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Increasing the dose of GnRH at a synchronized timed AI increases pregnancy rates in *Bos indicus* influenced cattle

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Abstract

Compared to *Bos taurus* cattle, *Bos indicus* influenced cattle have a history of decreased pregnancy rates following artificial insemination (AI). These decreased pregnancy rates among *Bos indicus* influenced cattle may be attributed to their higher excitability response to stressful situations, which can result in increased circulating cortisol that can delay or suppress ovulation. This experiment was designed to evaluate the effect of an increased GnRH dose at a synchronized timed artificial insemination (TAI) on pregnancy rates in *Bos indicus* influenced cattle. Over two years, *Bos indicus* influenced heifers (n = 50) from four locations, *Bos taurus* heifers (n = 123) and lactating *Bos indicus* influenced cows (n = 83) were inseminated with conventional semen using the CO-Synch+CIDR protocol. Heifers were inseminated between 48 to 56 h and mature cows between 56 to 66 h of last PGF2α administration. Non-lactating Brahman cows (n = 32) were also synchronized in the above manner and inseminated between 56 to 66 h with sexed *Bos indicus* influenced semen. All cows were randomly selected to receive either 100 µg (n = 144) or 200 µg (n = 144) of GnRH at insemination and examined via ultrasonography for pregnancy ~30 days post-TAI. The administration of 200 µg of GnRH at the time of AI to *Bos indicus* influenced cattle significantly ($P < 0.004$) increased pregnancy rates (0.43 ± 0.05) compared with 100 µg of GnRH (0.21 ± 0.04). This pattern of increased pregnancy rates in the 200 µg GnRH group occurred at all locations and in all cow types. Among *Bos taurus* heifers, the increased dose of GnRH at the time of AI did not affect pregnancy rates; 200 µg (0.49 ± 0.06) compared with a 100 µg dose (0.55 ± 0.06). These results indicate that increasing the dose of GnRH at the time of AI can increase synchronized pregnancy rates in *Bos indicus* influenced cattle, but not among *Bos taurus* heifers.

Keywords: artificial insemination, *Bos indicus*, GnRH, increased GnRH, stress.

Introduction

Psychological stress at or near the time of artificial insemination (AI) can result in decreased pregnancy rates among rodents (Christian, 1971), primates (Chen *et al*., 1992; Norman *et al*., 1994), sheep (Smart *et al*., 1994) and cattle (Welsh and Johnson, 1981; Stoebel and Moberg, 1982). Additionally, adrenocorticotropin (ACTH) challenges during this time are reported to block ovulation in the pig (Liptrap, 1970; Schilling and von Rechenberg, 1973) and in the cow (Liptrap and McNally, 1976; Stoebel and Moberg, 1979). This disruption of ovulation may be more exaggerated in *Bos indicus* influenced breeds than *Bos taurus* breeds of cattle. The pregnancy rates among *Bos indicus* influenced breeds are much lower (30 to 45%) in response to estrus synchronization and AI (Saldarriaga *et al*., 2007; Zuluaga *et al*., 2008) compared with *Bos taurus* cattle (Lamb *et al*., 2001; Larson *et al*., 2006). One factor contributing to this difference in pregnancy rates may be the different levels of stress experienced. The *Bos indicus* influenced breed exhibits a greater increase in cortisol during routine handling procedures compared with *Bos taurus* cattle (Zavy *et al*., 1992; Grandin, 1997).

An increase in cortisol during estrus can prevent or delay ovulation resulting from impaired LH release from the anterior pituitary (Daley *et al*., 1999). Glucocorticoids have been reported to decrease LH release in rodents (Ringstrom and Schwartz, 1985), primates (Dubey and Plant, 1985; Melis *et al*., 1987; Saketos *et al*., 1993) and domestic animals (Fonda *et al*., 1984; Dobson and Smith, 1995). A similar pattern has also been reported when pituitary cells cultured *in vitro* released reduced amounts of LH in response to glucocorticoid administration (Padmanabhan *et al*., 1983; Kamel and Kubajak, 1987; Baldwin *et al*., 1991). This reduction in LH may be mediated via glucocorticoid receptors present in the GnRH neurons of the hypothalamus (Ahima and Harlan, 1992) and gonadotroph cells in the anterior pituitary (Kononen *et al*., 1993). Although circhoral administration of GnRH has been reported to restore a normal release of LH in the presence of elevated glucocorticoids (Dubey and Plant, 1985), this is not practical or cost-effective in the cattle industry. The objective of this experiment was to determine if increasing the dose of GnRH at the time of artificial insemination would improve pregnancy rates in *Bos indicus* influenced cattle.

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Materials and Methods

This experiment was conducted over a 2 yr time period and across five different locations, utilizing a variety of *Bos indicus* influenced cattle (n = 165) and *Bos taurus* cattle (n = 123) from various commercial farms. Ovulation in all heifers and cows was synchronized using the CO-Synch+CIDR protocol. Each heifer or cow was administered 100 µg of GnRH (OvaCyst, AgriLabs, St. Joseph, MO) and received a CIDR (Pfizer Animal Health, New York, NY) implant on day 0. On day 7, the CIDR implant was removed, and each heifer or cow received 25 mg of PGF2α (Lutalyse, Pfizer, Kalamazoo, MI). All heifers were inseminated between 48 to 56 h, and all cows were inseminated between 56 to 66 h post-PGF2α with frozen-thawed conventional or frozen-thawed sex-sorted semen (Bremer *et al.*, 2004). In location 1 (Southeast Texas), a total of 19 Beefmaster heifers ranging from 14 to 18 months in age and weighing a minimum of 345 kg were used for AI with conventional semen from a Red Angus bull and were maintained on bermuda grass pasture supplemented with ad libitum grass hay. These heifers were randomly allotted to the control (n = 10) or treatment group (n = 9). In location 2 (Southeast Texas), a total of 32 mature, nonlactating, Brahman cows ranging in age from 3 to 6 yr were randomly allotted to control (n = 16) or treatment group (n = 16) AI with sex-sorted Brahman semen for the X-bearing chromosome (2,000,000 sperm per straw) and were maintained on bermuda grass pasture supplemented with mineral blocks and ad libitum hay (Table 1). In location 3 (Southeast Texas), Brangus heifers (n = 5) ranging in age from 14 to 18 months were randomly allotted to control (n = 2) or treatment group (n = 3), inseminated with conventional Angus semen and maintained on bermuda grass pasture. In location 4 (West Texas), a total of 83 mature, lactating, Beefmaster cows ranging in age from 3 to 10 yr, at a minimum of 50 days post-partum, were randomly allotted to control (n = 40) or treatment group (n = 43) for insemination with conventional semen from Beefmaster bulls and were maintained on buffel grass supplemented with Mix 30 (Agridyne, LLC). In location 5 (Southwest Louisiana), a total of 26 Brahman cross-bred heifers between the ages of 13 to 18 months and weighing between 355 to 464 kg were randomly allotted to either control (n = 16) or treatment group (n = 10) for AI with Beefmaster or Angus semen. Additionally, a total of 123 *Bos taurus* heifers (Angus) ranging in age from 12 to 16 months were randomly allotted to either control (n = 60) or treatment group (n = 63) for insemination with conventional semen from either an Angus or Charolais bull. In location 5, all cattle were maintained on ad libitum corn silage supplemented with 1.8 kg cracked corn and 0.68 kg of protein/mineral (33% Beefmaker, O'Neal's Feeder Supply Co., DeRidder, LA) once daily on a 12,000 m^2 asphalt base feedlot. Table 1 summarizes data on all the treatment animals across locations.

At AI, control heifers or cows received 100 µg of GnRH and treatment heifers or cows received 200 µg of GnRH. All inseminations were conducted by two experienced AI technicians. At ~30 days post-timed AI, all heifers or cows were examined per rectum with an Aloka 500-V Ultrasound (Corometrics, Wallingford, CT) with a 5 MHz probe to determine pregnancy. Pregnancy was defined in this study as the presence of a live viable fetus with a heartbeat.

All statistical analyses were performed with SAS (SAS Institute Inc., Cary, NC) using a general linear model (GLM) with Duncan's and LS Means (least squared means) post-hoc test to determine statistical differences. The independent variables used in statistical comparisons were treatments, location, AI technician, cow vs. heifer, breed type, semen and the interactions of all variables. Non-significant variables were eliminated from the model in a step-wise fashion.

Table 1. The location, number, breed type, age of animals and the semen type used.

Location	n	Breed	Type	Age	Semen type
1 Texas	19	BM	Heifer	14 to 18 mo	AN, conventional
2 Texas	32	BR	Cow	3 to 6 yr	BR, sexed-X
3 Texas	10	BA	Heifer	14 to 18 mo	AN, conventional
4 Texas	83	BM	Cow[1]	3 to 10 yr	BM, conventional
5 Louisiana	26	XB[2]	Heifer	13 to 18 mo	AN, conventional
5 Louisiana	123	AN, XB[3]	Heifer	12 to 16 mo	AN, conventional

[1]Lactating cows; [2]*Bos indicus* heifers; [3]*Bos taurus* heifers.
BM = Beefmaster, BR = Brahman, BA = Brangus, XB = Brahman crossbred, AN = Angus.

Results

Since there were no ($P > 0.05$) differences in mean ± SEM pregnancy rates among locations, conventional or sexed semen or between technicians, all data were combined. Among pooled data for *Bos indicus* influenced cattle, administration of 200 µg of GnRH at TAI increased ($P < 0.03$) pregnancy rates (0.43 ± 0.05) compared with 100 µg of GnRH (0.21 ± 0.04; Table 2).

Table 2. Pregnancy rates in *Bos indicus* cattle receiving 100 µg or 200 µg of GnRH at time of insemination.

Treatment	Pregnancy Rate ± SEM
100 µg GnRH	0.21 + 0.04[a]
200 µg GnRH	0.43 + 0.05[b]

[a,b]Values with differing superscript within a column are different (P < 0.003).

Among *Bos indicus* influenced heifers, the administration of 200 µg of GnRH at time of AI increased (P < 0.05) pregnancy rates (0.63 ± 0.10) compared with 100 µg of GnRH at time of AI (0.29 ± 0.09). However, among *Bos tarus* heifers, there was no increase (P < 0.60) in pregnancy rates between those receiving 100 µg of GnRH (0.55 ± 0.06) compared with those receiving 200 µg of GnRH at TAI (0.49 ± 0.06; Table 3).

Table 3. *Bos taurus* pregnancy rates comparing 100 µg or 200 µg of GnRH at time of insemination.

Treatment	Pregnancy Rate ± SEM
100 µg GnRH	0.55 + 0.06[a]
200 µg GnRH	0.49 + 0.06[a]

[a]Values with same superscript within a column are not different (P > 0.05).

Discussion

In cattle, an estrus characterized as prolonged (Erb *et al*., 1976), having a delayed preovulatory LH release (Gustafsson *et al*., 1986; Albihn, 1991) or a higher than normal systemic cortisol concentrations (Bage *et al*., 2000, 2001) tends not to result in successful breedings. Delayed ovulation can result in inappropriate insemination timing relative to ovulation, however, the administration of GnRH at time of insemination has been reported to improve the likelihood of ovulation occurring in a timely manner following administration (Silcox *et al*., 1993; Pursley *et al*., 1995). But, increased cortisol concentrations near estrus can impair the ability of normal levels of GnRH to induce ovulation by blocking the LH release (Liptrap and McNally, 1976; Stoebel and Moberg, 1979; Daley *et al*., 1999). This endocrinological pattern likely exists during synchronized AI in *Bos indicus* influenced cattle.

When using CO-Synch+CIDR and TAI, the pregnancy rate is nearly 30% higher in *Bos taurus* (Lamb *et al*., 2001; Larson *et al*., 2006) compared with *Bos indicus* influenced cattle (Saldarriaga *et al*., 2007; Zuluaga *et al*., 2008). This may be a result of decreased or delayed ovulation rates among *Bos indicus* influenced cattle, as they tend to have higher cortisol concentrations due to chute stress (Zavy *et al*., 1992; Grandin, 1997). The increased dose of GnRH is believed to increase the LH surge sufficiently to provoke ovulation despite the elevated cortisol concentrations. If this is the case, then the increased dosages of GnRH could result in more synchronized AI and ovulation.

By increasing the dosage of GnRH to 250 µg at the time of AI in repeat-breeder dairy cattle, pregnancy rates were increased (Morgan and Lean, 1993) and a significant increase in LH concentration occurred within 2 h of 250 µg administration compared with 50 µg or 100 µg of GnRH (Mee *et al*., 1993). A similar 100% improvement in pregnancy rates has been reported in *Bos indicus* crossbred dairy cow repeat breeders when administered 10 or 20 µg of a GnRH analogue (Buserelin acetate; Kharche and Srivastava, 2007). However, when administered an increasing dose of a GnRH analogue (buserelin acetate) of 8 to 12 µg in normal Zebu (*Bos indicus*) cattle, there was no increase pregnancy rate (Fernandes *et al*., 2001). It may be possible that the 12 µg dosage was not capable of overcoming a cortisol-induced blocked LH release, whereas the 20 µg of burserelin was capable. When the GnRH dose was increased among normal dairy heifers and cows, the pregnancy rate did not increase (Fricke *et al*., 1998; Yamada *et al*., 2002; Karimi *et al*., 2007). This may be due to a lesser stress response among *Bos taurus* compared to *Bos indicus* influenced cattle during routine stressors such as a chute restraint. This event would indicate that *Bos indicus* influenced cattle and those who suffer from more stress would likely have lower pregnancy rates due to delayed or blocked ovulation and could possibly benefit from increasing the dose of GnRH at insemination.

The improvement in pregnancy rates from a 200 µg dose of GnRH was shown to exist in all locations among heifers and mature cows as well as when used with conventional and sex-sorted semen. In this study, the improved pregnancy rates of *Bos indicus* influenced cattle resulting from the administration of 200 µg of GnRH at time of AI were comparable to normal pregnancy rates among *Bos taurus* cattle. Future research should evaluate the total number of *Bos indicus* cattle ovulating in response to increased GnRH doses at AI as well as the mean time to ovulation from GnRH administration in these cattle.

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