

# Effect of Ghrelin on Plasma Concentration of Gonadotropins in the Female Sannan Goats

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**G**hrelin is a 28 amino acid peptide which is secreted from the brain and the gastrointestinal system. Recent studies indicate that ghrelin negatively affects energy balance in most mammals. Based on its neuron distributions in hypothalamus, ghrelin coexists with many other neurons. Therefore, ghrelin controls different physiological actions on many different tracts; one of the physiological actions is its effect on gonadotropins secretions. The present investigation was carried out to analyze the potential involvement of ghrelin in the control of gonadotropin secretion.

**Materials and Methods:** Forty female Sannan goats were randomly divided into two groups; each group received daily injections of either 1 or 2 mg ghrelin/kg of body weight (BW) into the jugular vein for ten days. Blood samples were collected every 30 minutes, for two hours after injection of ghrelin, for three days before first injection, till three days after last injection; the samples were assayed for plasma FSH and LH concentration by the Radioimmunoassay (RIA) technique.

**Results:** Injections of 1 and 2 mg ghrelin/kg BW decreased the mean plasma concentration of LH throughout the injection period in all animals in the two groups. The results indicated that ghrelin significantly decreased mean plasma concentration of LH in the female Sannan goats ( $p < 0.01$ ); it however had no significant effect on the mean plasma concentration of FSH.

**Conclusions:** It was concluded that while ghrelin has no effect in the secretion of FSH, it does

have an inhibitory effect on LH.

**Key Words:** Ghrelin; Follicle stimulating hormone; Luteinizing hormone; Sannan goat

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## Introduction

Ghrelin is a 28-amino acid peptide identified as the endogenous ligand for the growth hormone secretagogue (GHS) receptor. Ghrelin is primarily expressed in the stomach and hypothalamus, and stimulates GH secretion in humans and rats.<sup>1-4</sup> Using in situ hybridization and RNase protection assays, the strongest expression of GHS-R mRNA was detected in several hypothalamic nuclei, including the anteroventral preoptic nucleus, anterior hypothalamic area, suprachiasmatic nucleus, anterolateral hypothalamic nucleus, ARC, PVN, and tuberomammillary nucleus.<sup>5,6</sup> Based on its neuron distributions in hypothalamus, ghrelin coexists with many other neuron; ghrelin has been shown to affect a number of different systems including stimulation of GH, ACTH, cortisol and prolactin release, feeding, gastric acid secretion, gastric motility, cell proliferation, inhibition of sympathetic activity, decrease of blood pressure and promotion of sleep.<sup>7</sup> One of the physiological actions of ghrelin is its effect on gonadotropins secretions. Several studies have shown that changes of ghrelin alter

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mean plasma concentrations of LH and FSH in rats, humans and rhesus monkeys; injections of ghrelin have been documented to decrease mean plasma LH concentrations in rats and ovariectomized rhesus monkeys.<sup>8-</sup>

<sup>11</sup> Furthermore, conflicting evidence exists about the direct effect in vivo of ghrelin on gonadotropin in nonruminants, e.g. injections of ghrelin had no effect on FSH and LH secretion in humans.<sup>12</sup> These changes of gonadotropin secretion due to increased ghrelin may be due to the effect of a negative energy balance which occurs simultaneously with ghrelin concentration; in conditions of negative energy balance, such as fasting for anorexia nervosa, high plasma levels of ghrelin are associated with decrease in LH secretion. The physiological effect of negative energy balance is mostly observed in nonruminants animals such as rats and humans<sup>13,14</sup> Most studies about the effect of ghrelin on LH and FSH secretions were done in nonruminants subjects. It is well established that plasma ghrelin level elevates during fasting, and is accompanied by low plasma glucose concentrations and a negative energy balance. The metabolism of ruminants differs from that of nonruminants;<sup>15</sup> it is hence assumed that the control of glucose concentration and energy balance in ruminants is different to that of nonruminants. Ruminants rarely have a fall in low plasma glucose concentrations and are less susceptible to a negative energy balance.<sup>16</sup> Based on these assumptions, the aim of this study was to determine whether alterations of ghrelin have any effect on LH and FSH concentrations in ruminants.

## Materials and Methods

**Animals and experimental schedule:** Forty Sannan female goats (weighing 45±2.5 kg) were housed in controlled chambers at a constant temperature of 25°C, 70% humidity and fed at libitum. The goats were randomly

divided into two groups; animals in both groups received 1 or 2 mg ghrelin/kg BW everyday for ten days. Blood samples were collected every 30 minutes after injection of ghrelin, for two hours from three days before the first injection till three days after the last injection, by venoject tubes from the jugular vein. Samples were kept on ice until centrifuged (3000 rpm 15 min). Plasma was frozen at -20°C, until use for FSH and LH assays.

**Hormone assays:** Ovine LH was used as the standard to produce antiserum Tabeshyarnoor Co. (Industrial City of Bu-Ali, Hamadan, Iran) and for producing labeled I125 antigen. A seven-point standard curve ranging from 0.4 to 10 ng LH was used. An average assay binding of 40% was achieved using an initial 1:20000 dilution of LH antiserum for LH assays. The inter- and intra-assay variations were 6% and 9% respectively. For FSH assay, ovine FSH (TYN-OFSH) and antiserum against FSH, produced by Tabeshyarnoor Co. (Industrial City of Bu-Ali, Hamadan, Iran), Ovine FSH (TYN-OFSH) was used for iodination. A seven-point standard curve ranging from 0.5 to 8 ng FSH was used. An average assay binding of 40% was achieved using an initial 1:10000 dilution of FSH antiserum for FSH assays. The inter- and intra assay variations were both 8%.

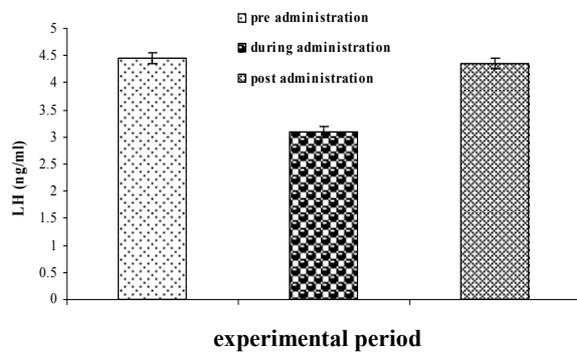
The ovine LH antibody used had a sensitivity of 99.9% and cross-reactivity with the FSH was less than 0.01. The ovine FSH antibody used had a sensitivity of 99% and cross-reactivity with the LH was less than 0.01.

**Statistics:** We used repeated measurements test for comparison among different groups, containing two ghrelin doses, three experimental periods including pre administration, during administration and post administration. Data were subjected to analyses of variance using the SAS.8.2 (SAS, 2001), and the treatment means were compared using the Duncan test.

## Results

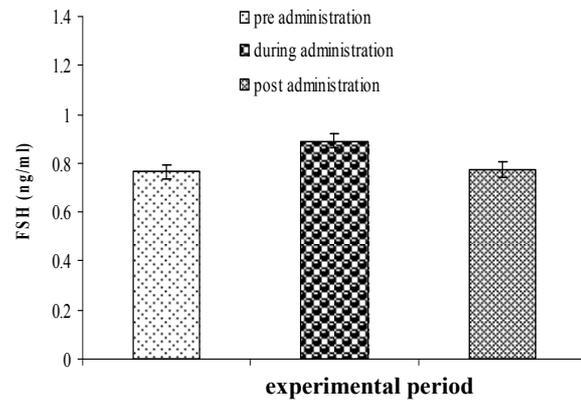
Effect of ghrelin injection on the mean plasma concentration of FSH and LH in three experimental periods:

The mean plasma LH concentration decreased in the experimental period of during administration in comparison with experimental period of pre administration; then, it increased in the period of post administration. Ghrelin caused up to 44% decrease in mean plasma concentration of LH in the experimental period during administration compared to the experimental period of pre administration, a decrease that was significant ( $p < 0.01$ ) (Fig. 1).



**Fig. 1.** The mean plasma concentration of LH in three experimental periods (the experimental period of pre administration (days 1-3) the experimental period of during administration (days 4-11), and the experimental period of post administration (days 12-14).

Injection of ghrelin did not cause significant changes in the mean plasma FSH concentration. In the administration period, ghrelin caused up to 16% increase in mean plasma FSH concentration in comparison to re-experimental administration period; it then decreased in the period of post administration. Although, ghrelin caused up to 16% increase in FSH concentration, the decrease was not significant (Fig. 2).

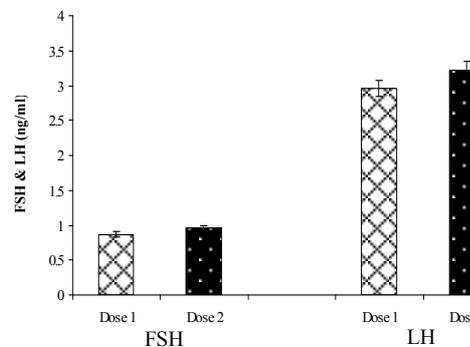


**Fig. 2.** The mean plasma concentration of FSH in three experimental periods: The preadministration experimental period (days 1-3), during administration experimental period (days 4-11) and post administration experimental period (days 12-14).

Therefore, the results of this experiment indicate that ghrelin may decrease the mean plasma LH concentration in female Sannan goats; however it has no significant effect on the mean plasma FSH concentration.

### Effect of ghrelin doses on FSH and LH

Results of statistical analysis showed no significant difference between the two doses of ghrelin for LH and FSH (Fig. 3).



**Fig. 3.** Effect of different doses of ghrelin on the mean plasma concentration of FSH and LH

## Discussion

The data of the present study indicate that ghrelin affects gonadotropin secretion in a different manner, a result that revealed for the first time that administration of ghrelin into the jugular vein in female Sannan goats decreases LH secretion, whereas it has no effect on their FSH secretion. Secretion of FSH and LH is dissociated in many physiological and experimental conditions.<sup>17-</sup>

<sup>19</sup> In rat gonadotropes, two distinct subsets of secretory granules have been described (small dense granules rich in LH and large lucent granules rich in FSH).<sup>20</sup> It is possible that ghrelin differentially modulates the intracellular signals activated by LHRH, thus triggering the release of the different granules.<sup>8</sup>

The present study showed that ghrelin does have an effect on LH, whereas it does not affect FSH, results in agreement with those of other studies.<sup>8-11</sup> There is only one study in humans, which indicates that Ghrelin has no effect in either of these two hormones.<sup>12</sup>

The pathways by which peripherally secreted ghrelin may affect LH secretion are not yet fully understood. There is considerable circumstantial evidence to suggest that NPY (neuropeptide Y) and AGRP (agouti related polypeptide) may mediate the inhibitory effect of ghrelin on the LH secretion. NPY and AGRP are collocated within the same neurons of the hypothalamic arcuate nucleus,<sup>4</sup> an area that is viewed as an important site in the control of food intake.<sup>21,22</sup> Ghrelin administration in the rodent increases the synthesis of both NPY and AGRP,<sup>17</sup> both peptides exert a powerful

orexigenic effect when infused centrally in the rodent and the rhesus monkey.<sup>18,19,23,24</sup> AGRP and NPY are highly up-regulated by food restriction,<sup>4</sup> a condition known to suppress pulsatile LH release in humans and other species.<sup>21,25,26</sup> Finally, data from OVX monkeys have shown that NPY or AGRP injections into the third ventricle inhibit pulsatile LH.<sup>10,27</sup> Moreover, it is possible that ghrelin exerts its inhibitory effect on LH secretion via GH (Growth Hormone). Ghrelin increases GH secretion, and elevated GH decreases LH secretion.<sup>12,28</sup> Insulin can mediate decrease of LH secretion. Ghrelin decreases insulin secretion,<sup>11,32</sup> which leads to inhibition of LH secretion.<sup>14,20,2-31</sup>

Ghrelin can stimulate appetite throughout inhibition of leptin,<sup>33</sup> and it is possible that ghrelin mediates its inhibitory effect on LH in this way as well, since leptin stimulates LH secretion,<sup>34,35</sup> and ghrelin inhibits leptin secretion.<sup>35</sup> Hence it is possible that decrease in leptin secretion leads to decrease in LH secretion. It can be said that the effect of leptin on LH secretion is the same as NPY and AGRP, because leptin exerts its inhibitory effect on appetite through an inhibitory effect on NPY and AGRP.<sup>36</sup>

In conclusion, Ghrelin has no effect in the secretion of FSH but it does have an inhibitory effect on LH.

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