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Embryo yield in dairy cattle after superovulation with Folltropin or Pluset

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ABSTRACT

Two commercial FSH products were compared in a retrospective study on 3990 commercial superovulations and embryo recoveries in dairy heifers and cows. In addition, the 56-day nonreturn rate of 19,400 embryos produced with these two preparations was analyzed. Embryo collections were performed during a 16-year period from donors of Holstein and Ayrshire breeds. Folltropin (Vetoquinol S.A., Lure cedex, France) group (Group F) consisted of 2592 superovulations, of which 80% were performed on heifers and 20% on cows, and Pluset (Laboratorios Calier, S.A., Barcelona, Spain) group (Group P) of 1398 treatments, of which 66% and 34% were on heifers and cows, respectively. Total number of recovered structures, number of transferable embryos, and the proportion of unfertilized ova (UFO) and degenerated embryos were analyzed. Distribution of embryos into quality grades (1–3) and developmental stages (4–9) according to the IETS classification guidelines and means for each collection were evaluated. The proportion of low-responders having fewer than five corpora lutea and yielding fewer than five embryos or ova was investigated for each treatment. Group P yielded 1.1 recovered structures more than Group F ($P < 0.001$). Consequently, however, the number of transferable embryos did not differ among the groups, being 7.0 and 7.1 in Groups F and P, respectively. Instead, there was an increase in the number of UFO from 2.0 in Group F to 3.0 in Group P ($P < 0.001$). The quality of embryos and the developmental stages were similar between the groups and there was no difference in the proportion of low-responding donors in Group F and Group P. Also, there was no difference in the nonreturn rate after transfer of embryos originating from donors superovulated with Folltropin or Pluset. It was concluded that equal numbers of transferable embryos and pregnancies can be achieved with Folltropin and Pluset.

1. Introduction

The success of superovulation and embryo recovery in cattle depends on numerous donor-related, environmental, and management factors. During the approximately 40-year evolution of superovulation in this species, the number of viable embryos recovered per donor has not elevated appreciably. Instead of increasing the number of transferable embryos, the improvements achieved in this field have facilitated the convenience of protocols (e.g., synchronization of follicular waves, fixed-timed AI) and animal welfare and management ease (e.g., slow-release formulations of FSH). The most significant limiting factor in the success of superovulation has been and continues to be the unpredictability, due to high between-individual variability, in the ovarian response to gonadotropin stimulation. A fundamental source of this variation is the gonadotropin-responsive follicular

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population present in the ovaries during the initiation of the treatment [1,2].

Among several properties of the donor female, environmental conditions such as heat stress [3] and several management factors also contribute to the outcome of superovulation. Management, including nutrition, the superovulation protocol, and semen quality, as well as timing and competence of AI all contribute to the success rate [4–6]. Today, the most frequently used superovulation protocols use purified FSH extracted from porcine or ovine pituitaries. This has been the practice since FSH displaced eCG in the late 1970s. The long half-life and high LH activity of eCG give rise to problems such as prolonged ovarian stimulation and deviant endocrine profiles, unovulated follicles, and compromised embryo quality. These disadvantages led to the use of FSH as the standard approach for superovulation in cattle. In some studies, purified FSH with a low amount of LH has shown to be beneficial for embryo quality compared with high-LH content in the superovulatory treatment (see review by Bo and Mapletoft [7]).

In addition to the type of gonadotropin used, other treatment protocol factors have an impact on the outcome of superovulation. The variation in FSH bioactivity among production batches can affect the success of superovulation [8]. The standard approach is to administer FSH over a period of 4 to 5 days, but prolongation of the treatment period to 6 to 7 days may be beneficial [9–11]. Diluting FSH in hyaluronan for a slow-releasing action, and thereby reducing the number and interval of injections required has proven to be an alternative for the traditional twice-daily intramuscular administration in certain types of donors, but not in dairy cows [12–14].

At present, there are at least three commercial products of porcine pituitary FSH for bovine superovulation approved in the veterinary market of European Union and one in North America. The LH: FSH ratio among these products varies from nearly negligible to equal amounts of each hormone. In Folltropin, the LH: FSH ratio is approximately 0.12, whereas in Pluset the LH: FSH ratio is 1.0. The range of FSH-products on the market is rather limited and in most European countries only one product is licensed. A practical question regarding the possible superiority of one product over another can plague embryo transfer practitioners and farmers. Comparison of these two products by Kelly et al. [15] revealed more ovulations and a higher number of recovered structures in heifers treated with multiple injections of Pluset compared with Folltropin. However, this increase was primarily a result of more unfertilized ova (UFO) and degenerated embryos, not transferable embryos. More freezable quality embryos were recovered from Folltropin-treated heifers. To our knowledge, this is the largest dataset published on *Bos taurus* breeds.

The objective of this retrospective study was to compare the outcome of embryo recovery in a large-scale commercial dataset after superovulation with Folltropin or Pluset, using a standard protocol of eight injections in a decreasing dose. In addition, data on transfers of embryos produced with Folltropin or Pluset were analyzed for the nonreturn rate.

2. Materials and methods

2.1. Donors and superovulation protocol

Data of 3990 superovulations and subsequent embryo collections during a 16-year period (2000–2015) from Finnish dairy farms and embryo transfer station were analyzed. Donor animals were cows (n = 975) and heifers (n = 3015) of Holstein and Ayrshire breeds. Superovulation was induced by eight intramuscular injections of FSH Folltropin (Vetoquinol S.A., Lure cedex, France) or Pluset (Laboratorios Calier, S.A., Barcelona, Spain), at 12-hour intervals over 4 days, beginning from 9 to 12 days after the onset of standing estrus. Folltropin group (Group F) consisted of 2592 superovulations, of which 80% were performed on heifers and 20% on cows, and Pluset group (Group P) consisted of 1398 treatments, of which 66% and 34% were heifers and cows, respectively. The breed distribution among the groups was 59% Ayrshire, 41% Holstein in Group F and 78% Ayrshire and 22% Holstein in Group P. Declining doses of 630 IU (Folltropin) or 850 IU (Pluset) of FSH in total was administered for cows, whereas heifers received 420 to 490 IU (Folltropin) or 500 to 600 IU (Pluset). The manufacturers' recommendation dose for dairy cows is 700 IU of Folltropin and 800 to 1000 IU of Pluset. The dose for cows was 90% and 85% of manufacturers' recommendations for groups F and P, respectively. For heifers, the dose was adjusted taking into consideration the age and size of the donor, varying from 67% to 77% of cows' doses of Folltropin and 59% to 70% of Pluset doses. The donors were treated with a standard dose of $PGF_{2\alpha}$ or a synthetic agonist along with the sixth (cows) or seventh (heifers) FSH treatment.

2.2. Artificial inseminations and semen

Inseminations were initiated 12 hours after the onset of standing estrus. The insemination protocol for conventional semen consisted of two inseminations 9 to 15 hours apart. When sex-sorted semen was used, predominantly three inseminations were performed, using a deep-uterine insemination technique. Two straws of sex-sorted sperm, containing 2 million sperm in each straw, were deposited into uterine horns at both insemination events. On the third insemination only one straw was used by dividing it into half for each uterine horn. Sex-sorted semen was used for 365 donors (9.1% of collections). Despite the fact that sex-sorted semen affects the embryo yield [16], these collections were not excluded from the data because their proportion was relatively small, they did not exceed 13% of collections in any of the groups, and they were distributed quite evenly into the groups.

2.3. Embryo collection and evaluation

Embryos were collected 7 days after inseminations by transcervical uterine flushing using a standard protocol and commercially available media. After collection, embryo morphology was assessed under a stereomicroscope (× 60 magnification) and embryos were classified according to the IETS classification guidelines [17] for quality (grades 1–3)