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The Effect of GnRH Agonist (Buserelin) Treatment of Awasi Ewes on Day 12 Post-mating on Plasma Oestradiol Concentrations

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Abstract: The main objective of this study was to examine the effect of treatment of Awasi ewes with GnRH agonist (buserelin) on day 12 post-summer induced mating on oestradiol concentrations. Ewes were divided into two groups (15 ewes/group). All ewes were synchronized for estrus using intravaginal progestagen sponges removed on day 12. Soon after sponges removal, all ewes were given an intramuscular 500 IU injection of PMSG. Natural mating was made by the introduction of two adult fertile rams (fitted with raddles) to each group one day after PMSG injections. Twelve days after the start of mating, only ewes marked by the rams were injected intramuscularly with 10 micrograms of buserelin (12 ewes-treated), or injected with normal saline (10 ewes-control). Blood samples were taken from these ewes for the measurement of oestradiol concentrations at -36, -24, -12 h and immediately before the start of treatment at 0 hour and at 12, 24 and 36 h post-treatment. No significant differences were found in oestradiol concentrations before and after treatment with buserelin (1.78 ± 0.10 and 1.66 ± 0.11 pg mL⁻¹, respectively). However, post-treatment oestradiol concentrations were found to be significantly higher ($p < 0.05$) in the control group compared to pre-treatment values (2.07 ± 0.15 and 1.78 ± 0.10 pg mL⁻¹, respectively). Furthermore, post-treatment oestradiol concentrations were found to be significantly higher ($p = 0.025$) in the control compared to buserelin treated ewes (2.07 ± 0.15 and 1.66 ± 0.11 pg mL⁻¹, respectively). These results indicate that treatment of Awasi ewes with buserelin 12 days post-summer induced mating suppressed the increase in plasma oestradiol concentrations.

Key words: Sheep, summer mating, GnRH analogue

INTRODUCTION

The rapid expansion in the sheep industry in Saudi Arabia had lead to the introduction of intensive and semi-intensive farming systems for all the farm species. The sheep industry constitutes around 50% of the total livestock market in Saudi Arabia and therefore, contributes considerably to meat production in the country. Factors such as anestrus during the summer which coincide with high environmental temperatures contribute largely to ewe's low fertility. Heat stress has been suggested to be a major factor for lower fertility in a number of species, including cattle (Ingraham *et al.*, 1974), goats (Ozawa *et al.*, 2005), rats (Takashi *et al.*, 2005) and ewes (Bolet, 1986). There are some suggestions that high environmental temperatures causes a decrease in fertility by affecting the animal's hormonal or embryonic systems (Gwazdauskas *et al.*, 1973; Putney *et al.*, 1988). Moreover, there are some data to suggests that defective luteal function and low progesterone concentrations during the early stages of pregnancy constitute a main factor in early loss of embryos (Ashworth *et al.*, 1989). Efforts have been made to improve conception rates, litter size and fetal

growth through progesterone supplementation after natural mating and artificial insemination (Davis *et al.*, 1986; McMillan, 1987; Ashworth *et al.*, 1989; Kleemann *et al.*, 1994; Kleemann *et al.*, 2001; Wallace *et al.*, 2003).

Treatment of ewes with buserelin (GnRH agonist) 12 days after mating has been found to improve fertility in ewes, does, cows and mares (Cam and Kuran, 2004a; McMillan *et al.*, 1986; Cam and Kuran, 2004b; Macmillan *et al.*, 1985; Newcombe *et al.*, 2001). It is suggested that treatment with buserelin may improve fertility rates in cows and ewes through improving the activity of the corpus luteum and its progesterone secretion needed for the maintenance of pregnancy (Macmillan *et al.*, 1985; Rettmer *et al.*, 1992; Basiouni and Homeida, 2005) or through its effect to form accessory corpora lutea (Beck *et al.*, 1996), decreasing oestradiol concentrations and consequently improving embryo survival by weakening the luteolytic mechanism (McMillan *et al.*, 1986; Beck *et al.*, 1996) However, all of the previous experiments dealing with treatment of ewes or cows with buserelin were conducted in areas where environmental temperatures never exceeded 35-40°C, i.e.,

in comparatively less stressful conditions than one could expect during summer months in Saudi Arabia. Treatment with buserelin was found to improve lambing rates under the extremely hot summer conditions in Saudi Arabia (Basiouni and Homeida, 2005).

The aim of this study, therefore, was to investigate the effect of treatment of Awasi ewes with buserelin on day 12 post-mating on plasma oestradiol concentrations in an attempt to explore the mechanism by which fertility of ewes is improved by buserelin treatment of ewes on day-12 post mating.

MATERIALS AND METHODS

This experiment was conducted at King Faisal University Research Station (Al-Hofouf, Saudi Arabia) during the month of August 2003 (average minimum and maximum temperatures were 26.6 and 49°C, respectively, average relative humidity were 24%). Thirty multiparous anestrus ewes of Awasi breed were divided into two groups (15 ewes/group). All ewes were synchronized for estrus using intravaginal progestagen sponges (Chronogest®, Intervet, UK Ltd) that were removed on day 12. All ewes were given an intramuscular 500 IU injection of PMSG (Intervet, UK Limited) soon after sponges removal. One day after PMSG injections, two rams were introduced to each treatment group. These rams were fitted with raddles. Twelve days after rams introduction, only ewes mated were injected intramuscularly either with 10 µg of buserelin (Buserelin, Hoescht UK Ltd) (n = 12), or normal saline (n = 10). Blood samples were collected from all ewes by jugular venepuncture at -36, -24, -12 and immediately before the start of treatment at 0 h and at 12, 24 and 36 h after the treatment. Soon after collection, all blood samples were centrifuged immediately, plasma separated and stored at -20°C until assayed for oestradiol. Plasma oestradiol concentrations were determined by radioimmunoassay using DPC Kits (oestradiol double antibody, KE2D, Diagnostic Product Co., Los Angeles, Ca, USA) by the method of Meikle *et al.* (1997). The limit of sensitivity of the assay was 0.77 pg mL⁻¹. The inter- and intra-assay coefficients of variation were 13.9 and 8.33%, respectively.

T-test was used to compare mean oestradiol concentrations between the treatment groups using SAS (SAS, 2001).

RESULTS

Radioimmunoassay for plasma oestradiol concentrations showed no difference in the mean oestradiol concentrations between the two treatment

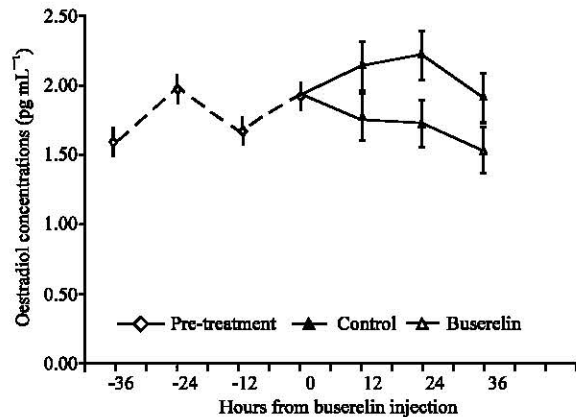


Fig. 1: Oestradiol concentrations (\pm SE) before and after treatment with buserelin injection given at time 0 h

groups before treatment with buserelin (1.78 ± 0.10 pg mL⁻¹) (Fig. 1). Furthermore, no differences were found in the mean oestradiol concentrations before and after treatment with buserelin in the buserelin treated group (1.78 ± 0.10 and 1.66 ± 0.11 pg mL⁻¹, respectively). However, in the control group, post-treatment oestradiol concentrations were found to be significantly higher ($p < 0.05$) compared to pre-treatment values (2.07 ± 0.15 and 1.78 ± 0.10 pg mL⁻¹, respectively). Post-treatment oestradiol concentrations were also found to be significantly higher ($p = 0.025$) in the control compared to buserelin treated ewes (2.07 ± 0.15 and 1.66 ± 0.11 pg mL⁻¹, respectively).

DISCUSSION

In the present study, the changes in plasma oestradiol concentrations resulted from buserelin treatment of Awasi ewes 12 days post-summer induced mating are similar to those reported in previous studies in both, cows and ewes (Rettmer *et al.*, 1992; Mann and Lamming, 1995a; Beck *et al.*, 1996). However, in this study plasma oestradiol concentrations did not differ between pre and post-treatment values in the buserelin treated ewes while post-treatment oestradiol concentrations increased significantly in the control group compared to pre-treatment values which may suggest that treatment with buserelin in this study may caused a suppression of an increase in oestradiol concentrations in the buserelin treated group rather than a decrease in its concentrations as it is reported previously (Beck *et al.*, 1996; Macmillan and Thatcher, 1988). Previous studies have suggested that the decrease in oestradiol concentrations as a result of treatment with buserelin may improve embryo survival

through the suppression of luteolytic mechanism (Beck *et al.*, 1996; Macmillan and Thatcher, 1988). Moreover, in Awasi ewes, Basiouni and Homeida (2005) found that among ewes that returned to estrus after treatment at day 12 post-mating, only ewes treated with buserelin at the previous cycle has a significantly higher plasma progesterone concentrations during the luteal phase of both, the first and the second estrous cycle. Any reduction in oestradiol concentrations during this time will reduce prostaglandin F2 α release and thus suppress or delay luteolysis (Mann and Lamming, 1995b; Beck *et al.*, 1996; Pate, 2003; Thatcher *et al.*, 2003). Therefore, any decrease or suppression of an increase in oestradiol secretion resulted from buserelin treatment at day 12 post-mating may increase embryo survival during this critical period of maternal recognition of pregnancy.

In the present study, significantly lower post-buserelin treatment oestradiol concentrations (compared to control) 12 days post-mating may contribute towards embryo survival and improve fertility that has been reported previously after such a treatment in a number of studies (Cam and Kuran, 2004a; McMillan *et al.*, 1986; Cam and Kuran, 2004b; Macmillan *et al.*, 1985; Newcombe *et al.*, 2001).

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