Hypogonadism in Heat Stressed Goats: Poor Responsiveness of the Ovary to the Pulsatile LH Stimulation Induced by Hourly Injections of a Small Dose of GnRH

Yukio KANAI, Noritoshi YAGYU and Takashi SHIMIZU

*Institute of Agriculture and Forestry, University of Tsukuba,
Tsukuba-shi, Ibaraki 305, Japan*

Abstract. An experiment was designed to determine if hypogonadism in heat stressed animals is associated with reduced endocrine function of the pituitary or the ovary. A long day-induced anestrus goat model in which ovulatory follicular growth is known to be induced by hourly injections of a small dose of GnRH was used. Ten anestrus animals, divided into two groups of 5 each, were treated with GnRH (3 ng/kg body weight) for 48 h under two temperature conditions, 25 C (Control) or 38 C (Heat stress). Changes in plasma concentrations of LH and estradiol were monitored at 2 to 4 h-intervals. In addition LH was monitored every 15 min for 16 h starting at 4 h before the first GnRH injection. GnRH treatment resulted in a prompt and sustained increase in plasma estradiol concentrations accompanied by a preovulatory LH surge within 24 h of GnRH treatment in all control animals. Heat stress abolished such ovarian responses in 4 out of 5 animals with the estradiol concentrations remained unchanged or increased slightly (7.7 pg/ml vs 2.0 pg/ml, $P < 0.01$). Before GnRH treatment, the frequency but not the amplitude of the LH pulse was higher in the heat stressed group (0.75/4 h vs 2.75/4 h, $P < 0.05$). The active pulsatile secretion was associated with slightly lowered estradiol concentration during this period (2.4 pg/ml vs 1.4 pg/ml $P < 0.05$). GnRH-treatment resulted in a synchronized pulsatile secretion of LH in both groups with the frequency of LH pulses being controlled at once every hour (3.67/4 h vs 4.17/4 h). The amplitude of GnRH-induced LH pulse (0.81 ng/ml vs 1.62 ng/ml, $P < 0.01$) as well as the baseline (0.73 ng/ml vs 2.02 ng/ml, $P < 0.05$) and mean concentrations (1.19 ng/ml vs 2.81 ng/ml, $P < 0.05$) of LH were significantly higher in the heat stressed group. These results demonstrate that heat stress abolishes GnRH-induced ovulatory responses in anestrus goats not mediated by a decreases in pituitary LH secretion, but rather by reducing responsiveness of the ovary to LH.

Key words: Hypogonadism, Heat stress, Ovarian responsiveness to LH, Goat.

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It is generally accepted that heat stress interferes with ovarian function and thereby depresses the reproductive performance of farm animals raised in hot environments [1–3]. Ovulatory failure and anestrus have been shown to be common obstacles to animal production in the trop-

ics and subtropics [4, 5]. In spite of the awareness of such detrimental effects of heat stress, the mechanisms by which heat stress affects the ovarian function are still poorly understood. A decrease in gonadotropin secretion has been often claimed to be a major cause of hypogonadism in heat stressed animals. This is based on evidence that exposure of animals to experimentally modified

hot environments caused a significant decrease in plasma LH concentration [6, 7]. However, there are conflicting findings that suppression of the estrus manifestation and/or decrease of estradiol secretion in heat stressed animals were not necessarily accompanied by a decrease in LH secretion [8, 9]. Therefore, it also appears probable that the responsiveness of the ovary to gonadotropins may decrease under heat stressed conditions.

The ovarian function such as follicular growth or estradiol secretion in mammals is under the control of inter-related endocrine functions of the hypothalamo-pituitary-gonadal axis. Heat stress may affect the axis at all levels either directly or indirectly. If this is the case for hypogonadism in heat stressed animals, it is difficult to know which part(s) of the hypothalamo-pituitary-gonadal axis is primarily affected by heat stress, even though hypogonadism is associated with significant changes in the plasma concentrations of pituitary gonadotropins and/or ovarian steroids.

The purpose of the present study was to determine if hypogonadism in heat stressed animals, as evidenced by the low ability of the ovary to secrete estradiol, is mediated through a decrease in pituitary LH secretion or associated with poor responsiveness of the ovary to circulating LH. In order to synchronize the follicular growth and subsequent increase of estradiol secretion, long day-induced anestrus goats were used in the present study and the frequency of pulsatile LH secretion was adjusted to a constant rate by giving them hourly injections of a small dose of GnRH. This treatment is known to be sufficient to induce LH pulses and ovulatory follicular growth in anestrus ewes [10].

Materials and Methods

Animals and long day treatment

Cycling Shiba goats (Japanese native breed) which are non-seasonal breeders but known to be photo-sensitive [11], were exposed to a long day-photoperiod (16 L: 8 D) from the beginning of April. After the initiation of the long day treatment, plasma progesterone levels were monitored twice a week to confirm when the animals became anestrus. Anestrus was defined as a period during which plasma progesterone levels consistently remained below 1 ng/ml.

Temperature treatments and hourly GnRH injections

Ten animals selected at 45 to 95 days of anestrus were divided into 2 groups (Control, n=5 and Heat-stress, n=5) and moved to climatic chambers where the temperature, relative humidity and photoperiod were adjusted to 25 C, 50% and 16 L: 8 D, respectively. After 7 days of adaptation, one week before the commencement of GnRH treatment (Day 0), the room temperature of the heat stressed group was gradually increased to reach 38 C on Day-1 with the relative humidity and photoperiod remaining unchanged to avoid acute sunstroke. A synthetic GnRH analogue (Conceral, Takeda Chemical, Osaka), diluted with sterile saline (100 ng/ml), was injected through a jugular vein catheter at a constant dose of 3 ng/kg of body weight once every hour. Hourly administration of GnRH was started at 8:00 on Day 0 and continued for 48 h.

Blood sampling and hormone assays

Blood samples for LH determination were collected through an indwelling jugular-catheter every 2 h during the GnRH treatment (all animals) and every 15 min for 16 h starting at 4 h before the first GnRH injection (4 out of 5 animals in each group). Blood samples for estradiol determination were collected in a similar manner but every 4 h throughout the GnRH treatment. Additional blood samples for progesterone determination were also collected twice a week before the GnRH treatment and once daily from Day 0 to Day 7. Plasma hormone concentrations were measured by specific radioimmunoassays as described elsewhere [9, 12]. Intra- and inter-assay coefficients of variations for LH, estradiol and progesterone were 9.6 and 13.5%, 10.3 and 14.5%, and 12.7 and 12.9%, respectively.

Statistical analysis

Data were expressed as the mean $\pm$ SEM and the difference between means were analyzed by student's *t*-test where it was appropriate. The characteristics of the pulsatile LH secretion during serial blood sampling periods were assessed using the PULSAR pulse analysis program [13].

Results

Under heat stress conditions, the rectal tempera-

ture and respiration rate significantly ($P<0.001$) increased by 2.3 C and 5.5 times, respectively, compared with those under control conditions (38.8 C and 29.3 times/min). Whereas daily food intake decreased ($P<0.05$) by 57% and daily water intake increased ($P<0.05$) 5.0 times (27.1 g and 48.6 ml/kg of body weight for the controls, respectively). The acute heat stress thus induced severe systemic responses but there were no clinical symptoms of sunstroke.

Ovarian responses to hourly injections of GnRH as determined by the increase of plasma estradiol level and the occurrence of preovulatory LH surge

In the control group, GnRH treatments resulted in a prompt and sustained increase in plasma estradiol concentrations. This increase in estradiol was accompanied by a preovulatory LH surge in all animals. Such ovarian responses were not observed in the heat stressed group except for one animal (#39) in which a slight increase of estradiol was detected followed by a far-delayed onset of LH surge (Fig. 1). Mean plasma concentrations of estradiol and the characteristics of the preovulatory LH surge are summarized in Table 1. The mean estradiol concentration after GnRH treatment but before LH surge was significantly ($P<0.01$) lower in the heat stressed group than in the control group. Although estradiol levels before GnRH treatment remained at the basal levels, there was also a significant ($P<0.05$) difference between the 2 groups. Plasma progesterone levels increased to more than 1 ng/ml on Day 4 in all animals in the control group and on Day 6 in the 1 heat stressed animal

which responded with delayed LH surge (data were not shown), indicating that ovulation took place in these animals. Plasma progesterone concentrations before GnRH treatment (the first 2 weeks under chamber conditions) consistently remained below 0.3 ng/ml in all animals in both groups.

Fig. 1. Changes in plasma LH and estradiol concentrations in long day-induced anestrus goats treated with hourly i.v. injections of a small dose of GnRH (3 ng/kg body weight) under 2 temperature conditions.

Table 1. Effects of heat stress on the ovulatory responses of long day-induced anestrus goats treated with hourly i.v. injections of a small dose of GnRH^{a)}

	Control (25 C)	Heat stress (38 C)
No. of animals ovulated/treated	4/4	1/5
Interval to LH surge (h)	20.5 ± 2.0	40
Magnitude of LH surge (ng/ml)	57.2 ± 5.9	56.4
Plasma estradiol levels (pg/ml):		
Before GnRH treatment	2.4 ± 0.4	1.4 ± 0.3*
During GnRH treatment ^{b)}	7.7 ± 0.9	2.0 ± 0.4**

Data were expressed as the mean ± SEM.

a) GnRH was administered once every hour at a dose of 3 ng/kg body weight for 48 h.

b) Data after LH surge were excluded.

* **: The value significantly differs from that of control group at $P<0.05$ and $P<0.01$, respectively.

Pituitary responses to hourly injections of a small dose of GnRH

Plasma profiles of LH in response to hourly injections of GnRH are shown in Fig. 2 and the characteristics of the pulsatile secretion of LH are summarized in Table 2. Pulsatile secretion of LH during the pre-GnRH treatment period tended to be highly active in heat stressed animals. The response of LH release to hourly injections of GnRH also appeared to be greater in the heat stressed group (Fig. 2). During the pre-GnRH treatment period, the frequency of the LH pulse was significantly higher in the heat stressed group and this was associated with higher values of baseline and mean concentrations of LH in this group (Table 2). Whereas the amplitude of the LH pulse was not affected. Hourly injections of GnRH resulted in a short-lived increase of LH secretion and a number of these GnRH-induced LH pulses were not different between the two groups. However, the baseline, the amplitude of the induced LH pulses and mean concentrations of LH were significantly ($P<0.05$) higher in the heat stressed group.

Discussion

The results of the present study demonstrated that heat stress abolished the ovulatory responses induced by repeated injections of a small dose of

GnRH in anestrus goats by diminishing the responsiveness of the ovary to the pulsatile stimulation of LH, rather than by decreasing the

Fig. 2. Profiles of plasma LH concentration in long day-induced anestrus goats treated with hourly i.v. injections of a small dose of GnRH (3 ng/kg body weight) under 2 temperature conditions. Asterisks represent the LH pulse identified by the PULSAR pulse analysis program.

Table 2. Characteristics of the pulsatile secretion of LH in long day-induced anestrus goats treated with hourly i.v. injections of a small dose of GnRH^{a)}

	Control (25 C)	Heat stress (38 C)
Before GnRH treatment		
No. of LH pulses per 4 h	0.75 ± 0.22	2.75 ± 0.42*
Amplitude (ng/ml)	1.29 ± 0.21	1.46 ± 0.16
Baseline concentration (ng/ml)	0.23 ± 0.07	1.15 ± 0.24*
Mean concentration (ng/ml)	0.43 ± 0.08	1.69 ± 0.26**
During GnRH treatment		
No. of LH pulses per 4 h	3.67 ± 0.14	4.17 ± 0.17
Amplitude (ng/ml)	0.81 ± 0.08	1.62 ± 0.27*
Baseline concentration (ng/ml)	0.73 ± 0.13	2.02 ± 0.44*
Mean concentration (ng/ml)	1.19 ± 0.11	2.81 ± 0.48*

Data were expressed as the mean ± SEM.

Characteristics of the pulsatile secretion were assessed using the PULSAR pulse analysis program.

a) GnRH was administered once every hour at a dose of 3 ng/kg body weight for 48 h.

*, **: The value significantly differs from that of control group at $P<0.05$ and $P<0.01$, respectively.

pituitary LH secretion in response to GnRH.

In the present study, animals in the heat stressed group were exposed to gradually elevating temperatures before starting hourly GnRH injections to avoid the occurrence of acute sunstroke syndrome. This procedure, however, resulted in a slight but significant decrease in estradiol concentration at the basal level during the pre-treatment period, indicating that the follicular development at the time of GnRH treatment might not have been uniform between the control and heat stressed groups. Therefore, the failure of the ovulatory responses in the heat stressed group may be ascribed in part to a difference in follicular development at the commencement of GnRH injections.

The frequency of LH pulses during the pre-treatment period was much higher in the heat stressed group. This would be explained by the slightly lowered estradiol levels in this group, since it has been well established that a negative feedback effect of estradiol on the hypothalmo-pituitary axis increases during the anestrus season in ewes [14] and goats [15]. It is undeniable in the present study that a decrease in basal estradiol levels in the heat stressed animals during the pre-treatment period made it difficult to determine if the heat stress directly affected the pulsatile LH secretion other than by the negative feedback influence of ovarian estradiol. However, since the frequency of LH pulses in heat stressed animals drastically increased in response to a slight decrease in the basal level of estradiol, it would seem unlikely that heat stress exerts a significant suppressive effect on the pulsatile LH secretion. This assumption is supported by a previous finding that in ovariectomized ewes the rhythmicity of the LH secretion showed little change under heat stressed conditions similar to those adopted in the present study [16].

During the GnRH treatment period, the amplitude of LH pulses but not the frequency was much greater in the heat stressed group than in the controls with the baseline and mean LH concentrations sustained at higher levels in this group. The magnitude of the preovulatory LH surge observed in one animal of the heat stressed group was similar to that of the control animals. These results suggest that heat stress did not reduce the ability of the pituitary to secrete LH either in tonic or in surge mode secretion. This agrees with a previous study in cyclic cows [8] and ours in goats [9] in which the basal and surge levels of LH concentra-

tion were little affected by heat stress despite plasma estradiol concentrations being significantly lower during the follicular phase. However, there are controversial findings in the literature as to the effect of heat stress on LH secretion in cyclic or ovariectomized animals: unchanged [17, 18], decreased [6, 7, 19] or increased [20]. These discrepancies probably depend on several factors such as differences in the species, the severeness of heat stress, physiological state of the animals (e.g., intact or ovariectomized) and the frequency of blood sampling. The characterization of these factors requires further study.

In the present study, while the failure of ovulatory follicular growth in heat stressed animals, or little increase of estradiol secretion, appears to be attributable to poor responsiveness of the ovary to circulating LH, it remains to be known how heat stress reduces the ovarian responsiveness. FSH is known to be an important hormone which stimulates the early stages of follicular growth and steroidogenesis [21]. It has also been shown that heat stress reduces FSH secretion after an i.m. injection of GnRH in cyclic cows [19]. These facts imply that a decrease in FSH secretion can not be precluded as a possible cause of hypogonadism under heat stressed conditions. However, it is unlikely that the hypogonadism observed in the present study was associated with changes in FSH secretion. In our previous study heat stress did not reduce FSH secretion either in cycling or ovariectomized goats [22]. It has also been shown that pulsed injection of purified LH alone can induce ovulatory follicular growth in seasonally anestrus ewes [23, 24]. This ovulatory response is similar to that produced by pulsed GnRH injection [10].

Although direct evidence is not available at present, it has been suggested that the increase in prolactin [25] or ACTH secretion [2] may have some implications in heat stress-related hypogonadism. Prolactin is known to increase several times under hot environmental conditions [16, 26] and it has been shown that prolactin inhibits the ovarian aromatase activity and thereby suppresses estradiol secretion [27–29]. The induction of hyperprolactinemia either by prolactin injection in sows [30] or TRH injection in ewes [31] reduces estradiol secretion with little change in LH and FSH secretion in the ewe. On the other hand, it has been shown that administration of ACTH during the follicular

phase results in a decrease in estradiol secretion and a delay in the preovulatory LH surge in cows [32]. These phenomena are similar to those observed in heat stressed animals. Therefore, the activation or disturbance of the hypothalamo-pituitary-adrenal axis by heat stress [1, 8] may also be associated with heat stress-related hypogonadism. Alternatively, it is possible that heat stress acts at the ovarian level to reduce estradiol secretion indirectly by decreasing the ovarian blood flow. The uterine blood flow, which shares a common arterial blood supply in part with the ovary, has been shown to decrease in heat stressed ovariectomized ewes [33] and cows [34]. It seems of interest to determine by further study whether these factors are involved in the mechanism by which heat stress causes hypogonadism.

In conclusion, heat stress suppresses ovulatory

follicular growth induced by pulsed GnRH injection in anestrus goats not mediated by a decrease of pituitary LH secretion. Instead, the failure of the ovulatory response in heat stressed animals appears to be associated with poor responsiveness of the ovary to LH.

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