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Use of LH or eCG in the Last Day of Superestimulatory Treatment

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ABSTRACT

In the last decades several hormonal treatments to induce multiple ovulation and embryo transfer (MOET) have been developed. Tight control of the time of ovulation allowed the use of fixed-time artificial insemination (FTAI) in embryos donors, facilitating animal management. Although, protocols that allow FTAI have evolved and yield as much embryo as conventional protocols that requires estrus detection, substantial increase in viable embryo production has not been observed in superestimulated bovine cattle. The present mini-review put emphasis on superstimulatory protocols in which the last two doses of pFSH are replaced by eCG or LH. Recent results indicate that an extra LH stimulus (using eCG or LH), on the last day of P-36 superestimulatory treatment, seems to improve transferable embryo yield in both *Bos taurus* and *Bos indicus* cattle.

Keywords: embryo transfer, superovulation, bovine, FSH, LH, eCG.

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I. INTRODUCTION

Bovine females have been the subject of several researches aiming to improve exploitation of gametes. Gordon [19] reported that offspring calves posses more than 100.000 oocytes in their ovaries, which will generate 0.01% of viable products, i.e., 10 descendents during their reproductive lives. Along with artificial insemination, multiple ovulation and embryo transfer (MOET) and *in vitro* production of embryos are useful tools to increase gametes production [25].

Several treatments to induce multiple ovulations in cattle have been recommended [4,3,6,10]. Equine chorionic gonadotropin administered alone [13] or in combination with eCG antiserum [14], FSH from swine, ovine or equine pituitary extracts [16], or recombinant bovine FSH [22], are some of the hormones that have been utilized to induce superovulation in the cow.

It has been reported that the presence of a dominant follicle at the beginning of the superstimulatory treatment decreases embryo production [18,23]. A few strategies have been developed to avoid the presence of the dominant follicle when initiating superstimulatory treatments, such as starting FSH on the first day of the estrous cycle [20,34], aspirating the dominant follicle or all follicles larger than 5 mm in diameter [8], or synchronizing the follicular wave with estradiol and progesterone [9,11].

Bo et al. [9,11] have shown that the use of progesterone in association with intramuscular administration of estradiol promotes follicle atresia and induces emergence of a new follicle wave approximately 4 days after treatment. To avoid the presence of the dominant follicle, the FSH superstimulatory treatment is initiated at the beginning of the new follicle wave *i.e.*, 4 days after insertion of the progesterone intravaginal device and administration of estradiol. Two days after the first FSH treatment, a luteolytic dose of PGF2α is administered and 12 hours later the intravaginal device is removed. Donor cows are artificially inseminated 12 and 24 hours after estrus detection. Seven days later, the embryos are collected, classified and cryopreserved or transferred.

It has been suggested that follicles that did not ovulate following FSH superstimulation did not develop normally, or did not possess sufficient LHR to be activated by the preovulatory LH surge [39,15,21]. Thus, it has been proposed that postponing the preovulatory LH surge may be a method of increasing embryo production [15,21], and allowing for FTAI in superstimulatory protocols [4,5,6].

Barros & Nogueira [4] examined the efficacy of different protocols in which the expected time of ovulation was postponed by 6 to 12 hours and ovulation was induced by administration of LH or GnRH [4,28]. Although these protocols did not increase the quantity of viable embryos significantly when compared to estrus detection protocols, it was possible to control the ovulation time with the hormonal treatments, allowing the use of FTAI. From these experiments, a new protocol was developed, called the P-36 protocol [5]. In this protocol, the intravaginal progesterone releasing device was left in place for up to 36 hours after PGF2α administration (that is why the protocol was called P-36) and ovulation was induced with exogenous LH, administered 12 hours after withdrawal of the progesterone device (*i.e.*, 48 hours after PGF2á administration). Since ovulation occurs between 24 and 36 hours after LH administration [23], FTAI was scheduled for 12 and 24 hours after LH, avoiding the necessity of estrus detection. The effectiveness of the P-36 protocol has been confirmed [4-6], and more recently, an average of 13.3±0.75 total structures and 9.4±0.63 viable embryos, with a viability rate of 71.0% (1279/1807) following 136 embryo collections in Nelore cows has been reported [30]. These results were comparable to those reported in studies in which Nelore cows were inseminated 12 and 24 hours after onset of behavioral estrus [27,28].

Protocol P-24, a variation of the P-36 protocol in which the progesterone device is removed 24 hours after PGF2a and LH is administered 24 hours later (48 hours after PGF2a), has been utilized in Nelore females with comparable results to those obtained with P-36 protocol [6,40].

Although donors are typically inseminated twice, 12 and 24 hours after administration of LH or GnRH in the P-36 protocols [5,28], it is possible to use a single FTAI. The use of a single straw semen did not significantly reduce the viability rate when FTAI was scheduled at 16 hours after LH administration (viability rates of 52.4 and 58.3%, respectively; [6]) for one *vs* two FTAI in the P-36 protocol. Nevertheless, special attention must be paid to both quality and quantity of semen that is used for a single FTAI in superstimulated donors, in order to avoid a decline in viable embryo production [5,30].

Another modification of the P-36 protocol has been examined in Bonsmara [2] and Nelore cattle [1], in which the last two FSH doses were replaced by administration of eCG. It was expected that on the last day of the

superstimulatory treatment protocol most of the follicles had already acquired LH receptors [17] and would grow in response to eCG due to its capacity to stimulate both LH and FSH receptors [26]. As a consequence, this superestimulatory protocol with eCG would increase ovulation rate and embryo production. Nelore cows (n = 20) were randomly allocated to two groups: P-36 protocol and P-36/eCG protocol, and each animal received both treatments in a crossover experimental design. The animals in the P-36 group received the "original" P-36 treatment protocol, while those in the P-36/eCG group had the last two pFSH treatments replaced by two 200 IU doses of eCG (i.m.). The number of follicles with a diameter >6 mm at the time of administration of pLH (21.1 ± 2.9 *vs* 15.3 ± 2.1) and of the total number of embryos/ova recovered (10.0 ± 1.5 *vs* 6.7 ± 1.2, P < 0.03) were greater in animals that received eCG [1]. These results indicate that the administration of eCG stimulated final follicle growth, possibly due to the presence of LH receptors in follicles larger than 7 mm [31,37], resulting in a greater number of follicles capable of ovulating in response to the administration of exogenous LH. Consequently, eCG increased the total number of ova/embryos recovered, but there was no significant increase in the mean number of viable embryos from cows treated with eCG (7.3 ± 1.20 *vs* 5.1 ± 1.10); however, the total number of viable embryos produced by cows treated with the P-36/eCG protocol (146 *vs*102) suggests that it is advantageous to replace the last two doses of pFSH with eCG. Similar positive results were recently obtained in 47 Brangus females using P-36/eCG (10.9 ± 1.5 viable embryos) as compared to P-36 (7.1 ± 1.4; [33]). On the other hand, Sartori *et al.* [36] reported that there was no increase on embryo production after using eCG in a P-24 protocol (3.7 ± 0.5 viable embryos) as compared to the P-24 protocol alone (4.9 ± 0.7) in Nelore heifers. However, the same authors have recently found in Sindhi females that adding eCG to the P-36 protocol increased the number of viable embryos (6.5 ± 1.2 *vs* 2.4 ± 0.7; P < 0.01; [24]). These data indicate that adding eCG to the P-36 protocols seems to be advantageous, and its use on the P-24 protocol needs to be further investigated. The difference between these two protocols is that in the P-24 protocol the progesterone device is removed 12 hours earlier than in the P-36 protocol, and, consequently, may allow earlier LH secretion that could advance oocyte maturation and disrupt fertilization following FTAI.

Taking into account that consecutive use of eCG reduces bovine embryo production [7], presumably due to antibody production against eCG, replacement of eCG by LH in the last day of superestimulatory treatment, was recently investigated. Rosa et al. [35] studied embryo yield in Angus cows (n = 22) treated with P-36/LH60 (control group), P36/eCG (the last two FSH doses were replaced by two 200 IU doses of eCG), P36/LH (the last two FSH doses were replaced by two 1.0 mg doses of LH), and P36/FSH+LH (the last two FSH doses were immediately followed by two 1.0 mg doses of LH). They observed that replacement of eCG by LH (group P36/LH), resulted in a decline (P < 0.05) in transferable embryos, when compared to the others groups. However, addition of LH to the last two doses of pFSH (P36/FSH+LH) improved embryo quality and numerically increased the total embryo yield (87) when compared to Control (43) and P36/LH group (13), and was similar to P36/eCG (67). These results indicate that eCG can not be replaced by LH (2.0 mg) in Angus cows. However, FSH+LH may be used instead of eCG, in the last day of the superestimulatory treatment.

In order to test if a higher dose of LH would have beneficial effect on embryo production, Oliveira et al. [32], allotted Nelore cows (n = 25) in 4 groups: P-36, P-36/eCG (the last two FSH doses were replaced by two 200 IU doses of eCG), P36/LH2.0 (the last two FSH doses were replaced by two 1.0 mg doses of LH), and P-36/LH4.0 (the last two FSH doses were replaced by two 2.0 mg doses of LH). Each donor was superestimulated four times and received all treatments (crossover). In spite of the tendency of lower ovulation rate in LH2.0 as compared to eCG group (50.6 *vs* 67.8%, respectively, P = 0.06), there was no significant difference among treatments P-36 (3.3 ± 0.7), P-36/eCG (4.5 ± 0.5), P-36/LH2.0 (3.7 ± 0.8) and P-36/LH4 (4.2 ± 1.0) for viable embryo yield. It was concluded that eCG can be replaced by pLH (4.0 mg), in the last day of superestimulatory treatment of P-36 protocol, without affecting viable embryos yield in Nelore cows. Overall, these experiments indicate that an extra LH stimulus (using eCG or LH), on the last day of P-36 superestimulatory treatment, seems to improve transferable embryo yield in both *Bos taurus* and *Bos indicus* cattle. Additional experiments are warranted to confirm and amplify these findings.

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