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Buserelin Improves Ovulation-Embryo Synchrony Compared With hCG After eCG-Induced Superovulation in Donor Sows

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ABSTRACT

This study directly compares hCG and buserelin as ovulation-inducing agents following eCG-based superovulation in weaned donor sows, focusing on their practical impact on ovulation-embryo synchrony under field conditions. Fifty-five multiparous sows received 1000 IU eCG 24 h after weaning and were treated at onset of estrus with either 750 IU hCG or 10 μg BU. Embryos were recovered on Day 6 after estrus. Ovulatory response, embryo recovery, fertilization rate and viable embryo number did not differ between treatments. Embryo quality parameters were also similar. However, BU-treated sows exhibited improved ovulation-embryo synchrony, with a higher proportion of animals presenting embryos at the blastocyst stage compared with hCG-treated sows ($p = 0.0051$). In conclusion, BU maintains embryo yield and quality while improving ovulation-embryo synchrony compared with hCG in eCG-superovulated donor sows.

1 | Introduction

Efficient porcine embryo transfer (ET) programmes require donor sows capable of producing a high number of developmentally synchronized embryos within a predictable time window (Martinez-Serrano and Martinez 2025). In weaned multiparous sows, eCG-induced superovulation enhances ovulatory response and embryo yield, with ovulation commonly being induced at estrus onset using hCG (Angel et al. 2014). Although effective, hCG acts directly on ovarian LH receptors and has a relatively prolonged live-time effect, that is biological activity, which may contribute to variability in ovulation timing and embryonic stage at recovery (Ziecik et al. 2021). In addition, fluctuations in the availability of veterinary hCG have highlighted the need for alternative ovulation-induction strategies in superovulated donor sows.

GnRH agonists, such as buserelin (BU), induce ovulation indirectly by stimulating endogenous LH release from the pituitary, more closely resembling the physiological preovulatory LH surge (Knox 2015). While BU has been shown to narrow the ovulation window in insemination protocols (Falceto et al. 2023), its effect on ovulation-embryo synchrony under eCG-based superovulation remains unclear. The present study compared hCG and BU for ovulation induction following eCG-based superovulation in weaned sows, testing the hypothesis that BU would improve ovulation-embryo synchrony without compromising ovulatory response or embryo yield.

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2 | Materials and Methods

All procedures were conducted in accordance with Directive 2010/63/EU on the protection of animals used for scientific purposes and were approved by the Ministry of Livestock, Fisheries and Aquaculture of the Region of Murcia, Spain (A13230503).

2.1 | Animals

The study was performed under commercial farm conditions (Agropor SL, Murcia, Spain). A total of 55 multiparous sows (parity 2–6) were selected at weaning based on body condition score (2.8–3.2 on a 5-point scale) and reproductive performance (previous farrowing rate and prolificacy > 90% and 12 piglets born, respectively), with no differences among the groups. Animals were housed individually under ambient conditions of controlled humidity and temperature facilitated by a forced ventilation system in a conventional mechanically ventilated confinement facility and fed a standard commercial diet with ad libitum access to water.

2.2 | Superovulation, Estrus Detection and Insemination

At weaning, sows were randomly assigned to one of two superovulation treatments. All females received 1000 IU eCG intramuscularly 24 h after weaning. Estrus detection was subsequently performed daily between 07:00 and 09:00 h by experienced personnel, starting 24 h after weaning, and the onset of standing estrus was defined as Day 0. Ovulation was induced at the onset of estrus with either 750 IU hCG (hCG group; $N = 24$) or 10 μg BU (BU group; $N = 23$), both administered intramuscularly. Only sows exhibiting estrus within 3–5 days post-weaning were inseminated and included in embryo analyses. The distribution of the weaning-to-estrus interval among these sows was 40.4%, 48.9% and 10.6% for intervals of 3, 4 and 5 days after weaning, respectively. Sows exhibiting estrus outside this interval were excluded from embryo analyses and considered only for pregnancy efficiency calculations.

Sows were inseminated intracervically twice at 0 and 24 h after standing estrus with 3×10^9 spermatozoa in 90 mL BTS; semen motility was > 70% and morphological abnormalities < 15%.

2.3 | Embryo Recovery and Evaluation

On Day 6 after the onset of estrus, sows underwent laparotomy (Martinez et al. 2015). The ovaries were examined and corpora lutea (CL) counted to determine ovulatory response. Follicular cysts (≥ 1–2 cm, without ovulatory signs) were recorded individually (Gonzalez-Ramiro et al. 2021). Polycystic ovaries were defined as ovaries containing more than eight large follicular cysts and lacking visible corpora lutea. Structures were recovered by flushing with recovery medium (Funahashi et al. 2000), and classified under a stereomicroscope as viable embryos, unfertilized oocytes, or degenerated embryos. Recovery rate was calculated as the number of total structures recovered divided by the number of CL. Embryos were staged according to IETS criteria (Martinez et al. 2025). Compacted morulae and unhatched blastocysts graded as excellent or good were considered viable and transferable. Fertilization rate was calculated as viable embryos relative to total recovered structures. Pregnancy was confirmed by the presence of ≥ 4 viable embryos at laparotomy on Day 6. Pregnancy rate was calculated based only on sows exhibiting estrus within 3–5 days post-weaning, which were considered eligible embryo donors. Pregnancy efficiency was calculated relative to the total number of weaned sows assigned to each treatment group. Sows exhibiting estrus outside the 3–5 day interval were inseminated for routine herd management but were not considered eligible embryo donors and were therefore classified as non-pregnant for the purpose of pregnancy efficiency calculations, reflecting their loss as potential donors in a practical ET programme. Embryo production efficiency was expressed as the number of viable or transferable embryos per total weaned sow. Ovulation–embryo synchrony was assessed from the proportion of donor sows presenting embryos at the morula or blastocyst stage on Day 6 after estrus. Total cell number (TCN) and apoptosis were assessed in full blastocysts using a TUNEL assay (Brison and Schultz 1998) according to manufacturer's instructions. Nuclei were counterstained with Hoechst 33342, and apoptosis was expressed as the proportion of TUNEL-positive nuclei relative to total nuclei.

2.4 | Statistical Analysis

Data were analysed using SPSS software. Continuous variables were evaluated for normal distribution prior to parametric testing. Normally distributed data were compared using Student's *t*-test. Proportional data were analysed using chi-square with Yates correction when appropriate. Results are presented as mean ± standard deviation (SD) or percentages. Statistical significance was set at $p < 0.05$.

3 | Results

Eight of the 55 sows (14.5%) did not exhibit estrus within 3–5 days after eCG treatment and were excluded from embryo analyses. In the remaining 47 sows, the weaning-to-estrus interval averaged 3.7 ± 0.6 days, with estrus occurring mainly on Days 3 (39.1%) and 4 (50.0%), and less frequently on Day 5 (10.9%). Pregnancy rate and pregnancy efficiency were unaffected by treatment (Figure 1A). The incidence and number of ovarian cysts were comparable between groups, and no polycystic ovaries were observed (Figure 1B). Ovulatory response remained within the same range across treatments (33.6 ± 7.9 vs. 35.4 ± 7.6 CL per sow for hCG and BU, respectively; Figure 1C). Similarly, embryo recovery, fertilization rate, and viable embryo numbers were maintained irrespective of treatment (Figure 2A,B).

All viable embryos recovered on Day 6 were classified as compacted morulae or unhatched blastocysts and were therefore considered transferable. Developmental distribution, however, differed between treatments. In the BU group, 91.3% of sows presented embryos at the blastocyst stage, whereas hCG-treated sows showed a more balanced distribution between morulae and blastocysts ($p = 0.0051$; Figure 2C). Embryo quality was

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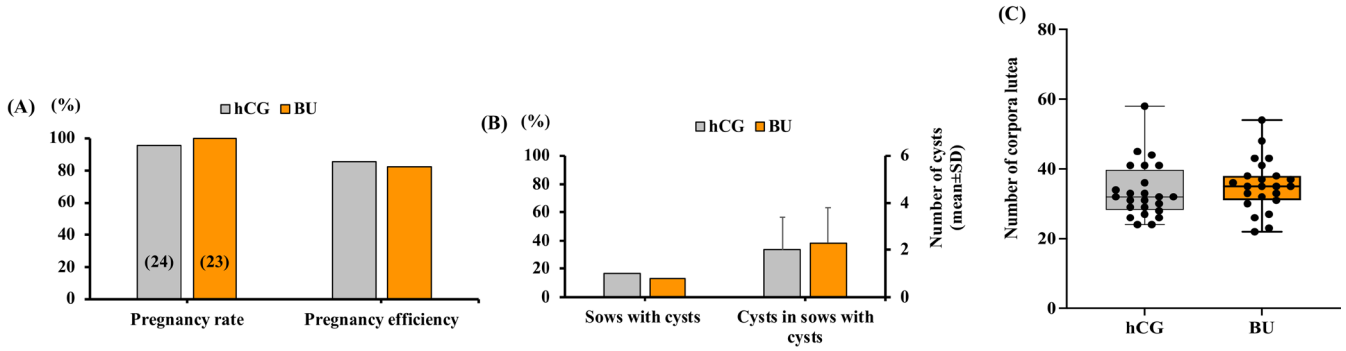


FIGURE 1 | Reproductive performance following ovulation induction with hCG or buserelin (BU) in eCG-superovulated donor sows. (A) Pregnancy rate and pregnancy efficiency. Numbers in parentheses indicate the number of sows per group. (B) Incidence of ovarian cysts and mean number of cysts per sow presenting cysts. (C) Ovulatory response expressed as number of corpora lutea (CL) per sow. Data are presented as box-and-whisker plots showing the median, minimum and maximum values, with individual data points displayed.

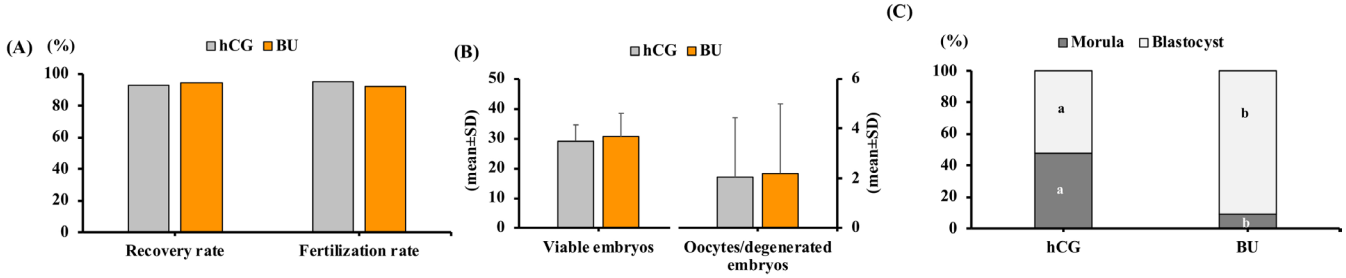


FIGURE 2 | Embryo production parameters and developmental synchrony following ovulation induction with hCG or buserelin (BU). (A) Embryo recovery rate and fertilization rate. (B) Mean number of viable embryos and unfertilized oocytes/degenerated embryos per pregnant sow. (C) Proportion of sows presenting embryos at the morula or blastocyst stage at Day 6. Different letters within the same embryonic stage indicate significant differences between treatments.

unaffected by treatment, with similar TCN (80.9 ± 13.8 vs. 87.1 ± 13.1), apoptosis rate (1.4% ± 1.1% vs. 1.6% ± 1.0%), and proportion of blastocysts containing apoptotic cells (39.4% vs. 41.7%) in both groups.

4 | Discussion

The present study shows that replacing hCG with BU after eCG-based superovulation maintains ovulatory response, fertilization rate, embryo yield and quality in weaned sows, while improving developmental synchrony at Day 6.

The superovulatory response in both groups agrees with previous reports on eCG protocols in weaned sows (Angel et al. 2014). Both treatments resulted in comparable pregnancy rates and similar proportions of viable high-quality embryos, as indicated by TCN and apoptosis parameters, supporting BU as an alternative to hCG, in line with findings from fixed-time insemination studies (Driancourt et al. 2013). BU treatment resulted in a more uniform embryonic distribution, whereas hCG was associated with greater stage dispersion. Because the embryonic stage at recovery reflects ovulation timing (Soede and Kemp 1997), slight differences in the ovulatory window, likely related to the distinct hormonal responses induced by hCG and BU, may explain the stronger synchrony observed in the BU group. This interpretation is consistent with previous studies reporting a relatively predictable ovulatory response following buserelin administration (Pearodwong et al. 2019). Because estrus was detected once daily under commercial farm conditions, an error of up to 24 h in assigning the onset of standing estrus cannot be excluded. However, the same procedure was applied to both treatment groups and therefore any potential effect on embryo stage interpretation would be expected to affect both groups similarly. Although the weaning-to-estrus interval is known to influence reproductive physiology in sows, previous studies conducted under comparable superovulation conditions have shown that weaning-to-estrus intervals between 3 and 5 days do not affect embryo collection efficiency or embryo developmental competence (Martinez et al. 2020). Therefore, the differences in ovulation–embryo synchrony observed in the present study are more likely attributable to the ovulation-inducing treatment than to variation in the weaning-to-estrus interval. Improved synchrony may simplify ET scheduling and batch consistency, which are important practical considerations in commercial systems (Martinez et al. 2016). This suggests the use of BU as an operational rather than biological advantage.

Beyond biological considerations, practical aspects also support the use of alternatives to hCG. Periodic limitations in the availability of veterinary hCG highlight the importance of alternative ovulation-induction protocols. The comparable reproductive performance observed with BU supports its use as a substitute in superovulated donor programmes.

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In conclusion, BU represents a reliable alternative to hCG for ovulation induction after eCG-based superovulation, maintaining embryo yield while improving ovulation-embryo synchrony.

Author Contributions

A.L.-J., I.P. and E.A.M. conceived and designed the study. A.L.-J., M.G.-C., H.G.-R., M.A.G., C.C., I.P. and E.A.M. performed the experiments. A.L.-J., I.P. and E.A.M. analysed and interpreted the data. A.L.-J. wrote the primary manuscript. H.R.-M., E.A.M. and I.P. revised, discussed and edited the manuscript. All authors revised and approved the manuscript for publication. H.R.-M., I.P. and E.A.M. obtained the funding to carry out the study.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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