Theriogenology 119 (2018) 275–281

Contents lists available at ScienceDirect

Theriogenology

journal homepage: www.theriojournal.com

Effect of season on dairy buffalo reproductive performance when using P4/E2/eCG-based fixed-time artificial insemination management

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ARTICLE INFO

Article history:
Received 20 March 2018
Received in revised form
5 July 2018
Accepted 6 July 2018

Keywords:
Buffalo
Reproduction
Artificial insemination
Estrus synchronization
Seasonality

ABSTRACT

This study aimed to compare the reproductive efficiency of dairy buffaloes subjected to TAI protocols based on progesterone, estrogen, and equine chorionic gonadotrophin (P4/E2+eCG) during the fall/winter (n = 168) and spring/summer (n = 183). Buffaloes received an intravaginal P4 device (1.0 g) plus estradiol benzoate (EB; 2.0 mg im) at a random stage of the estrous cycle (D-12). Nine days later (D-3), the P4 device was removed and buffaloes were given $PGF_{2\alpha}$ (0.53 mg im sodium cloprostenol) plus eCG (400 IU im). GnRH (10 µg im buserelin acetate) was administered 48 h after P4 device removal (D-1). All animals were subjected to TAI 16 h after GnRH administration (D0). Frozen-thawed semen from one bull was used for all TAI, which were all performed by the same technician. Ultrasound examinations were performed on D-12 and D-3 to ascertain cyclicity (presence of CL), D-3 and D0 to measure the diameter of the dominant follicle (ØDF), D+10 to verify the ovulation rate and diameter of the corpus luteum (ØCL), and D+30 and D+45 to detect pregnancy rate (P/AI 30d and 45d, respectively) and embryonic mortality (EM). Fetal mortality (FM) was established between 45 days and birth, and pregnancy loss between 30 days and birth. There were significant differences between fall/winter and spring/summer only for cyclicity rate [76.2% (128/168) vs. 42.6% (78/183); P = 0.02]. The others variables did not differ between the seasons: ØDF on D-3 (9.6 ± 0.2 mm vs. 9.8 ± 0.2 mm; P = 0.35); ØDF on D0 (13.1 ± 0.2 mm vs. 13.2 ± 0.2 mm; P = 0.47); ovulation rate [86.9% (146/168) vs. 82.9% (152/182); P = 0.19]; ØCL on D+10 (19.0 ± 0.3 mm vs. 18.4 ± 0.3 mm, P = 0.20); P/AI on D+30 [66.7% (112/168) vs. 62.7% (111/177); P = 0.31]; P/AI on D+45 [64.8%% (107/165) vs. 60.2% (106/176); P = 0.37]; EM [1.8% (2/111) vs. 3.6% (4/110); P = 0.95]; FM [21.9% (18/82) vs. 8.0% (7/87); P = 0.13]; and PL [23.8% (20/84) vs. 12.1% (11/91); P = 0.13]. In conclusion, dairy buffaloes present similar reproductive efficiency in fall/winter and spring/summer when subjected to P4/E2/eCG-based protocol for TAI.

1. Introduction

The buffalo species shows seasonality during shorter days (estrus during fall/winter) and typical reproductive seasonal behavior which intensifies with increasing distance from the equator [1–4]. This physiological characteristic adversely affects the buffalo dairy industry and results in seasonal calving and a dramatic variation of milk supply throughout the year [1,2,5]. Consequently, the availability of buffalo milk for the industry during the fall/winter can reach three times the available amount in the spring/summer [6,7].

Protocols for synchronization of follicular waves and ovulation to timed artificial insemination (TAI) based on the combination of progesterone, estradiol, and equine chorionic gonadotropin (P4/E2+eCG) are able to bypass the reproductive seasonality of female buffalo [3,4,8–13]. Thus, the buffalo industry might be able to

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https://doi.org/10.1016/j.theriogenology.2018.07.004

benefit from genetic improvements arising from AI as well as overcome reproductive seasonality, resulting in calving throughout the year [8,10,14–16].

Although satisfactory pregnancy rates (~50%) were obtained in synchronized buffaloes with TAI P4/E2+eCG-based protocols during the spring/summer [8,9,11], some studies in Italy have shown high rates of pregnancy loss (PL) when buffaloes were inseminated during this period [17]. Rates of embryonic mortality (EM) around 10–45% (between 25 and 45 days after AI) [18–25] and fetal mortality (FM) around 10–15% (between 45 and 70 days after AI) [22–24] have also been reported during the nonbreeding season. Although lower rates of EM between 30 and 43 days (~5%) have been described in Brazil, this data is limited to buffaloes inseminated after estrus detection only during the fall/winter seasons [26].

Some studies were designed to compare the reproductive efficiency of buffaloes during different reproductive seasons [21,27]. However, these Italian researchers were restricted to describing P/AI and EM only up to 45 days of gestation, and using a TAI protocol based on GnRH and PGF2α (Ovsynch). This protocol presents severe P/AI impairment during spring/summer in relation to fall/winter when applied to grazing buffaloes kept under tropical conditions (6.9% vs. 48.8%) [28]. Therefore, there are no studies that describe the real effects of reproductive seasonality on pregnancy rates (P/AI), EM, fetal mortality (FM), and general pregnancy loss (PL) of dairy buffaloes during different reproductive seasons.

Thus, the aim of the present study was to compare the ovarian responses and reproductive performance of dairy buffaloes subjected to TAI protocol based on P4/E2+eCG during the fall/winter and spring/summer. The present hypothesis was that the buffaloes subjected to P4/E2+eCG-based protocol for TAI during the spring/summer showed a detrimental effect on follicular, luteal, and P/AI responses, as well as higher EM, FM, and PL rates compared with buffaloes receiving the same protocol during the fall/winter.

2. Materials and methods

2.1. Ethical considerations

This research (protocol number 2240/2011) was conducted in accordance with the Ethical Principles in Animal Research adopted by the “Ethics Committee in the Use of Animals” of the School of Veterinary Medicine and Animal Science of University of Sao Paulo and was approved on 06/22/2011.

2.2. Farms and animals

The experiment was conducted in five commercial dairy buffalo farms of the Ribeira Valley (Farms 1, 2, 3, 4, and 5) located in the State of Sao Paulo (Table 1), Brazil (southern hemisphere). All farms included in our experiment had already performed synchronized ovulation programs for timed-AI, so there was previous infrastructure to perform TAI management, and distribution of births throughout the year. A total of 351 crossbreed Murrah and Mediterranean buffaloes (37 primiparous and 314 multiparous) were used and maintained exclusively on *Brachiaria* spp. grass, with free access to water. One hundred sixty-eight buffaloes were used during fall/winter (n = 168; winter solstice - May to July) and 183 during spring/summer (n = 183; summer solstice - November to January). At each season of the year, all healthy females that were available for reproduction were included in the study. Data on days in milk (DIM; 111.1 ± 8.8 days in fall/winter and 144.3 ± 8.6 days in spring/summer) and body condition scores (BCS; 3.3 ± 0.0 in fall/winter and 3.3 ± 0.0 in spring/summer) of the females were recorded on the first day of the TAI protocol (Day -12). The BCS scale was from 1 to 5, where 1 corresponded to emaciated animals and 5 corresponded to obese animals [1].

Table 1
Number of dairy buffalo cows subjected to P4/E2/eCG-based protocol for TAI during fall/winter and spring/summer according to farm (1, 2, 3, 4, and 5) and parity (primiparous and multiparous).

Farm	Breeding season				Total
	fall/winter		spring/summer		
	Primiparous	Multiparous	Primiparous	Multiparous	
1	0	25	0	26	51
2	2	12	10	15	39
3	0	70	9	68	147
4	9	12	6	14	41
5	1	37	0	35	73
Total	12	156	25	158	351

2.3. Synchronization of follicular wave emergence and ovulation protocol

At a random stage of the estrous cycle (D-12) (Fig. 1), buffalo cows received 2.0 mg of estradiol benzoate (EB; Sincrodiol®, Ourofino Agribusiness, Cravinhos, Sao Paulo, Brazil) intramuscularly (im) and an intravaginal P4 device (1.0 g of P4, Sincrogest®, Ourofino Agribusiness, Cravinhos, Sao Paulo, Brazil). Nine days after (D-3), the P4 device was removed and 0.53 mg im of sodium cloprostenol (PGF2α; Sincrocio®, Ourofino Agribusiness, Cravinhos, Sao Paulo, Brazil) and 400 IU im of equine chorionic gonadotropin (eCG; Novormon®, MSD Animal Health, Sao Paulo, Brazil) were administered. Ovulation was induced 48 h after the P4 device removal (D-1), using 10 μg im of buserelin acetate (GnRH; Sincroforte®, Ourofino Agribusiness, Cravinhos, Sao Paulo, Brazil). All animals were subjected to TAI 16 h after the GnRH administration (D0).

The inseminations were all performed by the same technician using semen from a single sire with proven fertility in previous TAI programs. All batches of semen used in this study were evaluated through computerized analysis of sperm cells (CASA), using the Hamilton Thorne system (Hamilton Thorne Research, Beverly, USA) used for cattle. The evaluations were conducted in the Andrology Laboratory of the Department of Animal Reproduction, University of Sao Paulo, Sao Paulo, Brazil.

2.4. Ultrasonographic evaluation

The females underwent ovarian follicular dynamic evaluation with a linear transducer of 5.0 MHz (Chison D600Vet, Shenzhen,

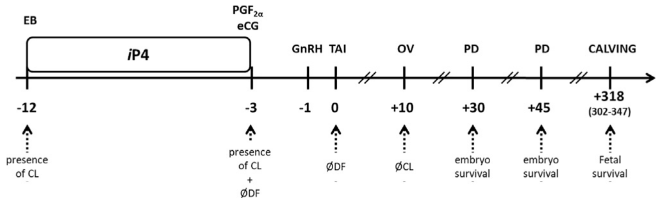

Fig. 1. Schematic diagram of TAI protocol based on P4/E2 plus eCG during fall/winter and spring/summer. EB – 2.0 mg of estradiol benzoate; P4 –1.0 g of intravaginal progesterone device; PGF2α – 0.53 mg of sodium cloprostenol; eCG – 400 IU of equine chorionic gonadotrophin; GnRH - 10 μg of buserelin acetate; TAI – timed artificial insemination; OV – ovulation rate; PD – pregnancy diagnosis; Calving – birth rate; Cyclicity – presence of corpus luteum on D-12 and D-3; ØDF – diameter of dominant follicle on D-3 and D0; ØCL – diameter of corpus luteum on D+10; Embryo survival – Pregnancy per AI (P/AI) on D+30 and D+45 after TAI.