ABSTRACT

Embryo transfer is an important assisted reproductive technique to increase the efficacy of reproduction in dairy cattle, although responses to superovulation therapies remain inconsistent. Stress can negatively affect reproduction in farm animals, and could be a factor in compromising ovulation induction and embryo transfer success due to intensive handling procedures. This study evaluated ovulation rates, development of functional corpora lutea (CL), embryo quality, and fecal glucocorticoid (FGM) and fecal progestagen (FPM) metabolite concentrations in response to two FSH superovulation protocols in crossbred Thai dairy cows. Cows were administered decreasing doses of FSH (200 mg) administered over 4 or 6 days (n=3/treatment). All females ovulated based on FPM profiles, but numbers of CL and embryos collected, and embryo quality varied across individuals. Nevertheless, similar numbers of transferable embryos (~ 4 each) were obtained for both 4- and 6-day FSH regimens. FGM concentrations during superovulation (Mid) (162.2 ± 8.4 ng/g) were greater than those before (115.8 ± 5.8 ng/g) or after (104.3 ± 9.8) treatment (P < 0.05), but there was no treatment difference. Observed increases in FGM indicate that reproductive manipulation can induce acute stress
responses; however, there were no apparent adverse effects on reproductive responses or embryo quality.

**Keywords:** Cow, Fecal glucocorticoids, Fecal progestagens, Stress, Superovulation

**INTRODUCTION**

Superovulation has been shown to increase the efficiency of embryo transfer and *in vitro* fertilization techniques in ungulate species, both domestic, such as sheep, goats (Cognié et al., 2003; Rahman et al., 2008) and cattle (Bó and Mapletoft, 2014), and non-domestic, including red deer (*Cervus elaphus*) (Bainbridge et al., 1995) and brown brocket deer (*Mazama gouazoubira*) (Zanetti et al., 2014). Twice daily intramuscular injections of decreasing follicle-stimulating hormone (FSH) doses over 4 days (total dose of 260-300 mg) has been a standard superovulation protocol for crossbred dairy cows in Thailand for more than 20 years (Sumretprasong et al., 2008; Bó and Mapletoft, 2014). It is typically combined with use of a progesterone-releasing device (CIDR-B) inserted intravaginally, which remains in place until the end of FSH treatment (Bó et al., 2008). PGF<sub>2α</sub> administration on the third day of FSH treatment has further been incorporated into the protocol to synchronize estrus, followed by artificial insemination (AI) at 12, 24 and 36 hours after standing heat. Finally, GnRH often is administered at the time of the first AI to ensure ovulation takes place.

A follicular wave is defined as the synchronous growth of a group of 8 – 41 small follicles (3 – 4 mm in diameter) from which a single follicle is selected to become dominant (Adams et al., 2008). This growth in cattle is FSH–dependent, with subordinate follicles becoming atretic when FSH concentrations are below the threshold needed to sustain development. Superstimulatory treatments with exogenous FSH prevent the regression of subordinate follicles, allowing small follicles to be recruited into the cohort of growing follicles (Bó et al., 2008). Although FSH is generally administered over 4 days, it has been proposed that longer treatments could increase recruitment by providing more time for smaller follicles to reach an ovulatory size and acquire the capacity to ovulate (Mapletoft and Bó, 2013). Assuming a growth rate of 1 – 2 mm per day, these follicles could be recruited by adding 2 – 3 days to the treatment protocol (Mapletoft and Bó, 2013). Indeed, lengthening the treatment protocol from 4 to 7 days without increasing the total amount of FSH administered increased the number of ovulations and transferable embryos in beef cows (García Guerra et al., 2012).

Problems with superovulation regimens often are related to highly variable ovarian responses, both within and across species and individuals (Cognié et al.,