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REGULAR ARTICLES

Pregnancy rate in water buffalo following fixed-time artificial insemination using new or used intravaginal devices with two progesterone concentrations

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Abstract

This study evaluated the pregnancy rate (PR) after timed artificial insemination (TAI) in water buffalo (*Bubalus bubalis*) during both non-breeding and breeding season, using either a new or reused intravaginal device (IVD) with two different progesterone concentrations. A total of 247 dairy buffalo cows were randomly assigned using a two-by-three factorial design and four replicates to the following groups: (1) new intravaginal device (IVD-New: DIB®, 1.0 g of P_4, $n = 51$ or CIDR®, 1.38 g of P_4, $n = 55$); (2) intravaginal device previously used once (9 days) (IVD-Used1x: DIB, $n = 40$ or CIDR, $n = 51$); or (3) intravaginal device previously used twice (18 days) (IVD-Used2x: DIB, $n = 27$ or CIDR, $n = 23$). On day 0, animals received the IVD plus 10.5 μg of buserelin acetate (GnRH) intramuscularly. On day 9, the devices were removed and 25 mg of $PGF_2\alpha$ plus 500 IU of eCG was given intramuscularly. On day 11 (48 h after IVD withdrawal), animals received 10.5 μg of GnRH and were artificially inseminated 8–12 h later. Data were analyzed using Proc Logistic of SAS®. Animals that received IVD-New-DIB, had a significantly higher PR (62.7%; $P = 0.0193$) compared to animals that received IVD-New-CIDR (40%). Pregnancy rate was not negatively affected by reusing both types of IVD. Overall PR (new and reused devices) was higher ($P = 0.0055$) in the DIB group (62.7%) compared to the CIDR group (45%). In conclusion, PR was higher in buffaloes treated with devices containing 1.0 g of P_4 (DIB®) compared to those receiving 1.38 g of P_4 (CIDR®). Reusing the intravaginal devices did not affect negatively PR/TAI, suggesting that P_4 concentrations within the TAI protocols in water buffaloes could be reduced, without impairing their fertility.

Keywords Water buffalo · *Bubalus bubalis* · Timed AI (TAI) · Progesterone · Reused devices

Introduction

Ovulation synchronization and timed artificial insemination (TAI) protocols in water buffalo have the advantage of eliminating the need of heat detection, which is characterized by low efficiency due to poor estrus behavior compared to cattle (Perera 2008). Furthermore, TAI can be used as a tool to improve cyclicity and reproductive performance of buffalo cows, especially during seasonal anestrus. These hormonal protocols are aimed to control follicular development and luteal function, and synchronize the ovulation, allowing TAI without the need of heat detection (Carvalho et al. 2016; Monteiro et al. 2016).

Protocols used during both breeding and non-breeding seasons in buffaloes are based on the use of devices impregnated with progesterone (P4), combined with GnRH (De Rensis et al. 2005) or Estradiol (E_2) (Baruselli et al. 2003; Carvalho et al. 2014; Monteiro et al. 2016). Most of these TAI programs have derived from adaptations of the hormonal protocols used in cattle, based on their follicular wave similarities (Baruselli et al. 1997; Perera 2008; Presicce et al. 2005), and hormonal profiles during the estrous cycle (Presicce 2007).

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In cows, it has been reported that higher P_4 concentrations during TAI protocols could compromise follicular development (Carvalho et al. 2008), ovulation, and pregnancy rates (Dadarwal et al. 2013). Progesterone concentrations during the buffalo cow's estrous cycle are similar to those in cattle (Presicce 2007). However, lower peak of P_4 has been described in buffaloes compared to cows (Perera 2011). This evidence suggests potential negative effects of high P_4 concentration in the IVD on the response to ovulation synchronization and TAI in buffaloes. Studies in Mediterranean and Murrah water buffaloes, synchronized with hormonal protocols based on IVDs containing high P_4 concentration (PRID®: 1.55 g and CIDR®: 1.38 g), GnRH, and $PGF_2\alpha$, reported lower pregnancy rates (De Rensis et al. 2005; Murugavel et al. 2009; Neglia et al. 2003) than those observed in buffaloes treated with IVDs containing lower (1.0 g) P_4 concentrations (Carvalho et al. 2016; Monteiro et al. 2016). Moreover, Carvalho et al. (2014) suggested that lower circulating P_4 concentrations (~1 ng/ml) may be adequate to promote normal ovarian follicular growth, ovulation, and pregnancy in buffaloes treated with TAI protocols based on P_4 IVD during seasonal anestrus. This suggestion is supported by the fact that buffalo cows treated with IVD containing low P_4 concentration (1.0 g) or a reused IVD (once and twice) were able to maintain adequate levels of circulating P_4 (~1 ng/ml), without detrimental effects on pregnancy rate (Carvalho et al. 2016; Monteiro et al. 2016).

We hypothesized that higher P_4 concentration contained in the IVD could reduce the pregnancy rates in water buffalo cows submitted to ovulation synchronization and TAI protocols. Thus, the aim of the present study was to compare the pregnancy rate after TAI in water buffalo (*Bubalus bubalis*) mature cows, using new or reused commercial IVD containing two different progesterone concentrations (DIB®: 1.0 g of P_4 vs. CIDR®: 1.38 g of P_4).

Materials and methods

Animals and management

The study was developed in a commercial water buffalo dairy farm located in the humid rainforest of Venezuela (N: 9° 0′ 35.93″, W: 71° 53′ 25.74″; AMSL: 4 m) during both the non-breeding (from February to June) and breeding seasons (from October to December). A total of 247 multiparous water buffalo cows (1 to 5 calvings), with body condition score between 3 and 3.5 (scale 1 to 5; where 1 = too thin and 5 = too fat; Alapati et al. 2010), were enrolled in this trial in four replicates (two in the non-breeding and two in the breeding season). Animals grazed Tanner grass (*Brachiaria arrecta*) with free access to water and supplemented with a mineral mix ad libitum. Only animals without apparent anatomical anomalies of the reproductive tract (evaluated by ultrasonography per rectum) were included. Data of days postpartum, body condition score (BCS), and parity were recorded on the first day of the TAI protocol (day 0). All cows were milked twice daily (3:00 AM and 2:00 PM) by an automatic milking machine, with the presence of calves to stimulate milk let down.

Experimental design, synchronization of ovulation, and timed artificial insemination protocol

This study was performed using a novel TAI protocol based on intravaginal P_4 device, combined with GnRH and $PGF_2\alpha$ for synchronization of ovulation. Animals received the hormonal treatments on a random day of their estrus cycle (Fig. 1). The experimental design was based on a factorial two-by-three arrangement. On the first day of treatment, animals were randomly assigned to one of the following groups: (1) new intravaginal device [IVD-New: DIB® (Syntex, Buenos Aires, Argentina), $n = 51$ or CIDR® (Eazi-Breed CIDR®, Zoetis Animal Health, Parsippany, NJ, USA), $n = 55$]; (2) intravaginal device previously used once (9 days) (IVD-Used1x-DIB, $n = 40$ or -CIDR, $n = 51$); or (3) intravaginal device previously used twice (18 days) (IVD-Used2x-DIB, $n = 27$ or -CIDR, $n = 23$). The intravaginal devices DIB and CIDR contained 1.0 and 1.38 g of P_4, respectively.

On day 0, animals received the IVD for 9 days and a dose of GnRH analogue (10.5 μg of buserelin acetate, Conceptal®, Intervet, MSD Animal Health, Madison, NJ, USA) intramuscularly (im). On day 9, IVDs were removed and 25 mg de $PGF_2\alpha$ (Lutalyse®, Zoetis Animal Health) plus 500 IU of eCG (Folligon®, Intervet, MSD Animal Health) was administrated im. On day 11 (48 h after IVD withdrawal), animals received 10.5 μg of GnRH (Conceptal®) im for inducing the ovulation and then they were artificially inseminated by a single AI technician 8–12 h later, using semen from a single Murrah bull of known adequate fertility (Fig. 1).

In order to prevent reproductive tract infections, the previously used IVDs were washed and disinfected for 15 min using a solution of 0.5% lauryl dimethyl benzyl ammonium bromide (Gerdex®, Producciones Rodeneza C.A., Caracas, Venezuela).

Ultrasound examination and pregnancy diagnosis

At the onset of the synchronization protocol (day 0), all cows were examined using ultrasonography per rectum to determine the uterine status and ovarian structures. Animals with any reproductive tract abnormality were not included in this study. Ultrasound examination was performed using a CTS 900V ultrasound scanner equipped with a linear rectal multi-

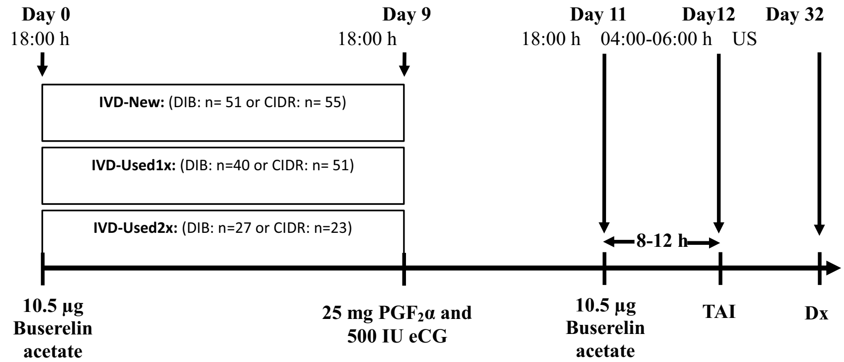

Fig. 1 Schematic diagram showing the P_4 + GnRH/PGF$_2\alpha$/GnRH-based protocol for synchronization of ovulation and timed artificial insemination (TAI) during both the non-breeding and the breeding season in dairy buffalo cows. IVD-New intravaginal device new. IVD-Used1x a device that had previously been used once for 9 days. IVD-Used2x a device that had previously been used twice (18 days). TAI timed artificial insemination, *Dx* pregnancy diagnosis, *US* ultrasonography

frequency probe (SIUI: Shantou Institute of Ultrasonic Instruments Co., Ltd., Guangdong, China).

Pregnancy diagnosis was performed by ultrasonography per rectum 30–32 days after TAI, through detection of an embryonic vesicle with a viable embryo (presence of heartbeat). Pregnancy rate was defined as the number of pregnant buffalo cows divided by the total number of buffalo cows submitted to TAI in each group.

Statistical analysis

Statistical analysis for pregnancy rate was performed using Proc Logistic of the Statistical Analysis System for Windows (SAS Version 9.3, 2001). Comparisons among treatment groups were performed using chi-square test. Statistical significant differences were considered at $P < 0.05$.

Results

Season (non-breeding and breeding season), BCS, days in milk (DIM), and parity at the beginning of the protocols did not affect pregnancy rates ($P >$ 0.05; data not shown). Mean for BCS and DIM were 3.15 ± 0.2 (BCS: 3 to 3.5) and 119.9 ± 17.4 days (DIM: 102 to 140), respectively. Median of parity was 3 (parity: 1 to 5).

Pregnancy rate (PR) obtained in each group after TAI, using either a new or reused IVD with two different P_4 concentrations, is shown in Fig. 2. The overall pregnancy rate (including new and reused IVD) was higher ($P = 0.043$) in buffalo cows in the DIB group than those in the CIDR group (62.7%, $n = 74/118$ vs. 45%, $n = 58/129$, for DIB and CIDR groups, respectively).

Moreover, animals that received a new DIB device (IVD-New-DIB) had a significantly higher ($P = 0.0193$) pregnancy rate compared to animals that received a new CIDR device (IVD-New-CIDR; 62.7%, $n = 32/51$ vs. 40%, $n = 22/55$, respectively; Fig. 2).

Likewise, logistic regression analysis showed a significant ($P = 0.0196$) effect of treatment groups on pregnancy rate (Table 1). The odds ratio (OR) for the occurrence of pregnancy indicated that buffalo cows belonging to the IVD-New-DIB and IVD-Used1x-DIB groups were 2.5 and 2.7 times more likely to become pregnant than buffalo cows in the IVD-New-CIDR group. Similarly, the OR for occurrence of pregnancy in buffalo cows in the DIB groups (new and reused DIB) was 2.1 times greater when compared to the CIDR groups (new and reused CIDR).

Discussion

This study showed that the type of P_4 IVD (CIDR vs. DIB) within ovulation synchronization and TAI protocols affects pregnancy rate in water buffalo cows. Pregnancy rate was greater in buffalo cows treated with IVD containing 1.0 g of

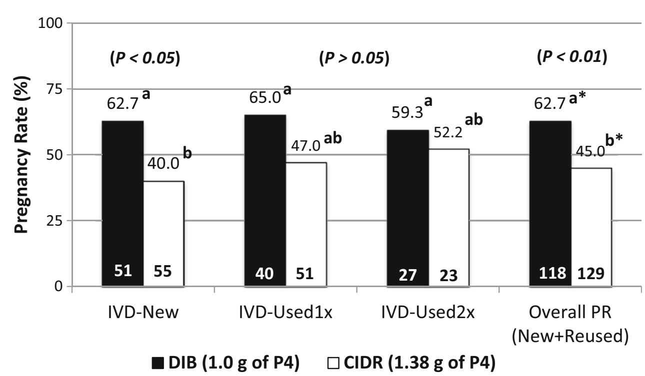

Fig. 2 Pregnancy rates in dairy buffalo cows after TAI using new (New) or reused (Used1x; Used2x) intravaginal devices (IVD) with two different progesterone concentrations (1.0 g vs.1.38 g of P_4). Superscript letters a and b mean among white or black chart bars with different superscripts differ ($P < 0.05$). Superscript letters a and b with asterisks mean white or black chart bars with different superscripts differ ($P < 0.01$). Overall PR overall pregnancy rate, considering new and reused devices

Table 1 Pregnancy rates after using new or reused intravaginal devices with two different progesterone concentrations (1.0 vs. 1.38 g) in water buffalo synchronized with two commercial (DIB® vs. CIDR®) devices

Item	Treatment groups	Pregnancy rate (%)	Odds ratio (OR)	95% (CI)	*P* value
CIDR	New vs. Used1x	40 vs. 47	1.3	0.61–2.88	0.46
	New vs. Used2x	40 vs. 52	1.6	0.61–4.35	0.32
	Used1xvs. Used2x	47 vs. 52	1.2	0.45–3.28	0.69
DIB	New vs. Used1x	63 vs. 65	1.1	0.46–2.61	0.82
	New vs. Used2x	63 vs. 59	0.8	0.33–2.24	0.76
	Used1xvs. Used2x	65 vs. 59	0.7	0.28–2.14	0.63
CIDR vs. DIB	CIDR-New vs. DIB-New	40 vs. 63	2.5	1.15–5.52	0.02
	CIDR-New vs. DIB-Used1x	40 vs. 65	2.7	1.19–6.48	0.02
	CIDR-New vs. DIB-Used2x	40 vs. 59	2.1	0.85–5.57	0.10
	CIDR-Used1x vs. DIB-New	47 vs. 63	1.8	0.85–4.17	0.11
	CIDR-Used1x vs.DIB-Used1x	47 vs. 65	2.0	0.89–4.89	0.09
	CIDR-Used1x vs.DIB-Used2x	47 vs. 59	1.6	0.63–4.20	0.31
	CIDR-Used2x vs. DIB-New	52 vs. 63	1.5	0.33–2.24	0.39
	CIDR-Used2x vs. DIBUsed1x	52 vs. 65	1.7	0.59–4.83	0.32
	CIDR-Used2x vs. DIB-Used2x	52 vs. 59	1.3	0.43–4.09	0.62
	CIDR-Overall vs. DIB-Overall	45 vs. 63	2.1	1.23–3.42	0.01

New intravaginal device (IVD-New: DIB®, 1.0 g of P_4, or CIDR®, 1.38 g of P_4. Intravaginal device previously used once (9 days) (IVD-Used1x: CIDR or DIB). Intravaginal device previously used twice (18 days) (IVD-Used2x: CIDR or DIB). *CI* confidence interval

P_4 (DIB®), compared to that obtained with IVD containing higher P_4 concentration (1.38 g of P_4, CIDR®). These differences could be attributed to differences in P_4 concentrations, hormone chemical composition, and rate of delivery or/and absorption between intravaginal devices.

It has been reported that high circulating P_4 concentration (from endogenous or exogenous sources) during the follicular phase is able to decrease LH pulse frequency (Forde et al. 2011), which may affect ovulation and pregnancy rates in cattle. Moreover, lower P_4 environment during growth of the ovulatory follicle at TAI increased the preovulatory follicle size and subsequent corpus luteum (CL) size and function, improving the pregnancy rates in beef cattle (Dadarwal et al. 2013). Despite hormonal profiles (progesterone concentration or LH pulse frequency) and follicular dynamics (including follicle and CL sizes) were not measured in the current experiment, it is possible to speculate that differences in pregnancy rate between the experimental groups could have been influenced by the initial P_4 concentration present in the devices (1.38 g of P_4 in CIDR® vs. 1.0 g of P_4 in DIB®).

Optimization of the dominant follicle size has been an important target in synchronization of ovulation and TAI protocols (Wiltbank et al. 2011; Baruselli et al. 2012). In beef cattle, the presence of larger follicles at the time of the TAI improves the rate of ovulation, which results in greater pregnancy rates (Perry et al. 2007; SáFilho et al. 2010). Increasing plasma progesterone concentrations and ovulation rate in cattle after TAI has been positively correlated with total CL or ovulatory follicle volume, indicating that CL size and function were influenced by the size of the ovulatory follicle (Echternkamp et al. 2009). In buffalo cows, the diameter of the preovulatory follicle has a positive impact on plasma estradiol concentration at estrus, subsequent progesterone profile, and conception rate (Pandey et al. 2011). Likewise, in *Bos indicus* beef cows (Nellore) treated with previously used twice IVD (1.0 g of P_4) produced larger dominant follicles (15.3 ± 0.4 mm; $P = 0.06$), compared to cows treated with new IVD (13.5 ± 0.8 mm) and previously used once IVD (14.9 ± 0.5 mm) (Sales et al. 2015).

In the present study, the animals treated with DIB had similar pregnancy rates than buffalo cows treated with IVD containing 1.0 g of P_4 plus E_2 and TAI during the breeding season under tropical conditions (64%; Monteiro et al. 2016), seasonal anestrous (52.7 and 55.9%; Carvalho et al. 2013 and Carvalho et al. 2014). Additionally, the study by Monteiro et al. (2016) showed slightly higher pregnancy rate in animals treated with IVD used once containing 1.0 g of P_4 (70.0%), than the values reported in the current experiment in animals treated with IVD used once containing also 1.0 g of P_4 (65%). Perhaps, these differences might have been influenced by season. The study by Monteiro et al. (2016) was performed during the breeding season, while the present trial was done in both non-breeding and breeding seasons, during which no seasonal effects were observed on pregnancy rate [68.18% ($n = 15/22$) vs. 61.11% ($n = 11/18$) for non-breeding and breeding seasons, respectively].

In contrast, other authors have reported lower pregnancy rates (around 40% in average) in both Mediterranean (De Rensis et al. 2005; Neglia et al. 2003) and Murrah buffaloes (Murugavel et al. 2009), using a combination of P_4, GnRH, and $PGF_2\alpha$ (PRID®: 1.55 g of P_4 and CIDR®: 1.38 g of P_4, respectively). It is possible to infer that differences in pregnancy rates observed in different studies using similar P_4-based IVD combined with GnRH and $PGF_2\alpha$ could be also due to variation in P_4 concentration, type of device used, protocol length and device permanence, timing of AI relative to ovulation, semen quality, management, environmental conditions, and breed.

The results of the current study also indicate that reusing the IVD (once and twice) in water buffalo cows do not have negative effects on pregnancy rate. Similar findings were reported by Carvalho et al. (2014), who found that reusing the IVD containing 1.0 g of P_4 did not affect negatively the PR in dairy buffalo cows, showing PR values of 55.9, 50.4, and 48.3%, for New, Used1x, and Used2x devices, respectively.

Despite reusing CIDRs (one or two times) that decrease device P_4 content (which might be similar to that in new DIB), pregnancy rate was numerically lower (no statistical significance) in the animals treated with used CIDRs compared to those receiving a new DIB (CIDR Used1x: 47%, CIDR Used2x: 52%; DIB New: 62%). In the present study, the sample size was small which could have affected the ability to detect minor differences in pregnancy rate. Other factors different from P_4 content in the IVD (i.e., P_4 chemical composition, vaginal absorption, hormone lifespan) might have also affected the pregnancy rate in buffalo cows treated with different P_4 devices.

Studies evaluating the potential negative effects of high P_4 concentration, different chemical composition, or absorption rate using different IVD models on the dominant follicle development and pregnancy rate in water buffalo are warranted. A study performed by Long et al. (2009) in ovariectomized cows to measure plasma P_4 concentrations on day 1 after CIDR insertion in animals treated with new CIDR containing 1.9 g of P_4, CIDR used once, or CIDR used twice showed a gradual decrease in P_4 concentration in all groups. Interestingly, plasma P_4 concentrations on the day after insertion were significantly higher ($P < 0.05$) in cows treated with used-CIDRs than the values at day 7 of the other treatment (Long et al. 2009). These findings could explain the absence of negative effect of reusing CIDR devices on pregnancy rate in the current experiment.

Decreasing the P_4 concentration in CIDR prescribed for water buffalo, or reusing the CIDR devices two or three times could enhance the pregnancy rate in water buffalo cows submitted to P_4-based ovulation synchronization and TAI protocols. An additional benefit of reusing the IVD is the reduction of the costs of associated with synchronization protocols for TAI programs in water buffalo.

The results obtained in the current trial recapitulate previous studies (Carvalho et al. 2014; Monteiro et al. 2016) and support our hypothesis that higher P_4 concentration contained in the IVD could affect the pregnancy rate in water buffalo cows. Based on these results, it is possible to infer that an optimal P_4 concentration in IVDs for buffalo cows submitted to TAI protocols could be ≤ 1.0 g. Additional studies in this regard are necessary and should be further investigated.

In conclusion, pregnancy rate was higher in buffaloes treated with the synchronization protocol using 1.0 g of P_4 (DIB®) compared to that obtained with 1.38 g of P_4 (CIDR®), which could be attributed to differences in P_4 concentration, chemical composition, or absorption rate. Likewise, reusing the intravaginal device (two and three times) did not affect negatively the pregnancy rate, suggesting that P_4 concentration in protocols for ovulation synchronization and TAI could be adjusted in this species.

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Compliance with ethical standards

Statement of animal rights All animals were cared for in accordance with acceptable practices as for the Care and Use of Agricultural Animals in Agricultural Research and Teaching (FASS 2010).

Conflict of interest statement The authors declare that they have no conflict of interest.

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