were used as a control group (did not receive the superovulation treatment). All the sows were inseminated in the same way and underwent a laparotomy for embryo collection on day 6 of gestation. The ovulation rate was calculated considering the average number of corpora lutea present in the ovaries (as shown in the *figure*). The effectiveness of the superovulation treatment in donor sows was determined based on the total number of viable versus non-viable embryos collected, and/or non-fertilized oocytes and follicular cysts.

The recovery rate was defined as the number of structures recovered with respect to the total number of corpora lutea.



A: Corpora lutea on day 6 of gestation; B: Follicular cyst; C: Embryos at the blastocyst stage; D: Embryos at the morula stage.

Embryo selection

Grade 1 or 2 embryos (excellent and good quality, respectively) in the morula or blastocyst stage on day 6 were classified as viable. The rest of the structures collected, including poorly developed and/or poor-quality embryos and oocytes were considered non-viable embryos and non-fertilized oocytes, respectively.

All the results are expressed as means ± SEM and the percentages were evaluated using Fisher's exact test. Continuous variables were evaluated using the Kolmogorov–Smirnov test to determine if they were normally distributed and were then compared using ANOVA, applying Bonferroni post-hoc tests. Pearson's correlation coefficient was used to evaluate associations between the number of corpora lutea and other continuous variables (number of viable embryos, non-viable embryos, non-fertilized oocytes and cysts). In all cases, differences were considered significant at $P < 0.05$.

Response to superovulation treatment

All of the reproductive parameters obtained for the control and superovulated sows in this study are represented in *table 1*. All the donors used became pregnant, and in all three donor groups, there was a significant correlation ($P < 0.05$) between the number of corpora lutea and viable embryos collected. However, the number of corpora lutea did not correlate with the quantity of non-viable embryos or non-fertilized oocytes obtained.

The recovery rate was very high and was similar in the three donor groups (range: from 88.0 ± 4.7 to 93.9 ± 3.4%). Moreover, the superovulation treatment did not increase the incidence of follicular cysts (range: 0.2 ± 0.1 to 0.9 ± 0.5), or the number of non-viable embryos or non-fertilized oocytes (range: 0.0 ± 0.0 to 2.2 ± 1.3). In contrast, more corpora lutea and viable embryos were collected in the superovulated donors than in the control donors ($P < 0.05$). The superovulated DanBred sows produced the most ($P < 0.05$) corpora lutea (37.7 ± 2.0) and viable embryos (31.6 ± 2.2). In turn, the Pietrain sows produced more ($P < 0.05$) corpora lutea (28.3 ± 4.5) and viable embryos (26.3 ± 2.6) than the Duroc sows (23.5 ± 1.0 and 21.5 ± 1.2, respectively). Nonetheless, with respect to the control sows, the superovulated DanBred and Pietrain sows produced similar quantities of corpora lutea (12.3 ± 1.9 and 10.5 ± 2.3, respectively) and this exceeded ($P < 0.05$) the amount produced by the Duroc donors (3.1 ± 1.0).

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	DanBred		Duroc		Pietrain	
	Control	Superov.	Control	Superov.	Control	Superov.
Pregnant sows, *N*	10	10	10	10	10	10
Corpora lutea	25.4 ± 1.6^{a}	37.7 ± 2.0^{b*}	20.4 ± 0.7^{a}	23.5 ± 1.0^{b*}	17.8 ± 1.0^{a}	28.3 ± 4.5^{b*}
Cysts	0.2 ± 0.1	0.5 ± 0.4	0.9 ± 0.5	0.6 ± 0.4	0.6 ± 0.4	0.7 ± 0.4
Viable embryos	24.0 ± 1.9^{a}	31.6 ± 2.2^{b}	17.3 ± 1.1^{a}	21.5 ± 1.2^{b}	15.6 ± 1.1^{a}	26.3 ± 4.6^{b}
Non-viable embryos/oocytes	0.0 ± 0.0	2.2 ± 1.3	0.5 ± 0.3	0.1 ± 0.1	0.3 ± 0.1	0.6 ± 0.3
Recovery rate (%)	93.9 ± 2.6	89.9 ± 2.1	88.0 ± 4.7	91.6 ± 2.4	89.5 ± 3.2	93.9 ± 3.4

Control: non-superovulated sows; Superov.: superovulated sows treated with 1,000 IU of eCG and 750 IU of hCG. The data are expressed as means ± SEM. The different superscripts shown for the same breeds indicate significant differences ($P < 0.05$).

Table 1. Reproductive parameters obtained in superovulated and non-superovulated DanBred, Duroc and Pietrain sows on day 6 of gestation.

These results show:

1. That the response to superovulation treatment was different between breeds, with DanBred sows showing the most pronounced treatment response.
2. That in every case, the donor-recipient ratio could be reduced with superovulation treatment (*table 2*), which is highly beneficial in terms of ET applications and for genetics companies.

Breed	Control sows	Superovulated sows
DanBred	1.2:1	0.9:1
Duroc	1.7:1	1.4:1
Pietrain	1.9:1	1.1:1

Table 2. Reduction in the donor-recipient ratio achieved via superovulation treatment with 1,000 IU of eCG and 750 IU of hCG in DanBred, Duroc, and Pietrain sows versus the non-treated control sows for non-surgical transfers performed with 30 embryos.

Conclusion

These results show that the superovulation treatment with eCG and hCG increased the efficacy of *in vivo* production and embryo collection from weaned DanBred, Pietrain and Duroc sows without affecting cyst formation, the number of non-viable embryos and/or non-fertilized oocytes obtained. In addition, although superovulation was effective in all three donor groups, the treatment response differed according to the genetic line considered.

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