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Half dose of prostaglandin F₂ α is effective to induce luteolysis in the synchronization of ovulation protocol for fixed-time artificial insemination in buffalo (*Bubalus bubalis*)

Meia dose de prostaglandina F₂ α é eficaz na indução da luteólise em protocolos de sincronização da ovulação para inseminação artificial em tempo fixo em bubalinos (*Bubalus bubalis*)

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Summary

The objective of this study was to evaluate the efficiency of half dose of PGF₂ α in the synchronization of ovulation protocol (GnRH/PGF₂ α /GnRH) for fixed-time artificial insemination in buffalo. Two hundred and sixty two buffaloes (³40 days postpartum) were divided in 2 groups (Group 1, n = 125, GnRH/½ dose de PGF₂ α /GnRH; Control Group, n = 137, GnRH/PGF₂ α /GnRH). Animals in Group 1 received 25mg of GnRH IM (Lecirelin, Gestran-plus®) and, seven days later, half dose of PGF₂ α IM (75mg d-cloprostenol, Preloban®). Two days after PGF₂ α injection, all animals received 25mg of GnRH IM (Lecirelin). Animals in Control Group received the same treatment, except for the dose of PGF₂ α (150mg, IM, d-cloprostenol, Preloban®). All animals were fixed-time inseminated 16 hours after the second GnRH injection. Blood samples were collected from 35 buffaloes in Group 1 and 30 buffaloes in Control Group on day of PGF₂ α and second GnRH injections to measure plasma progesterone concentrations. Conception rate was not influenced by PGF₂ α dosage (P>0.05). Conception rates were 48.00% (60/125) and 40.90% (56/137) in Group 1 and Control Group, respectively. It was observed that 88.60% (n = 35) of the buffaloes in Group 1 and 90.00% (n = 30) in Control Group presented luteolysis 48 hours after the PGF₂ α injection (P>0.05). In summary, present data show that both treatments result in acceptable conception rates. Therefore, the recommended dose of PGF₂ α can be reduced in 50.00% in the synchronization of ovulation protocol for fixed-time artificial insemination in buffalo.

Key-words
Synchronization of ovulation.
Dinoprost.
Buffalo.

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Introduction

The efficiency of synchronization of ovulation for fixed-time artificial insemination in buffalo was previously studied^{1,2}. Synchronization of follicle growth, as well as induction of ovulation with exogenous GnRH injections have resulted in satisfactory conception rates in fixed-time artificial insemination (TAI) programs in buffalo^{1,2}.

GnRH injection, in any phase of the estrous cycle, results in a peak of LH that promotes the ovulation of follicles > 9.0 mm³ or luteinization of non-viable follicles and emergence of a new follicle wave 2 or 3 days later⁴. With ovulation or luteinization of the dominant follicle, progesterone levels remain high and therefore an injection of PGF₂α is included on Day 7 of the protocol to induce luteolysis and allow the ovulation of the new dominant follicle³. The second GnRH injection is recommended 48 hours after PGF₂α injection for better synchronization of ovulation and benefit TAI⁵.

Several studies have demonstrated the efficiency of these protocols for synchronization of ovulation using reduced doses of PGF₂α in bovine^{6,7}. Chohan⁸ verified that reduced doses of PGF₂α promote luteolysis and induce estrus and ovulation in buffalo. Thus, the objective of this study was to assess the efficiency of half recommended dose of PGF₂α in the synchronization of ovulation protocol for fixed time insemination in buffalo.

Material and Method

Animals

The experiment was performed in properties in southeast Brazil during the breeding season of 2001 (autumn

and winter; BARUSELLI²). Two hundred and sixty two multiparous dairy Murrah buffaloes were selected according to postpartum period (≥40 days) and body condition score (BCS ≥ 3.0; scale 1 to 5). All animals were maintained in pasture regime with mineral salt support.

Experimental Design

Animals in Group 1 (n = 125) received 25mg of GnRH IM (Lecirelin, Gestran-plus®) on Day 0 and, seven days later (Day 7) 75mg of PGF₂α IM (d-cloprostenol, Preloban®). Two days after PGF₂α injection, these animals received 25mg of GnRH IM (Lecirelin). Animals in Control Group (n = 137) received the same treatment except for the dose of PGF₂α that was twice higher (150mg, IM, d-cloprostenol, Preloban®). All animals were fixed-time inseminated 16 hours after the second GnRH injection in both groups (Figure 1).

To avoid influence of semen on conception rate, semen batches were randomly divided between groups. Furthermore all inseminations were performed by the same person to minimize influence on the results. Conception was checked by transretal ultrasonography using a 5.0MHz probe (Pie Medical 480, Netherlands) 30 days after TAI.

Blood samples for hormonal analysis

At the moment of PGF₂α (Day 7) and the second GnRH (Day 9) injections, blood samples were collected from jugular vein to measure plasma progesterone concentration in 35 buffaloes in Group 1 (75mg of d-cloprostenol, Preloban®; half dose of PGF₂α), and 30 buffaloes in Control Group (150mg of d-cloprostenol, recommended dose of PGF₂α). Progesterone concentration was determinate by radioimmunoassay

(RIA) using a commercial kit (DPC, Diagnostic Products Corporation, USA). The sensibility of the test was 0.05 ng/ml and a intra assay coefficient of variation was 8.00%.

Statistical analysis

Chi-square test was used to analyze the binomial variables, percentage of buffaloes with concentration of progesterone <1.0 ng/ml and conception rate. ANOVA was used to evaluate the continuous variables (progesterone concentration) for repeated measures. T-test was used to evaluate differences between means.

Results

Evidence of luteolysis was observed 48 hours after PGF₂α injection in 88.60% (n = 35) of the buffaloes in Group 1 and 90.00% (n = 30) in Control Group (P>0.05; Table 1, Figure 2). Four animals (11.40%) in Group 1 and three (10.00%) in Control Group did not show decrease in progesterone concentration 48 hours after PGF₂α injection (Figure 3).

Conception rate was not affected by the decreased in PGF₂α dosage (P>0.05). Conception rate in Group 1 and in Control Group were 48.00% (60/125) and 40.90% (56/137), respectively (Table 2).

The decrease in treatment cost for each pregnant buffalo treated with half dose of PGF₂α was R\$8.81 (Table 3).

Figure 1

Treatment scheme for synchronization of ovulation for fixed-time artificial insemination in buffalo (Gestran-plus®, Tecnopec, São Paulo, Brazil; Preloban®, Hoescht Roussel Veterinária S.A, São Paulo, Brazil)

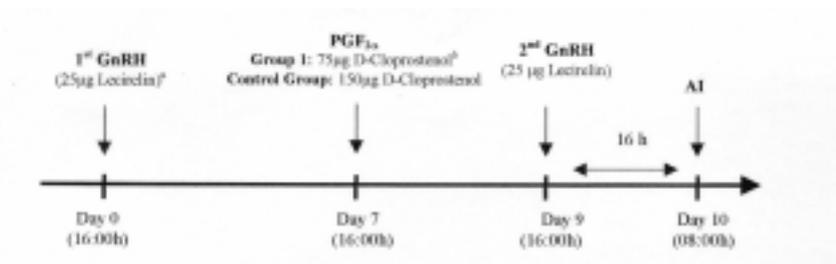


Figure 2

Mean plasma progesterone concentration (mean ± SD) at the moment of PGF₂α injection (d-cloprostenol) and 48 hours afterwards in synchronized buffaloes treated with GnRH/PGF₂α/GnRH that responded to PGF₂α (n = 58). São Paulo, 2001

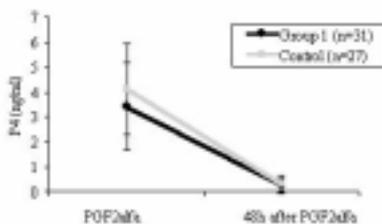


Figure 3

Mean plasma progesterone concentration (mean ± SD) at the moment of PGF₂α injection (d-cloprostenol) and 48 hours afterwards in synchronized buffaloes treated with GnRH/PGF₂α/GnRH that did not respond to PGF₂α (n = 7). São Paulo, 2001

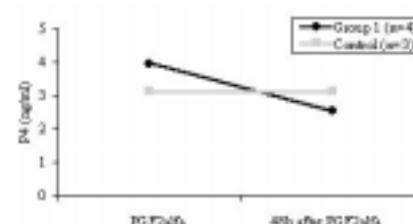


Table 1

Luteolytic response in buffaloes treated with different doses of $\text{PGF}_2\alpha$ during synchronization of ovulation protocol (GnRH/ $\text{PGF}_2\alpha$ /GnRH) for fixed-time artificial insemination. São Paulo, 2001

Groups	Dose de $\text{PGF}_{2\alpha}$	Number of animals with	
		P4 concentration > 1.0 ng/ml on Day 7 ($\text{PGF}_{2\alpha}$ injection)	Percentage of animals with P4 concentration < 1.0 ng/ml 48h after $\text{PGF}_{2\alpha}$ injection - % (n)
Group 1	75 mg	35	88.6 (31/35)
Control	150 mg	30	90.0 (27/30)
Total		65	89.2 (58/65)

*Concentration of progesterone < 1.0 ng/ml 48 after $\text{PGF}_{2\alpha}$ injection was considered luteolysis.

Table 2

Conception rate in buffaloes treated with different doses of $\text{PGF}_2\alpha$ during synchronization of ovulation protocol (GnRH/ $\text{PGF}_2\alpha$ /GnRH) for fixed-time artificial insemination. São Paulo, 2001

Groups	Dose of $\text{PGF}_{2\alpha}$	N° of Animals	Conception rate % (n)
Group 1	75 mg	125	48.0 (60/125)
Control	150 mg	137	40.9 (56/137)
Total		262	44.3 (116/262)

Table 3

Cost analysis of the protocol for synchronization of ovulation (GnRH/ $\text{PGF}_2\alpha$ /GnRH) for fixed-time artificial insemination in buffalo treated with recommended or half dose of $\text{PGF}_2\alpha$ (d-cloprostenol, 75mg vs. 150mg). São Paulo, 2001

Costs		Group 1 (75mg)	Control (150mg)
	Number of pregnant^a	60	56
Cost of $\text{PGF}_{2\alpha}$ ^b	For animal (R\$)	2.25	4.50
	For pregnant (R\$) ^c	4.69	11.01
Cost of protocol ^b	For animal (R\$)	8.85	11.10
	For pregnant (R\$)	18.44	27.25

^aNumber of pregnant buffaloes at ultrasonography evaluation 30 days after TAI.

^bEstimated costs of hormones. Cost of R\$3.30 for 25mg of Lecirelin and R\$4.50 for 150mg of d-cloprostenol.

^cCost for pregnancy was calculated dividing the total cost with hormones by the number of pregnant animals. Value of American dollar/real in the day of budget (US\$1.00 = R\$2.50).

Discussion

An adequate conception rate using the TAI was achieved in both experimental groups. Conception rates of buffaloes inseminated did not differ between groups (Group 1=48.00% vs. Control Group=40.90%, $P>0.05$). These results are similar to those obtained by Barros⁷, who evaluated the use of half dose of $\text{PGF}_2\alpha$ in bovine synchronized with the protocol GnRH/ $\text{PGF}_2\alpha$ /BE and verified similar pregnancy rates when compared to animals treated with the full dose. Reduced doses of $\text{PGF}_2\alpha$ have been efficient to induce luteolysis, estrus and ovulation in buffalo as shown by Chohan⁸.

The possibility to decrease the dose of $\text{PGF}_2\alpha$ without altering the efficiency of luteolysis contributes to reduce costs of the protocol for synchronization of the ovulation. $\text{PGF}_2\alpha$ injection represents a significant protocol' cost. However, if reduction of $\text{PGF}_2\alpha$ dosage is not efficient to induce luteolysis, conception will not occur, even if animal ovulate⁶.

In present study, luteolysis was hormonally verified in 88.60% of buffaloes in Group 1 and 90.00% in Control Group ($P>0.05$). The luteolysis rate observed agreed with SANTOS et al.⁶ that worked with bovine synchronized by the "Ovsynch" protocol (GnRH/ $\text{PGF}_2\alpha$ /GnRH).

Present data allow to

recommend half dose of PGF₂α (d-cloprostenol) to efficiently induce luteolysis, without affecting conception rate of synchronization protocol for TAI. Half dose of PGF₂α reduced treatment cost in R\$8.81 for each

pregnant buffalo. In summary, it was verified that half dose of PGF₂α is enough to induce luteolysis in synchronization of ovulation protocol for fixed-time artificial insemination in buffalo.

Resumo

O objetivo desse estudo foi avaliar a eficiência da meia dose de PGF₂α no protocolo de sincronização da ovulação (GnRH/PGF₂α/GnRH) para inseminação artificial em tempo fixo em bubalinos. Foram utilizadas 262 búfalas (³ 40 dias pós-parto), divididas em dois grupos (Grupo 1, n = 125, GnRH/ 1/2 dose de PGF₂α/GnRH; Grupo Controle, n = 137, GnRH/PGF₂α/GnRH). Os animais do Grupo 1 receberam 25 mg de GnRH IM (Lecirelina, Gestran-plus®) e, após sete dias, receberam 75mg de PGF₂α IM (d-cloprostenol, Preloban®). Dois dias após a aplicação da PGF₂α, esses animais receberam 25mg de GnRH IM (Lecirelina). Os animais do Grupo Controle receberam o mesmo tratamento, à exceção da dose de PGF₂α (150mg de d-cloprostenol, Preloban®). Todos os animais foram inseminados em tempo fixo 16 horas após a segunda aplicação de GnRH. Foram colhidas amostras de sangue de 35 búfalas do Grupo 1 e de 30 do Grupo Controle nos dias da aplicação de PGF₂α e da segunda dose de GnRH. As colheitas de sangue foram realizadas para avaliar as concentrações plasmáticas de progesterona. Os resultados demonstraram que a taxa de concepção não foi influenciada pela dose de PGF₂α (P>0,05). As taxas de concepção dos Grupos 1 e Controle foram de 48,00% (60/125) e 40,90% (56/137), respectivamente. Observou-se que 88,60% (n = 35) das búfalas do Grupo 1 e 90,00% (n = 30) do Grupo Controle apresentaram luteólise 48 horas após a aplicação de PGF₂α (P>0,05). Em conclusão, os resultados indicaram que ambos tratamentos proporcionaram aceitáveis taxas de concepção. Dessa forma, a dose recomendada de PGF₂α pode ser reduzida pela metade no protocolo de sincronização da ovulação para inseminação artificial em tempo fixo em bubalinos.

Palavras-chave

Sincronização da ovulação.
Dinoprost.
Búfalos.

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