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Effect of fixed-time embryo transfer on reproductive efficiency in high-producing repeat-breeder Holstein cows

C.A. Rodrigues[a], A.A. Teixeira[b], R.M. Ferreira[b], H. Ayres[b], R.F. Mancilha[a], A.H. Souza[b], P.S. Baruselli[b,*]

[a] SAMVET Embriões, Rua Getúlio Vargas 300, CEP 13560-000, São Carlos, SP, Brazil
[b] Departamento de Reprodução Animal, FMVZ-USP, Rua Prof. Dr. Orlando Marques de Paiva 87, CEP 05508-000, São Paulo, SP, Brazil

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ABSTRACT

The aim of the present study was to compare a synchronization of time of ovulation protocol for fixed-timed embryo transfer (FTET) with the usual administration of a single dose of prostaglandin associated with detection of estrus. Also, the effect of the presence of CL at the beginning of FTET protocol was evaluated. Lactating Holstein cows ($n = 651$) with three previous artificial inseminations were classified according to presence or absence of a corpus luteum (CL). Cows with a CL were randomly assigned to two additional treatments and submitted to embryo transfer after detection of estrus (PGF-Estrus) or FTET (FTET-CL). Cows without CL were allocated to the FTET-NoCL treatment. On a random day of the estrous cycle (Day 0), cows in the PGF-Estrus treatment ($n = 229$) were treated with 150 μg d-cloprostenol (PGF) i.m. followed by detection of estrus from Day 1 through Day 5 after PGF. Embryos were transferred 6–8 days after estrus detection. Cows in the FTET-CL ($n = 208$; presence of CL) and FTET-NoCL ($n = 214$; absence of CL) treatments received a norgestomet ear implant plus 2 mg estradiol benzoate (EB) and 50 mg progesterone i.m. on Day 0. On Day 8, the implant was removed and 400 IU eCG, 150 μg d-cloprostenol and 1 mg estradiol cypionate i.m. were administered. No detection of estrus was performed and Day 10 was arbitrarily considered as the estrus day. Ultrasonographic exams were performed in all recipients and only cows with a single CL ≥ 15 mm or multiple CL received a fresh or frozen–thawed embryo on Day 17. Pregnancy was diagnosed by ultrasonography at 30 and 60 days of pregnancy. When FTET and PGF-Estrus were compared, the proportion of cows receiving an embryo (recipients transferred-to-treated rate) was greater in the FTET-CL (75.0% (156/208) than in PGF-Estrus (34.5%, 79/229; $P < 0.0001$) treatment. Pregnancy rate (60 days) was also greater in FTET-CL (29.3%, 61/208) when compared to PGF-Estrus (16.2%, 37/229; $P = 0.001$). However, no differences were found in pregnancy loss [PGF-Estrus = 11.9% (5/42), FTET-CL = 9.0% (6/67); $P = 0.62$] and circulating progesterone concentration at embryo transfer [PGF-Estrus = 4.02 ± 0.52 ng/mL ($n = 25$), FTET-CL = 3.33 ± 0.32 ng/mL ($n = 27$); $P = 0.25$] among these treatments. The presence of CL at the beginning of FTET protocol resulted greater transferred-to-treated rate [FTET-CL = 75.0% (156/208) vs. FTET-NoCL = 61.2% (131/214); $P = 0.003$], but showed no effect on pregnancy rate at 60 days [FTET-CL = 29.3% (61/208) vs. FTET-NoCL = 22.9% (49/214); $P = 0.13$], pregnancy loss [FTET-CL = 9.0% (6/67) vs. FTET-NoCL = 2.0% (1/50); $P = 0.15$] and circulating progesterone concentration at ET [FTET-CL = 3.33 ± 0.32 ng/mL ($n = 27$) compared

* Corresponding author. Tel.: +55 11 3091 7674; fax: +55 11 3091 7412.
E-mail address: barusell@usp.br (P.S. Baruselli).

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to FTET-NoCL = 3.44 ± 0.40 ng/mL (n = 2 9); P = 0.82]. In conclusion, the protocol for synchronization of time of ovulation using norgestomet ear implant, EB and eCG increased recipients transferred-to-treated and pregnancy rates in high-producing repeat-breeder Holstein cows. Also, recipients without CL at the beginning of the time of ovulation synchronization treatment resulted in similar pregnancy rate as recipients with CL submitted to FTET protocol. Thus, the suggested protocol allowed the performance of FTET, without the need for detection of estrus, simplifying the reproductive management and increasing the reproductive efficiency in repeat-breeder Holstein recipients.

1. Introduction

Repeat-breeder cows are usually defined as sub-fertile animals without any anatomic or infectious abnormality and that require three or more services to become pregnant. These cows are characterized by poor fertilization rates (Graden et al., 1968; O'Farrell et al., 1983) and/or early embryonic loss (Linares, 1981; Gustafsson and Larsson, 1985). Thus, embryo transfer (ET) can be potentially used to minimize the eventual effects of uterine environment and lactation on early embryonic development, promoting greater pregnancy rates and, consequently, avoiding early embryonic death.

Success of embryo transfer depends on the efficiency of detection of estrus in recipients. However, only up to 50% of the cows in estrus are detected (Lyimo et al., 2000; Van Eerdenburg et al., 2002). Poorer results may be achieved when high-producing lactating cows are used as recipients, because of the deleterious effects of high milk yield increasing liver metabolism of estradiol and progesterone and consequently reducing expression of estrus and pregnancy rates (Sangsritavong et al., 2002; Lopez et al., 2004; Mann and Lamming, 2000). Several studies also reported a positive association between serum progesterone concentrations, embryonic development, capacity of the conceptus to secrete interferon-τ (Mann et al., 1999), and pregnancy rates in cattle (Santos et al., 2000; Baruselli et al., 2001; Binelli et al., 2001; Thatcher et al., 2001; Bó et al., 2002; Nasser et al., 2004).

One approach to overcome these problems associated with recipients reproductive efficiency is the use of hormonal protocols designed to control both follicular and luteal dynamics, abolishing the need for estrus detection for ET (Baruselli et al., 2000a,b, 2001; Tríbulo et al., 2000; Bó et al., 2001; 2002; Ferreira et al., 2006) Additionally, Rodrigues et al. (2007a) reported in a retrospective study that conception rates in repeat-breeder Holstein cows were greater after ET (41.7%; 1609/3858) than after artificial insemination [AI; 17.9% (1019/5693)], indicating that ET may be an effective alternative to achieve satisfactory conception rates throughout the year, especially during periods of heat stress.

Therefore, the aim of the present study was to compare the use of a single dose of PGF plus estrus detection with a FTET protocol in lactating Holstein repeat-breeder cows. The hypotheses were: (1) recipients treated for FTET would have greater transferred-to-treated and pregnancy rates than cows treated with a single dose of PGF associated with detection of estrus; (2) recipients submitted to FTET without CL at the beginning of the protocol would have similar reproductive efficiency (transferred-to-treated and pregnancy rates) of recipients with CL.

2. Materials and methods

2.1. Farm and animals

This experiment was conducted in a commercial farm in southwest Brazil (22°01′27″S/47°53′19″W) during the autumn (March through June) and winter (June through August), 2006. Lactating Holstein (*Bos taurus*) cows (n = 651; 206 primiparous and 445 multiparous) housed in free stall facilities were used. All recipients were considered repeat-breeders (having ≥three artificial inseminations) averaging 358.7 ± 152.6 (average ± S.D.) days in milk and milk production of 25.5 ± 8.2 kg/day (average ± S.D.). Cows were milked three times daily at approximately 8 h intervals and fed with silage as forage and a corn and soybean meal-based concentrate, sufficient to exceed the nutritional requirements of lactating dairy cows (NRC, 2001). All procedures, including injections, timed AI, ovarian ultrasonography and embryo transfer were approved by the Bioethic Commission of the School of Veterinary Medicine and Zootechny of University of São Paulo, São Paulo.

2.2. Experimental design

Non-pregnant females were evaluated by rectal palpation and classified according to the presence or absence of a CL. Cows with CL were randomly assigned to one of two treatments and submitted to embryo transfer after detection of estrus (PGF-Estrus) or FTET (FTET-CL). Cows without CL were allocated to the FTET-NoCL treatment and submitted to FTET. At a random day of the estrous cycle (Day 0), cows in the PGF-Estrus treatment (PGF-Estrus; n = 229) were treated with 150 μg of d-cloprostenol (PGF; Preloban®, Intervet, Brazil) i.m. followed by detection of estrus from Day 1 through Day 5 after PGF. Embryos were transferred 6–8 days after detected estrus. Cows in the FTET-CL (n = 208; presence of CL) and FTET-NoCL (n = 214; absence of CL) treatments received a norgestomet ear implant (Crestar®, Intervet, Brazil), 2 mg of estradiol benzoate (EB; Estrogin®, Farmavet, Brazil), and 50 mg of progesterone (Progesterona, Index Farmacêutica, Brazil) i.m. on Day 0. On Day 8, the implant was removed and 400 IU of eCG (Folligon®, Intervet, New Zeeland), 150 μg of d-cloprostenol (PGF; Preloban®, Intervet, Brazil) and 1 mg of estradiol cypionate (EC; E.C.P.®, Pfizer, Brazil) i.m. was administered. No detection of estrus was performed and