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Reducing treatments in cattle superovulation protocols by combining a pituitary extract with a 5% hyaluronan solution: Is it able to diminish activation of the hypothalamic pituitary adrenal axis compared to the traditional protocol?

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ABSTRACT

Traditional superovulation protocols that include multiple gonadotropin treatments are time-consuming and labor intensive. These protocols require multiple handling and restraining of embryo donors. This will likely increase the risks of injuries in both animals and humans and induce stress that may lead to a reduced superovulatory response. These are more evident when working with cattle that are rarely handled or raised on extensive grazing. The objectives of this experiment were to compare the efficacy of a split-injection protocol of porcine pituitary-derived porcine FSH (pFSH) preparation (slow release [SR] group) to the traditional 4-day treatment with pFSH administered twice daily (C group) and to determine the concentrations of cortisol in the hair as a marker of activation of the hypothalamic–pituitary–adrenal (HPA) axis during the two superovulatory treatments. Thirty-two heifers were stimulated twice in a 2 × 2 crossover design and compared for ovarian response and numbers and characteristics of recovered ova–embryo among treatments. No differences between SR and C groups were found in terms of percentage of responsive animals (100% vs. 93.8%) and ovulation rate (83.7 ± 1.1 vs. 79.5 ± 1.0%). A positive correlation was found between the number of follicles responsive to pFSH (2–8 mm) at the beginning of treatments and the superovulatory response, and no differences were found in these follicular populations between the two treatment groups. The numbers of CLs, ova–embryos, fertilized ova, transferable and freezable embryos recovered per cow were found to be significantly higher in SR compared with C group (14.0 ± 1.6 vs. 10.6 ± 1.0, 12.1 ± 1.6 vs. 7.6 ± 1.0, 11.1 ± 1.1 vs. 7.3 ± 1.0, 9.6 ± 1.4 vs. 6.6 ± 1.0, and 9.4 ± 1.4 vs. 6.0 ± 1.0 for SR and C group, respectively). The SR group produced also a significantly greater number of excellent- and/or good-quality embryos compared with the C group. The concentrations of cortisol in the hair at Days 14 and 21 were significantly greater in the C compared with the SR group ($P < 0.05$). In conclusion, these results indicate that the dilution of gonadotropin in a 5% hyaluronan solution, reducing the administration frequency, improves the quantitative and qualitative superovulatory response of Marchigiana

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heifers. Further studies using other breeds of cattle are needed to verify the results herein obtained and to confirm the lower activation of the hypothalamic–pituitary–adrenal axis caused in the donor by the split-injection protocol.

1. Introduction

Multiple ovulation and embryo transfer in cattle has been used for more than 4 decades in genetic improvement programs. However, the strong between-animal variability in the superovulatory response continues to be one of the major limiting factors affecting the development and the extensive use of multiple ovulation and embryo transfer in cattle breeding [1,2]. It is now well established that the main source of variability in the response to superovulatory treatments is the size of the pool of gonadotropin-responsive follicles present in the donor's ovaries [3]. In fact, there is a relationship between the number of small antral follicles at the time of initiation of FSH administration and the ovarian response to superovulatory treatment [4–7]. Nevertheless, superstimulation response in donor cattle can be influenced even by other factors related to animal (e.g., breed, age, reproductive history of the donor), environment [8], and the protocol used [9]. The differences in the protocol include the type of gonadotropin administered [10], route of administration [11], batch or dose used, and duration of the superovulation treatment protocol [9,12]. Three different types of gonadotropin have been used to induce superovulation in the cow, namely FSH–LH preparations, eCG, and human menopausal gonadotropin [9,13]. Generally, FSH is preferred to eCG, as FSH treatment results in high ovulation rates, with improved embryo quality and quantity in comparison to treatment with eCG [14,15]. However, when FSH is administered intramuscularly, to obtain a satisfactory superovulatory response, a twice-daily administration is required [15–17] due to its short half-life [18,19]. The labor involving multiple treatments over a period of days is an important factor in the overall cost of superovulation [20], combined with the variability and unpredictability of ovarian response to stimulation and the hormone costs; it can impair the profitability of conventional embryo transfer programs in cattle [21]. The need to repeat six to eight times the FSH administration also increases the possibility of failures due to mishandling and errors in the administration of treatments [22], particularly when used in animals not accustomed to be handled and with irritable temperament, such as zebu or some beef cows reared on free-grazing conditions [23]. Restraining beef cattle for multiple injections also increases the risk of injuries to both cattle and operators [24] and causes stress in donor cows, with subsequent decreased superovulatory response [25,26]. To overcome these drawbacks, several studies have been carried out over the years to reduce the number of administrations in the donors. Bó et al. [11,25], by using a single subcutaneous injection of porcine FSH (pFSH), were able to induce a superovulatory response comparable to that of the traditional eight injections protocol, even if the results change in relation to the site of injection and body condition of the donors. Likewise, most of simplified treatments, realized combining the pituitary extract with agents that cause the hormone to be released slowly over several days, presented some limitations [27]. Recently, Tríbulo et al. [28–30] have conducted a series of experiments administering one or two intramuscular (im) injections of pFSH diluted in a 10% or 5% hyaluronic acid solution, inducing an ovarian response and an embryos production comparable to the traditional approach.

Hyaluronan is a natural polysaccharide with a remarkable biocompatibility, and when used as a diluent, it can facilitate a sustained release of different drugs [31,32]. Despite the efficacy of the single administration protocol, the 10% of hyaluronan makes the solution viscous, and difficult to mix with pFSH and to extract from the vial [28]. The split-injection protocol demonstrated similar efficacy as the single-injection protocol, but the use of 5% hyaluronic acid allowed an easy mixing of the solution and the full recovery from the vial [30]. However, the various protocols proposed by Argentinian researchers [28–30] are not applicable in Europe for two main reasons. First, these authors have used estradiol 17β, which is banned from its use in European veterinary practice (Directives 96/22/EC and 2003/74/EC) [33]. Second, the hyaluronan formulation used in their trials is not approved for parenteral administration. A less expensive and ready-to-use hyaluronan formulation that has been approved for veterinary use, is available in the European veterinary market. Moreover, no experiment with hyaluronic acid as diluting agent has been conducted using pFSH formulation different from that previously used by these authors. On the basis of this consideration and having the need to superovulate Marchigiana heifers, an Italian beef breed that being typically reared on free-grazing condition is particularly restless, and with an irritable temperament, we decide to adapt the previously proposed slow release (SR) protocol [30] than European drug's legislation. One of the indications for the use of the slow-release treatment of pFSH is that it would cause less stress to the donor, however, to our knowledge, this has never been proven. The dosage of cortisol is frequently used to monitor activation of the hypothalamic–pituitary–adrenal (HPA) axis, but collection of biological samples frequently alters the cortisol concentration. Unlike other traditional matrices (serum, saliva, urine, feces) that provide a measurement of the cortisol concentration at a single time point or within 12 to 24 hours, the analysis of cortisol in hair is unaffected by either circadian variations in the hormone or factors inducing short-term variations [34].

The objective of this experiment was to compare the efficacy of a split-injection protocol of porcine pituitary-derived pFSH preparation to a standard superovulatory protocol with pFSH administered twice daily in Marchigiana beef heifers. Our hypothesis was that ovarian response and the number of good-quality embryos produced will be similar between heifers treated with a split-injection