Theriogenology 80 (2013) 642–647

Contents lists available at SciVerse ScienceDirect

Theriogenology

journal homepage: www.theriojournal.com

Timed artificial insemination should be performed early when used norgestomet ear implants are applied for synchronizing ovulation in beef heifers

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ARTICLE INFO

Article history:
Received 14 February 2013
Received in revised form 2 June 2013
Accepted 6 June 2013

Keywords:
Cattle
Bos indicus
Earlier ovulation
Estrous synchronization
Reproduction

ABSTRACT

The present study evaluated the effect of the type of norgestomet ear implant (new vs. used) on the ovarian follicular response (experiment 1) and pregnancy per artificial insemination (AI) (P/AI; experiment 2) of beef heifers subjected to an estradiol plus progestin timed artificial insemination (TAI) program. In experiment 1, 57 cyclic beef heifers were randomly assigned to one of two groups according to the type (new or previously used for 9 days) of norgestomet ear (NORG) implant. At the time of NORG implant insertion, the heifers were treated with 2 mg of intramuscular estradiol benzoate. Eight days later, the NORG implants were removed, and the heifers received an intramuscular administration of 150 μg of D-cloprostenol, 300 IU of equine chorionic gonadotropin, and 0.5 mg of estradiol cypionate. The heifers had their ovaries scanned every 12 hours from the time of NORG implant removal to 96 hours after verifying the occurrence and timing of ovulation. No difference (P = 0.89) was observed in the ovulation rates between the two treatments (new = 80.0%; 24/30 vs. used = 81.5%; 22/27). However, the heifers treated with a used NORG implant had (P = 0.04) higher proportion (36.4%; 8/22) of early ovulation (between 36 and 48 hours after NORG implant removal) compared with the heifers treated with a new NORG implant (8.3%; 2/24). In experiment 2, at the beginning of the synchronization protocol, 416 beef heifers were randomly assigned into two groups, as described in the experiment 1. Two days after the NORG implant removal, the heifers were reassigned to be inseminated at 48 or 54 hours after NORG implant removal. There was an interaction (P = 0.03) between the type of NORG implant and the timing of TAI on P/AI. The timing of insemination only had an effect (P = 0.02) on the P/AI when the heifers were treated with a used NORG implant [(TAI 54 hours = 41.9% (44/105) vs. TAI 48 hours = 58.6% (58/99)]. In conclusion, beef heifers synchronized with a used NORG implant plus estradiol exhibited a higher proportion of earlier ovulations, and TAI in these heifers should be performed 48 hours after removal of used NORG implants.

1. Introduction

The genetic gain is an important goal in cow-calf operations to increase the production per area and animal productive capacity. This progress can be achieved using reproductive technologies, especially artificial insemination

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0093-691X/$ – see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.theriogenology.2013.06.008

(AI). Thus, proportional enhancement of pregnant females using AI must be a target to reach a successful reproduction program by improving the service rates and pregnancy outcomes.

Despite commercial restrictions observed in some countries such as United States of America, New Zealand, and countries of European Union on the use of estradiol 17-beta and its ester-like derivatives to control the estrous and ovulation in cattle, its hormonal therapy can legally be used in South America. Furthermore, progesterone (P4) plus estradiol-based timed artificial insemination (TAI) protocol has been the most commercially used TAI synchronization protocol in Brazil [1–5]. In beef heifers, a common aspect among the estrus synchronization protocols for TAI is the insertion of an intravaginal device containing P4 or an ear implant containing norgestomet (NORG) plus administration of estradiol benzoate (EB; 2 mg intramuscular) on Day 0; an injection of prostaglandin (PG) $F_{2\alpha}$ on Day 8 or 9 at the moment of device withdrawal plus 300 to 400 IU of equine chorionic gonadotropin (eCG) [2,3,5,6]. Different ovulation inducers with similar efficiency can be used, such as estradiol cypionate (ECP; 0.5 mg intramuscular) at the moment of implant removal, EB (1.0 mg intramuscular) 24 hours after P4/progestin implant removal or GnRH at insemination [4]. TAI is generally performed 52 to 56 hours after device removal [1], and several experiments recently indicated the possibility of increasing this period to 48 to 60 hours after P4/progestin withdrawal [4,7,8].

Using a progestin/P4 implant more than once is a common practice to reduce the cost of estrus synchronization protocols for TAI. Previously used P4 implants provide a lower circulating concentration of P4 during the synchronization protocol [3,9,10]. The P4 has a significant effect on follicle development by regulating LH pulse frequency and indirectly controlling estradiol synthesis [11–14]. Lower circulating concentrations of P4 during the synchronization protocol could result in an increased frequency and amplitude of LH pulses, faster growth of the dominant follicle, and greater concentration of circulating estradiol during the proestrus [12,14,15]. The presence of a larger and mature dominant follicle leads to a short interval from the time of luteolysis or P4 source removal to the onset of estrus [12,16,17]. Therefore, heifers subjected to estrus synchronization protocols under lower levels of circulating P4/progestin (i.e., used implants) could be expected to have a larger follicle size at the time of implant removal, which could increase the occurrence of earlier ovulation, altering the window to perform TAI in cyclic beef heifers.

The optimal time at which insemination should take place relative to ovulation (Insemination-Oulation Interval) depends primarily on the lifespan of spermatozoa and on the viability of the oocyte in the female genital tract [18]. Several experiments have found that 6 hours is the minimum time needed for a viable sperm population capable of fertilization to pass through the oviduct [18–20]. Regarding the oocyte, the most desirable period for fertilization appears to be between 6 and 10 hours after ovulation [21], and the probability of conception decreases when AI is performed near the time of ovulation (less than 6–12 hours before ovulation; [22,23]). Additionally, the fertilization rate drastically decreases when AI occurs after ovulation [23].

Furthermore, the present study aimed to evaluate the timing of TAI (48 or 54 hours after NORG implant removal) according to the number of uses of the NORG implant (new or previously used for 9 days) on ovarian follicular dynamics (experiment 1) and pregnancy per AI (P/AI; experiment 2) in cyclic beef heifers. The hypothesis is that the optimum time to perform TAI depends on the NORG implant (new or used) used for the estrus synchronization protocol in beef heifers.

2. Materials and methods

2.1. Experiment 1: effect of a used NORG implant on ovarian follicular dynamics

2.1.1. Location and animals

This experiment was conducted in a state research farm (APTA—Alta Mogiana Regional Center) located in Colina, São Paulo, Brazil. All of the heifers were kept on *Brachiaria brizantha* pasture and given mineralized salt and free access to water. Data collection was performed during the 2010/2011 (group 1) and 2011/2012 (group 2) spring-summer (November–January) breeding seasons. A total of 57 cyclic Nelore (*Bos indicus*) beef heifers ranging between 20 and 24 months of age were assigned into two groups (group 1 [$n = 32$] and group 2 [$n = 25$]). The heifers were examined by transrectal ultrasonography to determine the presence of a CL on the first day of the synchronization protocol.

2.1.2. Experimental design

At a random stage of the estrous cycle, the heifers were randomly assigned to be treated with a new ($n = 30$) or a previously used NORG implant for 9 days ($n = 27$) (Crestar, MSD Animal Health, Boxmeer, Netherlands) and 2 mg of intramuscular EB (Estrogin, Farmavet, Sao Paulo, Brazil). After initial use, the used NORG implants were individually washed with water and soaked in a solution of chloride alkyl dimethyl benzyl ammonium (CB 30, Ouro Fino Agronegócio, São Paulo, Brazil) for ~10 minutes. Thereafter, the implants were dried using brown paper, thoroughly wrapped in aluminum paper, and stored at room temperature until use. Eight days after the insertion, the NORG implants were removed, and the heifers were given 150 μg of intramuscular D-cloprostenol (Preloban, MSD Animal Health), 300 IU of equine chorionic gonadotropin (eCG, Folligon, MSD Animal Health), and 0.5 mg of estradiol cypionate (ECP, Pfizer Animal Health, São Paulo, SP, Brazil).

2.2. Experiment 2: effect of a used NORG implant on pregnancy per AI

2.2.1. Location and animals

This experiment was conducted in a commercial beef farm located in Rio Verde do MT, Mato Grosso do Sul, Brazil. All of the heifers were kept on a *Brachiaria humidicula* pasture and given mineralized salt and free access to water. Data collection was performed during the 2010/2011 and 2011/2012 spring-summer (November–January) breeding seasons. A total of 416 cyclic Nelore (*B. indicus*) beef heifers aged between 20 and 30 months were used. The heifers were examined by transrectal ultrasonography to