Notas Científicas

The use of insulin to improve fertility of timed-inseminated postpartum suckled beef cows

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Abstract – The objective of this work was to evaluate the effect of insulin alone or in association with equine chorionic gonadotropin (eCG) on the fertility of postpartum beef cows subjected to synchronization. A total of 340 cows was subjected to fixed time artificial insemination. In the trial 1, the cows were subjected to temporary weaning (TW), while in the trial 2 the same protocol was tested without TW. The addition of an insulin injection to a progesterone/eCG-based protocol without TW increased the pregnancy rate of beef cows with body condition score (BCS) equal to or lower than 2.5. Insulin had no effect on cows submitted to TW or with BCS equal to or higher than 3.0.

Index terms: *Bos taurus*, eCG, insulin, FTAI, weaning.

Uso de insulina para aumento da fertilidade de vacas de corte pós-parto lactantes inseminadas

Resumo – O objetivo deste trabalho foi avaliar apenas o efeito da insulina ou dela em associação com gonadotrofina coriônica equina (eCG) na fertilidade de vacas em pós-parto submetidas à sincronização. Um total de 340 vacas foi submetido à inseminação artificial em tempo fixo. No experimento 1, as vacas foram submetidas ao desmame temporário (DT), enquanto no experimento 2, o mesmo protocolo foi testado sem DT. A adição de uma injeção de insulina a um protocolo baseado em progesterona/eCG sem DT aumentou a taxa de prenhez de vacas de corte com escore de condição corporal (ECC) igual ou menor que 2,5. A insulina não teve efeito em vacas submetidas a DT ou com ECC igual ou maior que 3,0.

Termos para indexação: *Bos taurus*, eCG, insulina, IATF, desmame.

The major challenge of the widespread use of fixed time artificial insemination (FTAI) is the high proportion of anestrous postpartum suckled beef cows in the herd. Among the alternatives that aimed to improve the use of FTAI and the pregnancy rates, the temporary weaning (TW) and the hormonal protocols are the most useful. In the tropical environment, the main protocols used to increase the use of artificial insemination (AI) include the use of progesterone releasing devices associated with equine chorionic gonadotropin (eCG) injection.

The eCG stimulates follicular growth through its follicle-stimulating hormone (FSH) and luteinizing hormone (LH) action (Murphy & Martinuk, 1991), improving the pregnancy rate (Sá Filho et al., 2009). Temporary weaning also increased pregnancy rate; however, eCG did not improve the pregnancy rate when used in association to TW (Sá Filho et al., 2009). These results show that eCG and TW are useful tools to improve pregnancy rate of postpartum cows, although they did not have an additive effect.

Insulin also plays an important role in follicular growth through its direct stimulatory effects on granulosa cell estradiol production and indirect stimulatory effects via amplification of gonadotropin action (Gong et al., 1994). Moreover, insulin injection in cows increased the steroidogenic capacity, the diameter of the largest follicle (Simpson et al., 1994), and the ovulation rate in response to a superovulatory program (Harrison & Randel, 1986). Evidence also shows that, in buffalos suffering from summer acyclicity, insulin pretreatment can increase the

diameter of the largest follicle and the estrous induction rate (Ramoun et al., 2007). Based on these information, it is possible that insulin injection in the moment of progesterone withdrawal in postpartum cows can improve the final follicular development, improving ovulation and, consequently, the pregnancy rate.

Considering these evidences, the objective of this work was to evaluate the effect of insulin alone or associated with eCG on the pregnancy rate of postpartum suckled beef cows subjected to FTAI with and without TW.

All procedures performed in the trials were approved by the Comitê de Ética em Experimentação Animal from the Universidade Federal de Pelotas.

For the trial 1, multiparous cows (Angus and Angus x Nelore, n = 148) were used in a commercial farm located in the Southern Region of Brazil (32º16' S, 52º32' W) during the breeding season (January). The cows were maintained under grazing conditions in a native pasture, for 50–90 days postpartum in a body condition score (BCS) of 3.0 (scale from 1, very thin, to 5, obese, at 0.5 increment; Houghton et al., 1990). In a random day of the estrous cycle, all cows received an intravaginal progesterone releasing device – 1.9 g, CIDR-B –, (InterAg, Hamilton, New Zealand) and 2 mg of estradiol benzoate – i.m., 1 mg mL^{-1}, Gonadiol –, (Syntex, Buenos Aires, Argentina). The day of CIDR insertion was considered Day 0. All cows received an injection of 12.5 mg of dinoprost – i.m., 5 mg mL^{-1}, Lutalyse –, (Pfizer, Paulínia, Brazil), a synthetic prostaglandin analogue, on Day 7. On Day 9, all cows were injected with 0.5 mg of estradiol cypionate – i.m., 2 mg mL^{-1}, ECP –, (Pfizer, Paulínia, Brazil) and CIDR was removed.

On Day 9, the calves were separated from their dams and the cows were divided in: control group (n = 55), which did not receive any additional treatment; insulin group (n = 38), which received a subcutaneous (s.c) injection of 125 international units (IU) of human recombinant insulin – s.c., 0.25 IU kg^{-1}, Novolin N –, (Novo Nordisk, Bagsvaerd, Denmark); eCG Group (n = 20), which received an injection of 400 IU of eCG – i.m., Novormon –, (Syntex, Buenos Aires, Argentina); and eCG + Insulin Group (n = 35), which received insulin and eCG as the previous groups.

For the trial 2, multiparous cows (Angus and Angus x Nelore; n = 192) were used in the same farm and under the same conditions described for trial 1 (February). The cows were between 50–90 days postpartum and had BCS between 2.0 and 3.5. The same base protocol from trial 1 was used. However, in trial 2 cows were not subjected to TW, and were divided into two groups: eCG (n = 92) and eCG + insulin (n = 100), which received insulin and eCG as cows from trial 1. The cows were also divided by BCS into two groups for further analysis: BCS≤2.5 (n = 108) and BCS≥3.0 (n = 88).

The AI was performed in the Day 11, 48 hours after the CwIDR removal for both trials. All cows were artificially inseminated with frozen semen from the same bull and by the same technician. Pregnancy diagnosis was performed 35 days after FTAI with an ultrasound Aloka SSD-500, 5 MHz probe (Aloka Inc., Tokyo, Japan).

All statistical analyses were performed with SAS 9.0 (SAS Institute, 2000). The analyses were done by χ^2 test, independently for each trial. For trial 2, the analysis was also performed through a comparison between the two groups of BCS.

The high pregnancy rate recorded in this study [trial 1, 72.3% (107/148); trial 2, 62.5% (120/192)] evidenced the proper efficiency of progesterone-based protocols that ranged around 45 to 70% of pregnancy (Baruselli et al., 2004; Colazo et al., 2004). However, this could be a detrimental factor for the hypothesis, since it is very difficult to increase the pregnancy rate above 70%.

The individual results for each group are presented in Table 1. The injection of insulin in addition to eCG

Table 1. Pregnancy rate of cows subjected to fixed time insemination with or without temporary weaning, for each treatment group[(1)].

Variable	With temporary weaning				Without temporary weaning			
	Body condition score = 3.0				Body condition score≤2.5		Body condition score≥3.0	
	Control	eCG	Insulin	Insulin + eCG	eCG	Insulin + eCG	eCG	Insulin + eCG
Pregnant	39	16	24	28	19	40	34	27
Total	55	20	38	35	48	60	44	40
Percentage	70.9	80.0	63.1	80.0	39.6a	66.7b	77.3b	67.5b

[(1)]Percentages followed by equal letters do not differ by χ^2 test, at 1% probability.

improved the pregnancy rate of cows with BCS≤ 2.5 in comparison to eCG alone in cows not subjected to TW, but not for cows with BCS≥3. In addition, insulin had no effect in cows subjected to TW and with BCS = 3.0. Other studies have shown a positive effect of insulin administration on ovarian follicular growth (Simpson et al., 1994; Ramoun et al., 2007) and ovulation rate (Harrison & Randel, 1986). However, these studies used daily injections of insulin and did not provide a useful approach to be applied in large beef herds subjected to FTAI as a single injection. A possible explanation for insulin being effective only in cows with BCS≤2.5 is that the serum insulin concentration is positively correlated to BCS (Leon et al., 2004); the injection of exogenous insulin in this group could act by compensating the reduced endogenous insulin concentrations, in the improvement of the final follicular growth and ovulation.

Another important conclusion of this study is that additional treatments to FTAI, in cows managed with TW and good BCS, do not seem to improve pregnancy rate. In that regard, postpartum cows cycling and with good BCS (Pinheiro et al., 2009) or subjected to TW (Sá Filho et al., 2009) had no increase in pregnancy rate in response to eCG injection. Similarly, there was no difference in the pregnancy rate between the control treatment and eCG groups with TW in this study, which highlights the good cycling status and BCS of these cows, as mentioned before. In the same way, the use of insulin did not have a positive effect when considering cows in good BCS and subjected to FTAI associated to TW.Therefore, the addition of insulin to a protocol based on progesterone and eCG without TW can increase the pregnancy rate of postpartum beef cows with BCS≤2.5. However, no beneficial effects of insulin are observed for cows subjected to TW or with BCS≥3.0.

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