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

C.E. Ferguson1,3, D.J. Kesler2, W. Storer1, M. Poole1, B. Pousson1, H. Richard1

1Department of Agriculture, McNeese State University, Lake Charles, LA, USA.
2Department of Animal Sciences, University of Illinois, Champaign-Urbana, IL, USA.

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 release from the anterior pituitary (Daley et al. 1985). 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 (Aihara and Harlan, 1992) and gonadotroph cells in the anterior pituitary (Kononen et al., 1993). Although chichoral 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.

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, adrenocorticotropic (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 (Aihara and Harlan, 1992) and gonadotroph cells in the anterior pituitary (Kononen et al., 1993). Although chichoral 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.
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 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 m² 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-time 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.

<table>
<thead>
<tr>
<th>Location</th>
<th>n</th>
<th>Breed</th>
<th>Type</th>
<th>Age</th>
<th>Semen type</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Texas</td>
<td>19</td>
<td>BM</td>
<td>Heifer</td>
<td>14 to 18 mo</td>
<td>AN, conventional</td>
</tr>
<tr>
<td>2 Texas</td>
<td>32</td>
<td>BR</td>
<td>Cow</td>
<td>3 to 6 yr</td>
<td>BR, sexed-X</td>
</tr>
<tr>
<td>3 Texas</td>
<td>10</td>
<td>BA</td>
<td>Heifer</td>
<td>14 to 18 mo</td>
<td>AN, conventional</td>
</tr>
<tr>
<td>4 Texas</td>
<td>83</td>
<td>BM</td>
<td>Cow¹</td>
<td>3 to 10 yr</td>
<td>BM, conventional</td>
</tr>
<tr>
<td>5 Louisiana</td>
<td>26</td>
<td>XB²</td>
<td>Heifer</td>
<td>13 to 18 mo</td>
<td>AN, conventional</td>
</tr>
<tr>
<td>5 Louisiana</td>
<td>123</td>
<td>AN, XB³</td>
<td>Heifer</td>
<td>12 to 16 mo</td>
<td>AN, conventional</td>
</tr>
</tbody>
</table>

¹Lactating 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.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Pregnancy Rate ± SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>100 µg GnRH</td>
<td>0.21 ± 0.04*</td>
</tr>
<tr>
<td>200 µg GnRH</td>
<td>0.43 ± 0.05</td>
</tr>
</tbody>
</table>

*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 taurus* 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.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Pregnancy Rate ± SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>100 µg GnRH</td>
<td>0.55 ± 0.06*</td>
</tr>
<tr>
<td>200 µg GnRH</td>
<td>0.49 ± 0.06</td>
</tr>
</tbody>
</table>

*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 buserelin 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.

**Acknowledgments**

The authors would like to thank the following companies; Pfizer Animal Health, Kalamazoo, MI and Teva Animal Health, St. Joseph, MO, who generously donated pharmaceuticals for use in estrous cycle synchronization during this experiment, Todd Henderson for insemination services and the following beef producers for participation in this study: Sam Parigi (Jefferson County Poor Farm), Gilbert Adams (Adam’s Beefmaster Farm), Lorenzo Lasater (Isa Cattle Co.).

**References**


Norman RL, McGlone J, Smith CJ. 1994. Restraint inhibits luteinizing hormone secretion in the follicular...


