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DIFFERENCES in PLASMA CONCENTRATIONS of LH and FSH in ANOESTRUOUS EWES TREATED with GnRH or a GnRH ANALOGUE

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ABSTRACT

This study was conducted using 16 anoestrous ewes to determine the effects of GnRH or an analogue of GnRH (A-N; Azagly-nafarelin; Intervet International BV, Boxmeer, The Netherlands) on secretion of LH and FSH. In the experiment, 7 treatments were used over 16 days in a randomised Latin square design. The treatments were saline, GnRH (0.1, 1 and 10 µg) or A-N (0.001, 0.01, 0.1 and 1 µg), desolved in 2ml saline then injected intravenously. Blood samples were collected at intervals of 15, 30 or 60 min from 60 min before until 360 min after the injection. In the experiment jugular venous blood was collected from an indwelling jugular venous catheter. The sensitivities of LH and FSH assays were 0.22±0.01 ng/ml and 0.055± 0.005 ng/ml respectively. For LH assay, intra-assay coefficient of variations for high, medium and low were 10.5, 11.1 and 11.4 and inter-assay coefficient of variation for high, medium and low were 20.2, 10.5 and 16.2, respectively. For FSH assay, inter-assay coefficient of variations for high, medium and low were 7.4, 7.6 and 12.0 and intra-assay coefficient of variations, or high, medium and low, were 4.8, 16.9, and 17.9, respectively.

According to the data, small doses of GnRH or A-N did not significantly effect plasma concentrations of FSH and LH , whereas higher doses of GnRH (10µg) and of A-N (1.0 µg) caused a significant increase in plasma FSH and LH concentrations. In addition, it has been seen that the A-N was 30 and 38 times more potent than GnRH for the induction of FSH and LH secretions, respectively.

Keywords: *GnRH, FSH, LH, Sheep*

KIZGINLIK MEVSİMİ DIŞINDAKİ KOYUNLARA YAPILAN GnRH veya GnRH ANALOĞU ENJEKSİYONUNUN FSH ve LH SALGISINDA SEBEP OLDUĞU DEGİŞİKLİKLER.

ÖZET

Bu çalışma GnRH ve GnRH analoğunun (A-N; Azagly-nafarelin; Intervet International BV, Boxmeer, The Netherlands) FSH ve LH salgısına etkisini incelemek için yapıldı. Kızgınlık dışındaki 16 koyun; random latin kare dizaynı kullanılarak 16 günden fazla bir süre içinde 7 değişik enjeksiyona tabi tutuldu. GnRH enjeksiyonu için 0,1; 1 ve 10 µg'lık dozlar ve A-N için ise: 0,001; 0,01; 0,1 ve 1 µg' lık dozlar 2 ml tuz çözeltisi içerisinde i.v verildi. Kan örnekleri enjeksiyondan 60 dakika önce ve 360 dakika sonra olmak üzere 15, 30 ve 60 dakika aralıklarla v. Jugularis'e yerleştilen kanuladan alındı. Plazmalardaki LH ve FSH hormonları RIA ile ölçüldü. Yapılan FSH ve LH analizleri için standartların oluşturulmasında kullanılan hormonların çapraz re-aktiviteleri biliniyordu. LH ve FSH ölçüm hassaslığı 0.22±0.01 ng/ml ve 0.055± 0.005 ng/ml olarak hesaplandı. LH analizinde ölçüm içi (Sıra ile az, orta ve yüksek miktarda hormon içeren kalite kontrol gurupları için) varyasyon katsayıları: 10,5; 11,1 ve 11.4 olarak bulundu. Ölçümler arası varyasyon katsayıları (Sıra ile az, orta ve yüksek) ise 20,2; 10,5 ve 16,2 olarak bulundu. FSH analizine ait ölçüm içi varyasyon katsayıları (Az, orta ve yüksek) 4,8; 16,9 ve 17.9 olarak hesaplandı. Ölçümler arası varyasyon katsayıları, az orta ve yüksek seviyede hormon içeren kalite gurupları için ise sıra ile 7.4, 7.6 ve 12.0 olarak bulundu.

Elde edilen verilere göre GnRH veya A-N küçük dozlar kullanılarak yapılan enjeksiyonu; LH ve FSH salgısını artırmada etkili olmadığı fakat yüksek dozlarda enjeksiyon LH ve FSH salgısını önemli ölçüde artırdığı belirlenmiştir. Ayrıca LH salgısını başlatmada A-N nin GnRH'den 38 kez ve FSH salgısını başlatmada ise GnRH'den 30 kez daha güçlü olduğu belirlenmiştir.

Anahtar Sözcükler: *GnRH, FSH, LH, Koyun*

INTRODUCTION

Gonadotrophin-releasing hormone (GnRH) from hypothalamus controls the secretion of LH and FSH from the anterior pituitary gland by which the ovaries are controlled. It is unlikely that very much hypothalamic GnRH reaches the ovary because of dilution in the peripheral circulation and its rapid degradation by enzymes (Tsafriri and Adashi, 1994) therefore it affects follicular growth through an effect on pituitary with the end result of LH and FSH synthesis. The amount of gonadotrophin secreted is a function of the amount of GnRH reaching the pituitary gland and the responsiveness of anterior pituitary gland to GnRH (Baird and McNeilly, 1981.; Kumar *et al*., 1997). This has been confirmed by active immunization against GnRH in sheep. Immunization of sheep against GnRH caused a decrease in LH and FSH concentrations and the absence of ovulation (Webb *et al.,* 1981; Brown *et al.,* 1995). Thus, in order to achieve appropriate gonadotrophin secretion to stimulate preovulatory follicular growth, GnRH analogues can be administered because of their long life and higher resistance to degradations. Therefore, they are generally used to stimulate ovarian function. Short-term treatment with a GnRH agonist does not generally suppress plasma concentration of FSH whereas chronic treatment with a GnRH agonist will eventually suppress plasma concentrations of FSH.(McNeilly and Fraser 1987; Picton *et al.,*1990; Picton *et al.,* 1991).

Pituitary gonadotrophins are important for the attainment of puberty in sheep and the progress of follicular development within the ovary. The external control of pituitary function by long acting releasing hormones (such as analogues) may help to shorten the time period between birth and puberty and also it may

facilitate folliculogenesis within ovary. Therefore, the aim of this study is to measure the effect of a A-N on pituitary secretions of LH and FSH.

MATERIALS AND METHODS

Animal

Sixteen cross bred sheep (Suffolk and Cheviot, 2-6 years old), with an average weight of 63 kg were purchased from a commercial company. The experiment carried out in spring time. The ewes were housed indoor and were penned together in groups of 3, 2 or 4 and hay fed and watered *ad libitum*.

GnRH

Human synthetic GnRH was purchased from Sigma chemical company (Cat; L-7134, St Louis, MO, USA). GnRH analogue, azagly nafarelin (A-N) obtained from intervet (A-N; Azagly-nafarelin; Intervet International BV, Boxmeer, The Netherlands)

Injections

The ewes were injected with saline (Control, n= 14) or different doses of GnRH (0.1, 1 and 10μg; n=13, n=13 and n=14 respectively) or a A-N, azagly nafarelin (1, 10, 100 and 1000 ng; n=13, n=13, n=14 and n=14) respectively. Blood samples (4 ml) collected at intervals of 15, 30 or 60 min from 60 min before until 360 min after the injection for 7 hours starting at 11:00 am.

Measurements of plasma LH and FSH concentrations

Labels

$Na^{125}I$ was obtained from Mr Michael Avella, Royal Veterinary College, UK. Ovine FSH and LH for iodination was obtained from NIDDK (Cat; AFP 4117A; Cat; AFP7071B, Beltsville, USA). These proteins were iodinated by using the Chloramine-T method (Hunter and Greenwood, 1964).

Reference preparation for LH

Ovine LH for use as the reference preparation obtained from NIDDK-NIH, oLH-26 (Cat; APF192279, Beltsville, USA). Standards were prepared in concentrations of 0.2 ng/ml to 100 ng/ml.

Reference preparation for FSH

Ovinea FSH (APB-4117A) was obtained from USA-BARC-Reproduction Lab-Beltsville, USA. Standards were prepared in assay buffer in concentrations of 0.05 to 50 ng/ml.

Measurement of Plasma LH level

Plasma LH concentrations were measured by RIA. The antibody for LH was developed in a rabbit (R 29) and kindly provided by Dr BK Campbell (University of Edinburgh, Centre for Reproductive Biology, Edinburgh. Rabbit IgG (Cat; S0022-220) and Normal Rabbit Serum (Cat; S030-220) was obtained from Scottish Antibody Production Unit, Low Hospital Carluke, Lanarkshire, Scotland.

100 μl of unknown plasma was placed in assay tubes containing 200 μl of assay buffer and 100 μl of the first antibody (R29) in duplicate and incubated for 2 days at 4 ^{o}C, 100 μl label was then added giving 12000-15000 CPM and the tubes were incubated at the same temperature for another 2 days. After two days, the tubes were added with the second antibody and the normal rabbit serum then tubes were incubated further 24h. After 24h, 1 ml 0.01 M phosphate buffered saline (PBS) was added and assay tubes were immediately centrifuged, at 2900 rpm for 1h. The tubes finally decanted and counted in a gamma counter.

The amount of LH was calculated by using computer software, Assay Zap Version 2.32, universal assay calculator, Copyright P. L. Taylor 1987-1992, Published and distributed by Biosoft 22 Hills Road, Cambridge CB2 IJP, UK).

Measurement of Plasma FSH level

Anti-ovine FSH developed in the rabbit (Cat; APF-C5288113, NIDDK-NIH, Beltsville, USA) was used at a dilution of 1/12000. Anti-rabbit IgG (second antibody) and normal rabbit serum were the same as used for LH assay. Buffers, incubation time and other assay procedures applied in FSH assay were also identical to the LH assay.

Assay parameters

The sensitivity of LH and FSH assays were 0.22±0.01 ng/ml and 0.055± 0.005 ng/ml respectively. For LH assay, intra-assay coefficient of variation for high, medium and low were 10.5, 11.1 and 11.4 and inter-assay coefficient of variation for high, medium and low were 20.2, 10.5 and 16.2 respectively. For FSH assay, inter-assay coefficient of variations for high, medium and low were 7.4, 7.6 and 12.0 and intra-assay coefficient of variations were 4.8, 16.9, and 17.9 respectively.

Statistical analysis

Data were analysed by using a randomised block design. Total variation, variation between groups, blocks and within groups were calculated. To identify which samples are significantly different at any time of sampling between the groups Last Significant Difference (LSD) method was used. Data are displayed on the figures as mean±sem.

RESULTS

The injection of 1 and 10 μg doses of GnRH caused significant increases ($P<0.05$) in LH secretion. Peak LH level for 0.1, 1 and 10 μg GnRH was 2.04±0.48, 5.01±0.83 and 12.55±2.66, respectively (Figure 1).

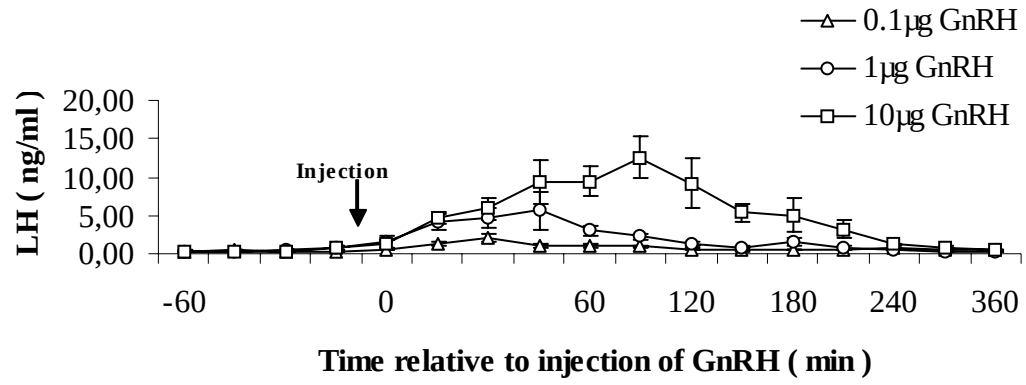

Figure 1. LH secretion was not increased after the injection of 0.1µg of GnRH. LH secretion was significantly increased after the injections of 1 and 10µg doses of GnRH.

Injection of A-N at low doses (1 ng and 10 ng) did not cause any significant changes in LH secretion (Figure 2). Even the doses were 10 times high (10ng A-N), LH secretion was still below the saline (control). Ewes showed some insignificant response to saline (P>0.05).

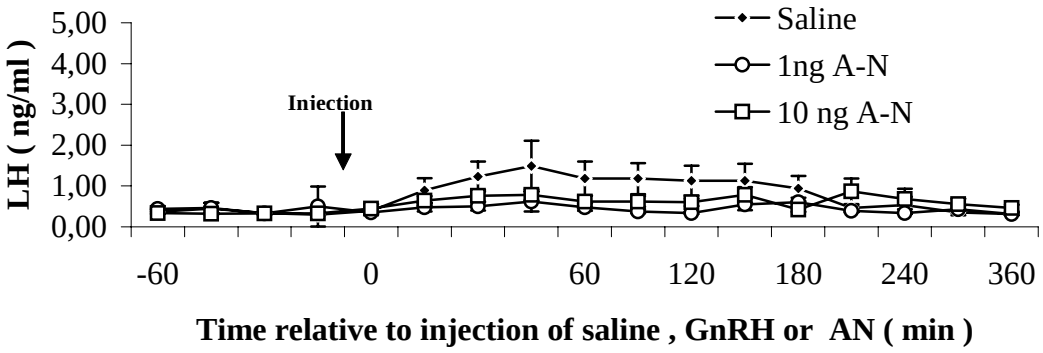

Figure 2. Small doses (1ng and 10ng) of GnRH analogue did not cause any change in LH secretion.

Higher doses of the A-N injection caused significant increases in LH pulses. Peak LH pulse concentrations for saline, 100 ng and 1000 ng A-N are 1.18±0.38, 6.60±1.80 and 46.21±6.82 ng/ml (mean±sem) respectively (Figure 3).

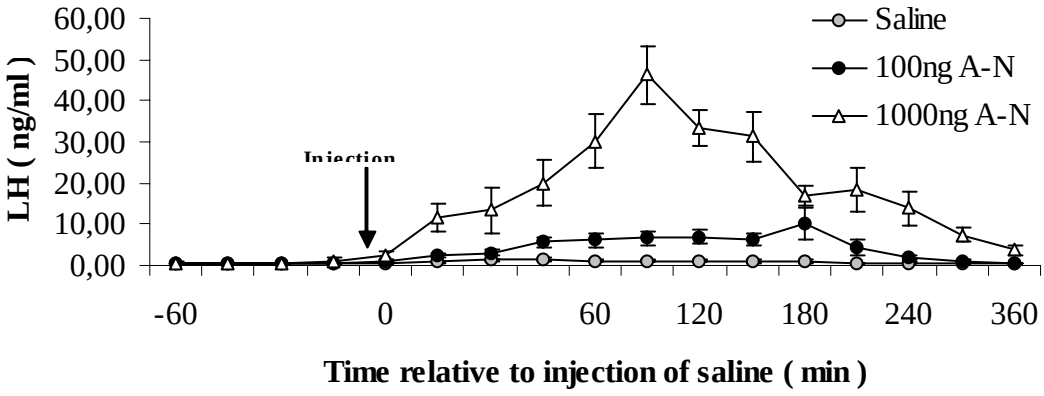

Figure 3. Injection of 100 and 1000ng GnRH analogue caused large increases in LH secretion (P<0.05)

Lower doses of GnRH or the A-N did not significantly increased secretion of FSH whereas higher doses significantly increased FSH secretion (Figures 4 and 5)

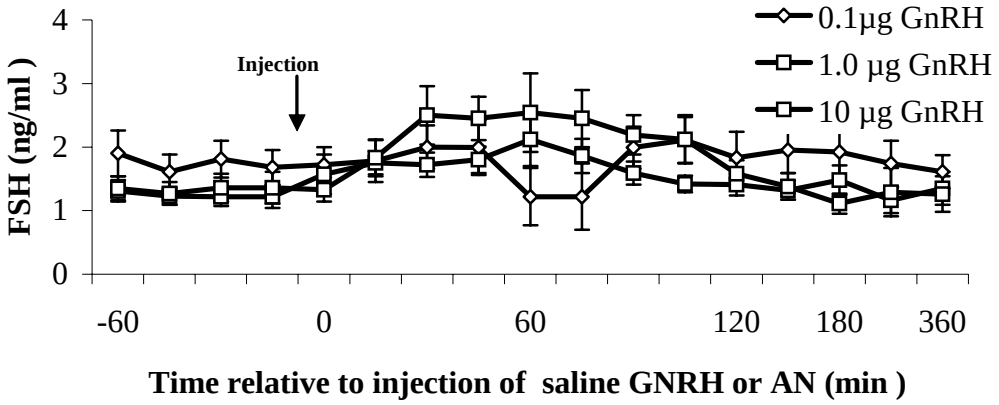

Figure 4. Changes in FSH concentration in response to different doses of GnRH.

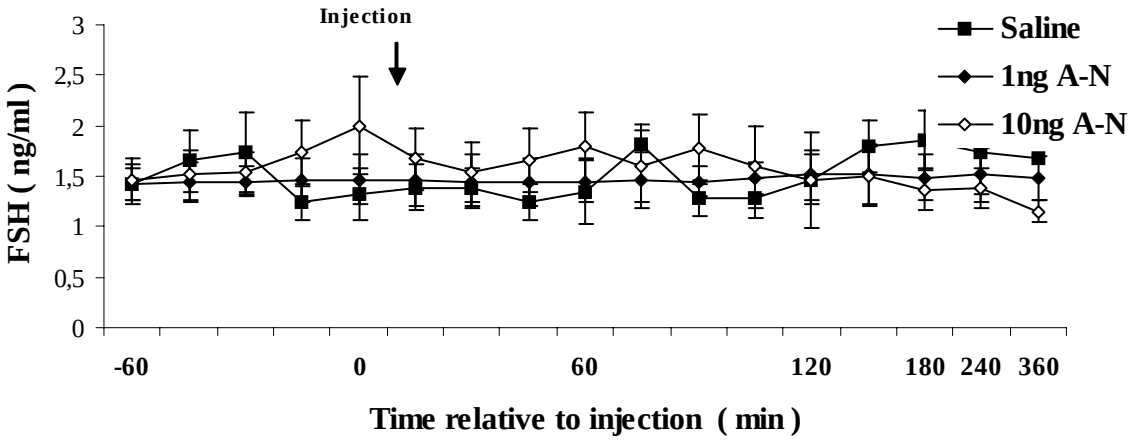

Figure 5. Injections of GnRH analogue (azagly-nafarelin), at the doses of 1 and 10 ng /ml, did not caused any significant effect, on plasma FSH concentrations.

FSH secretion was significantly affected by higher doses of A-N. Peak FSH levels obtained after the injections with saline, 100 and 1000 ng A-N were 1.82±0.5; 2.21±0.4 and 6.67±2.2 ng/ml (mean±sem) respectively (Figure 6).

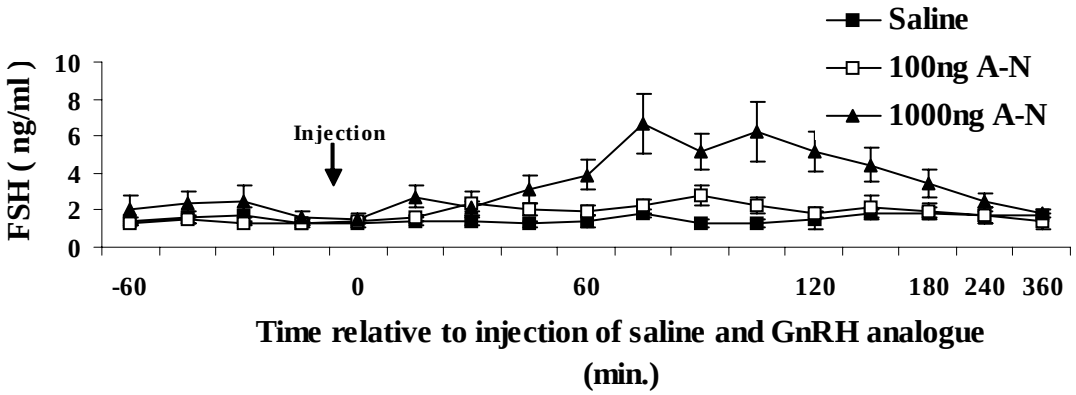

Figure 6. The injection of 1000 ng of the GnRH analogue significantly increased plasma FSH concentrations (P<0.05) while the injection of 100 ng A-N caused a smaller significant increase.

DISCUSSION

The data presented here, shows that the secretions of LH and FSH after the injections of GnRH or A-N were some differently affected. That is because the secretions of LH and FSH are differentially regulated by GnRH pulses. The mechanisms involved in regulation of gonadotrophin response of pituitary to GnRH,

are not understood. In an experiment (Molter Gerard *et al.,* 1999) ovariectomized ewes were passively immunized against GnRH and then the ewes were given pulsatile injections of saline or GnRH analogue for 48 h. Immunization against GnRH suppressed pulsatility of LH release and reduced the mean concentration of plasma FSH after three days. After immunization, pulsatile GnRH analogue replacement restored LH pulses whereas plasma concentrations of FSH did not changed.

Our data shows that lower doses of GnRH or A-N were not effective in inducing LH pulses whereas higher doses of A-N cause a large increases in LH secretion (Figures 1, 2 and 3). These were confirmed by the experiments on sheep that injection of rams with increasing doses of GnRH elicited a dose-dependent rise in LH secretion (Wu *et al.,* 1987). A similar result was obtained from female sheep which was injected with 125, 250 ng GnRH in saline. Mean plasma concentrations of LH were significantly higher in animals receiving 250 ng GnRH than those receiving 125 ng GnRH or saline (McLeot and Heresign, 1984). Clarke (1995) reported that injection of sheep with 500 ng GnRH in 4 pulses of 125ng at 10 min intervals caused a small increase in LH secretion with no preovulatory LH surge. Injection of a bolus amount of GnRH (1000 ng) induced a preovulatory LH surge, (Clarke, 1995).

Small doses of GnRH and the A-N were not effective in stimulating FSH secretion (Figures 4, 5 and 6). However, higher doses of GnRH (10μg) and A-N (1000 ng) caused a significant increase in plasma FSH concentration (Figure 4 and 6).

Short time positive effect of GnRH and its analogues on gonadotrohine secretion is well known. The results show that A-N is a good GnRH analogue which was 38 and 30 times more potent than GnRH in stimulating LH and FSH secretion respectively.

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