Oxygen and steroid concentrations in preovulatory follicles of lactating dairy cows exposed to acute heat stress


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Abstract

Maternal heat stress reduces oocyte competence for fertilization and post-fertilization development, but the mechanism is unknown. The present experiment investigated two potential mechanisms: (1) reduced oxygen delivery to the preovulatory follicle (due to increased thermoregulatory vascular perfusion of skin and respiratory tract); (2) reduced follicular steroid synthesis. These hypotheses were tested by measuring the fractional concentration of oxygen and concentrations of estradiol-17β and progesterone in follicular fluid of the preovulatory follicle of lactating Holstein cows. Estrous cycles were synchronized using GnRH on Day −9 and PGF$_{2\alpha}$ on Day −2. On Day 0, all cows without a CL and with a large preovulatory follicle were assigned to control or heat stress treatments for 1 d (beginning at 1030 h). Between 4 and 6 h after treatment (1430–1630 h), follicular fluid was aspirated by transvaginal puncture, and fractional oxygen concentration in follicular fluid of the dominant follicle was determined with a fluorometric fiber-optic oxygen sensor. There was no significant effect of heat stress on follicular fluid $P_{O2}$ or concentrations of estradiol-17β or progesterone among cows that had follicular fluid steroid concentrations considered typical of a preovulatory follicle. Follicular oxygen concentration was 6.9 ± 0.4% for control cows and 7.3 ± 0.3% for heat-stressed cows. Oxygen concentration tended to be inversely correlated to follicular diameter ($P = 0.09$). In conclusion, it was unlikely that reduced oocyte competence due to acute heat stress was caused by reductions in follicular concentrations of oxygen, estradiol-17β, or progesterone.

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1. Introduction

Fertility in lactating dairy cows decreases during hot months of the year [1–8] and after experimentally induced heat stress [9–11]. One cause for reduced fertility during heat stress is damage to the oocyte.

During the summer in warm regions of the world, oocytes show reduced competence to be fertilized and/or to support development to the blastocyst stage when subjected to in vitro fertilization [6,12–15]. In vivo, fertilization rate after AI in lactating cows was lower in summer than winter [16] and experimental application of heat stress to superovulated cows during the preovulatory period resulted in formation of embryos with reduced development potential [10].

One mechanism by which heat stress can result in oocyte damage involves disruption of oocyte function...
by elevated temperature. Indeed, culture of oocytes during maturation at elevated temperatures can reduce subsequent cleavage rate and blastocyst development rate following in vitro fertilization [17–20]. Furthermore, heat stress can compromise follicular steroid synthesis in vivo [21,22] and exposure of cultured follicular cells to elevated temperatures can reduce production of estradiol [21,23] and androstenedione and increase production of progesterone [23].

In addition, thermoregulatory responses by the lactating cow may perhaps indirectly compromise oocyte function by altering the follicular microenvironment during the preovulatory period, especially via reduced oxygen availability. One of the physiological responses to increased body temperature is redistribution of blood to the skin [24]. In lactating Holstein cows, evaporative heat loss from the skin has been estimated to represent approximately 85% of the total heat loss when ambient temperatures rise above 30 °C [25]. Increase in respiratory cycles is also a response to heat stress [26–29] and may result in redistribution of blood flow to muscles involved in respiratory activity, as in other species [30,31]. Increased blood flow to the periphery associated with thermoregulation concurrently reduced blood flow to the internal organs, including the reproductive tract. For example, blood flow to ovaries, cervix and oviduct from rabbits was decreased by 20–30% during heat stress, whereas vulvar blood flow increased 40% [32]. In another example, the blood flow to the uterus of ovariectomized cows treated with estradiol-17β was reduced by heat stress [33,34]. Perhaps reduced vascular perfusion of the preovulatory follicle during heat stress compromises oocyte function.

Studies from the human highlight the importance of follicular oxygen availability for oocyte function. In particular, increased follicular vascularity was correlated with increased pregnancy rate and perifollicular blood flow was positively correlated with follicular oxygen PO2 [35,36]. Also, follicular oxygen PO2 was associated with oocyte quality as determined by chromosomal structure, fertilization and subsequent development in vitro [36]. Oocytes derived from follicles containing ≤1.5% oxygen had approximately half the likelihood of reaching the 6–8 cell stage following insemination as oocytes from follicles with 3–5% oxygen [36].

The objective of the present study was to test whether acute heat stress causes a reduction in follicular fluid PO2 from the preovulatory follicle of lactating Holstein cows. Since heat stress has also been reported to affect follicular steroid synthesis [21,22], an additional objective was to determine acute effects of heat stress on follicular concentrations of estradiol-17β and progesterone.

2. Materials and methods

The experiment was conducted in September 2006, at the University of Florida Dairy Research Unit located near Gainesville, FL, USA (29°46'45.46"N, 82°24'58.53"W). The protocol used in the experiment was approved by the University of Florida Institutional Animal Care and Use Committee.

2.1. Cows

Lactating Holstein cows (n = 31) were used in a total of two replicates initiated 1 week apart (13 cows in the first replicate and 18 cows in the second replicate). Cows were fed a total mixed ration containing corn silage, corn grain and legume hay as the main ingredients (0.75 Mcal/kg of NE3 and 17.7% crude protein on a dry matter basis). Fresh water was available for both groups ad libitum. Cows were treated with Posilac® (Monsanto, St. Louis, MO, USA) according to manufacturer’s directions and were milked twice daily. Except for the day of follicular aspiration, cows were housed in free-stall barns containing fans and sprinklers.

2.2. Estrous synchronization and identification of cows with preovulatory follicles

For each replicate, estrous cycles were synchronized by 100 μg of GnRH (2 mL Cyostorelin®; Merial, Duluth, GA, USA) given i.m. on Day −9 and 25 mg PGF2α (5 mL Lutalyse; Pfizer, New York, NY, USA) given i.m. on Day −2. After being milked in the morning of Day 0 (the putative day of estrus), cows were subjected to transrectal ultrasonography using an Aloka 500 ultrasound unit equipped with a 5-MHz linear-array transducer (Aloka, Wallingford, CT, USA) to verify the presence of a dominant follicle and absence of a corpus luteum. Follicle diameter were measured and recorded. Of the 31 cows synchronized, three did not meet the ovarian criteria listed above and were excluded from the experiment. An additional six were not used in any of the analyses because follicular fluid was not retrieved as a result of accidental follicle rupture during the aspiration. Therefore, 22 cows were used in the experiment.

2.3. Heat stress treatments on Day 0

Cows with a preovulatory follicle on Day 0 were blocked by postpartum interval [<75 d (range = 68–75)...
procedure. Cows were restrained and given caudal epidural anesthesia at 1030 h. Follicular aspiration for ovum pick-up after initiation of heat stress (1030 h). Rectal temperature was recorded at 1-h intervals until follicular aspiration and immediately before follicular aspiration. Dry bulb temperature and relative humidity were recorded hourly at a height of 2.5 m.

2.4. Sampling of follicular fluid

Beginning at ~4 h after initiation of heat stress treatments (1430 h), follicular fluid was sampled using transvaginal follicular aspiration. Control and heat-stressed cows were sampled in alternating order. The entire sampling process required 2 h, and follicular fluid from the last cow sampled was obtained 6 h (1630 h) after initiation of heat stress (1030 h).

Follicular aspiration was performed by a modification of standard follicular aspiration for ovum pick-up procedure [37]. Cows were restrained and given caudal epidural anesthesia (5 mL of 2% (w/v) lidocaine; Pro Labs, St. Joseph, MO, USA) using an 18-gauge, 3.81-cm needle. Follicular fluid samples were collected using an Aloka 500 ultrasound device equipped with a needle guide and connected to a 5-MHz intravaginal sector transducer. The 20-gauge, 3.81-cm needle at the end of the guide was connected to a 5 mL syringe via a 70-cm length polyether ether ketone (PEEK) capillary tubing (outer and inner diameters of 0.16 and 0.05 cm, respectively; McMaster-Carr Supply Co., Atlanta, GA, USA) with Teflon compression fittings (Cole-Parmer, Vernon Hills, IL, USA) in a gas-tight system. After piercing the follicle with the needle, follicular fluid was aspirated manually into a 5 mL syringe.

2.5. Measurement of follicular oxygen concentration

Fractional concentration of oxygen was measured within 1–2 min of collection of follicular fluid by dynamic fluorescence quenching [38]. This technique has been used to monitor blood and tissue $P_{O_2}$ in a variety of animals and experimental settings [39–41]. Measurements were performed using a fluorometric fiber-optic oxygen sensor system (FOXY system; Ocean Optics, Inc., Dunedin, FL, USA) that utilized a USB-2000 linear diode-array fluorometer, LS-450 blue LED excitation source, and 300 μm optical fiber probe in a 0.16-cm outer diameter stainless steel jacket. The probe was coated with a ruthenium red compound that, upon excitation, emits fluorescence in inverse proportion to the $P_{O_2}$ in the sample, due to quenching effects of oxygen towards the fluorescent compound. Fluorescence was monitored on a laptop computer equipped with SpectraSuite spectroscopy software (Ocean Optics, Inc.). The probe tip was mounted in a low-volume (0.2 mL), flow-through chamber into which the fluid was injected via a syringe for measurement of fractional oxygen concentration. The oxygen sensor was maintained at ambient temperature, which was continuously monitored with an adjacent thermocouple. An opaque, black silicone coating on the optical fiber tip allowed two-point calibration with gases (air and 100% N2). The assay was validated by comparing readings for various concentrations of gaseous oxygen (0, 10.5, 21.0, 60.0 and 100%) with readings for follicular fluid that had been equilibrated with the same gas mixture. The accuracy of measurement [100 × (value for oxygen dissolved in follicular fluid/value for gaseous oxygen)] was 97.5, 92.9, 99.0, 106.1, and 113.5% for 0, 10.5, 21.0, 60.0, and 100% oxygen.

Upon collection, follicular fluid samples were immediately injected into the flow-through chamber and oxygen concentration was monitored until it was stable (typically <2 min). An additional 0.1 mL fluid was then injected to confirm that the reading was stable. The fluid was then withdrawn, placed in ice until storage at −20 °C (2–4 h later). Between each sample, the chamber was flushed with distilled water and the calibration points were checked.

Results are reported as fractional oxygen concentration to be consistent with similar literature describing oxygen partial pressure in the reproductive tract [36,42]. Conversion to $kP_a$ can be made using the following equation: $kP_a = \%O_2/100 \times 101.3$.

2.6. Measurement of plasma progesterone concentrations

A blood sample was collected from each cow immediately before follicular aspiration by coccygeal
venipuncture into evacuated heparinized 10-mL tubes (Becton Dickinson, Franklin Lakes, NJ, USA). After collection, blood samples were placed in ice until further processing at the laboratory (within approximately 2–4 h). Blood samples were centrifuged at 2000 × g for 20 min at 4 °C. Plasma was separated and stored at −20 °C until assayed for progesterone concentration. Progesterone concentrations were determined by radioimmunoassay (RIA) using the Coat-a-Count® progesterone kit (Diagnostic Products Corp., Los Angeles, CA, USA). The intra-assay coefficient of variation was 7.0% and the sensitivity was 0.11 ng/mL.

2.7. Determination of follicular fluid concentrations of steroids

Follicular fluid concentrations of estradiol-17β were determined by radioimmunoassay as described previously [43]. The intra-assay coefficient of variation was 13.0% and the sensitivity, defined as 95% of total binding, was 0.05 ng/mL. Follicular fluid concentrations of progesterone were determined by RIA as described previously [43]; the intra-assay coefficient of variation was 10.0% and the sensitivity, defined as 90% of total binding, was 1.7 ng/mL.

2.8. Determination of follicle class based on follicular steroid concentrations

For subsequent statistical analysis, the cows were separated post hoc into two follicle class groups based on steroid concentration: a high estradiol-17β group and a low estradiol-17β group. The high estradiol-17β group represented 16 of the 22 cows examined. Each cow in this group had a follicle with a high estradiol-17β concentration (>319 ng/mL) and low progesterone concentration (<243 ng/mL) [44], and was considered to have a preovulatory follicle. The remaining cows (6 of 22 cows) were placed in the low estradiol-17β group. Each had a follicle with a low estradiol-17β concentration (<102 ng/mL) and high progesterone concentration (>273 ng/mL). Cows in this group were considered to have either experienced an LH surge or to be cystic. The postpartum interval at the time of follicular aspiration for cows in the high estradiol-17β class were 81.8 ± 35.8 and 172.8 ± 27.6 d for control and heat stress groups, respectively, whereas cows in the low estradiol-17β class were 175.7 ± 43.2 and 184 ± 61.1 d for control and heat stress groups, respectively.

2.9. Statistical analysis

Data on diameter of the largest follicle on Day 0, rectal temperature at the time of follicular aspiration, follicular fluid PO2, and follicular fluid concentrations of estradiol-17β and progesterone were analyzed by least-squares analysis of variance using the GLM procedure (SAS for Windows, Version 8, 1999–2001, Cary, NC, USA). Effects included replicate, treatment (control vs. heat stress), follicle class (high vs. low estradiol-17β) and treatment × follicle class interaction. In addition, additional analyses were performed for data for cows in the high and low estradiol-17β classes separately. The estradiol-17β and progesterone concentrations exhibited heterogeneity of variance, so these data were log-transformed before analysis. Probability values are based on analysis of the transformed data, and results are presented as means ± S.E.M. for each subgroup.

Data on rectal temperature taken at hourly intervals at Day 0 were also analyzed by least-squares analysis of variance as described above, except that the analysis was a split-plot in time design with time and interactions with time included in the model. Cow was considered as a random effect and other main effects were considered as fixed. Cow (replicate × treatment) × time was used as the error for testing significance of the time effect.

Analyses of the relationship between follicle diameter and oxygen concentration were performed using the GLM procedure of SAS, with treatment as a class variable, linear, quadratic and cubic effects of follicle diameter, and with interactions of treatment and follicle diameter. Based on these analyses, the final statistical analysis was performed with the linear effect of follicle diameter as the only term in the model. A similar analysis was performed to determine the relationship between postpartum interval and oxygen concentration.

3. Results

3.1. Characteristics of cows at Day 0 before initiation of heat stress treatment

There was no difference in follicular diameter between control and heat-stressed cows (15.9 ± 1.2 and 16.0 ± 1.4 mm, respectively; P > 0.1). One heat-stressed cow from the high estradiol-17β follicle class had a plasma progesterone concentration of 1.5 ng/mL. All other cows in the analysis had a plasma progesterone concentration lower than 1 ng/mL.
3.2. Characteristics of heat stress treatment: dry bulb temperature, relative humidity and rectal temperature

Characteristics of the environment for control and heat-stressed cows and the resultant rectal temperatures are shown (Fig. 1). Dry bulb temperature increased and relative humidity decreased throughout the day for both control and heat stress pens (Fig. 1A). Temperature was higher and relative humidity was lower at all times for the heat stress pen compared to the control pen. Dry bulb temperatures and relative humidity were similar for both replicate days and are reported as averages from both replicates. Rectal temperatures were significantly lower for control cows than for heat-stressed cows. This was true whether examining rectal temperature at hourly intervals (Fig. 1B; temperature × time, \( P < 0.01 \)) or rectal temperature at the time of follicular aspiration (Fig. 1C, \( P < 0.01 \)). There was no interaction between treatment and follicle class on rectal temperature (\( P > 0.1 \)).

3.3. Follicular estradiol and progesterone concentrations

Follicular fluid estradiol and progesterone concentrations are shown (Table 1). As expected, estradiol-17\( \beta \) concentration was higher in the high estradiol-17\( \beta \) class as compared to cows in the low estradiol-17\( \beta \) group (\( P < 0.01 \)). In addition, progesterone concentration was lower in the high estradiol-17\( \beta \) class as compared to cows in the low estradiol-17\( \beta \) group (\( P < 0.01 \)). There was no effect of heat stress or follicle class × treatment interaction on the concentration of either steroid (\( P > 0.1 \)).

3.4. Oxygen concentration in follicular fluid

Follicular oxygen concentration ranged from 3.9 to 9.2\% and was not significantly affected by heat stress (Table 1). However, there was a follicle class × treatment interaction (\( P < 0.01 \)); there was no effect of heat stress treatment on \( P_{O_2} \) among cows in the high estradiol-17\( \beta \) class, but \( P_{O_2} \) was lower in the heat-stressed cows than control cows in the low estradiol-17\( \beta \) class. When the subset of cows with high estradiol-17\( \beta \) was analyzed separately, there was also no effect of heat stress on follicular oxygen concentration (\( P > 0.1 \)). Furthermore, among cows in the high estradiol-17\( \beta \) class, follicular oxygen concentration tended to be inversely related to follicular diameter (\( P = 0.09; \quad y = -0.20x + 10.24; \quad r^2 = 0.19 \)). There was no relationship between follicular oxygen concentration and postpartum interval.

4. Discussion

The objective was to determine the effect of acute heat stress on concentrations of oxygen, estradiol-17\( \beta \) and progesterone in the follicular fluid of the preovulatory follicle of the lactating dairy cow. The
The nature of the follicles in the low estradiol-17β class was unclear. Some may be cystic follicles, as there

Table 1
Effects of heat stress and follicle class [high estradiol-17β (>319 ng/mL) vs. low estradiol-17β (<102 ng/mL)] on ovarian follicular concentrations of estradiol-17β, progesterone and follicular PO2 in dairy cows

<table>
<thead>
<tr>
<th>Follicle class</th>
<th>Treatment</th>
<th>Estradiol-17β (ng/mL)a</th>
<th>Progesterone (ng/mL)b</th>
<th>Fractional oxygen concentration in follicular fluid (%)c</th>
</tr>
</thead>
<tbody>
<tr>
<td>High estradiol-17β</td>
<td>Control (n = 6)</td>
<td>1264.5 ± 84.3 (562–1852)</td>
<td>142.6 ± 12.7 (62–243)</td>
<td>6.9 ± 0.4 (4.9–8.5)</td>
</tr>
<tr>
<td></td>
<td>Heat stress (n = 10)</td>
<td>1707.9 ± 77.6 (319–2899)</td>
<td>103.7 ± 4.5 (43–194)</td>
<td>7.3 ± 0.3 (3.9–9.2)</td>
</tr>
<tr>
<td>Low estradiol-17β</td>
<td>Control (n = 4)</td>
<td>35.0 ± 4.0 (0.1–69)</td>
<td>1625.0 ± 423.5 (273–4102)</td>
<td>7.6 ± 0.5 (6.5–8.2)</td>
</tr>
<tr>
<td></td>
<td>Heat stress (n = 2)</td>
<td>51.0 ± 35.7 (0.21–101)</td>
<td>714.9 ± 55.2 (636–793)</td>
<td>4.6 ± 0.7 (4.5–4.6)</td>
</tr>
</tbody>
</table>

**a** Data are means ± S.E.M. and, in parentheses, the range of values. There was an effect of follicle class (P < 0.01).

**b** Data are means ± S.E.M. and, in parentheses, the range of values. There was an effect of follicle class (P < 0.01).

**c** Data are least-squares means ± S.E.M. and, in parentheses, the range of values. There was a follicle class × treatment interaction (P < 0.01).

main hypothesis was that reduction in blood flow to the ovary coincident with heat stress would reduce oxygen delivery to the follicle and result in lower oxygen availability to the follicle. Blood flow changes in response to heat stress within minutes [45] and it was expected, therefore, that the duration of heat stress employed would cause a period of attenuated blood flow to the ovaries of sufficient duration to reduce follicular fluid concentrations of oxygen. In contrast to this expected result, there was no effect of heat stress on follicular fluid PO2 in cows having follicular steroid profiles characteristic of the dominant preovulatory follicle.

Based on an increase in average rectal temperature of ~1.0 °C, the degree of heat stress applied in this study was similar to that which caused reduced blood flow to the reproductive tract in the rabbit and laying hen [31,32]. That heat stress did not reduce follicular fluid oxygen concentration suggested that mechanisms are present to maintain follicular PO2 in the face of systemic redistribution of blood flow during heat stress. For example, local adjustments in the microvasculature of the follicle may have ensured adequate oxygen delivery. The potential for regulation of blood flow to the follicle by oxygen was illustrated by reports that concentrations of oxygen in human ovarian follicles were negatively correlated with presence of the angiogenic factor interleukin-8 [46] and that hypoxia stimulated vascular endothelial growth factor production in porcine cumulus cells [47].

An alternative explanation for the lack of effect of heat stress is that the oxygen requirements of the follicle were low enough that an acute reduction in blood flow did not lead to a measurable decline in follicular PO2 during heat stress limited to only a few hours. Bovine cumulus cells use glycolysis extensively and it has been estimated that metabolism of these cells do not have a major impact on follicular oxygen content [48]. Perhaps effects of heat stress on follicular metabolism further reduced oxygen requirements of the follicle.

Although the current study did not detect an effect of 4–6 h of heat stress on follicular oxygen concentration, extended duration of heat stress (days or longer) may compromise follicular oxygen concentrations. The decision was made to focus on acute effects in the current experiment, due to experimental difficulties with a chronic heat stress model, which include potential confounding effects of heat stress on follicular growth [49–52]. It is important to note that acute heat stress during the preovulatory period (14 h) can compromise oocyte competence [10].

Intriguingly, heat stress did reduce follicular PO2 in the low estradiol-17β class. In these cows, which had low follicular estradiol-17β concentration and high follicular progesterone concentration, the PO2 for heat-stressed cows was 4.5–4.6% versus 6.5–8.2% for control cows. Although the follicle class × treatment interaction was significant, these data should be interpreted cautiously, as there were only six cows in the low estradiol-17β class. Nonetheless, it is possible that local regulatory mechanisms that maintain PO2 in preovulatory follicles (for example regulation of local blood flow) were absent in cows with low follicular estradiol-17β concentration. Consistent with this, follicles from the first follicular wave with estradiol-17β concentration higher than progesterone expressed more endothelial nitric oxide synthase (eNOS) and vascular endothelial growth factor (VEGF) proteins compared to follicles with estradiol-17β lower than progesterone [53]. Typically, eNOS and VEGF increase blood flow via vasodilation and angiogenesis, respectively [54–57].
are small follicular cysts characterized by lack of granulosa cells and with high progesterone concentrations and little estradiol-17β in the follicular fluid [58,59]. Another possibility is that the LH surge and follicular luteinization had been initiated in some cows in the low estradiol-17β group, since the LH surge is accompanied by a reduction in follicular estradiol-17β synthesis and an increase in progesterone synthesis [60–62].

Heat stress did not significantly affect follicular steroid concentrations. Although plasma estradiol-17β was decreased by heat stress in some experiments [49,63], Rosenberg et al. [64] found no effect of heat stress. Follicular estradiol-17β concentration was reduced in chronically heat-stressed cows [21,22] but not in acutely heat-stressed cows [65]. If steroid output was affected by heat stress in the present experiment, the difference during the experimental period was too small to cause a measurable change in the pool of steroids in the follicle.

To our knowledge, this is the first experiment describing the \( P_{O2} \) of the bovine follicle in situ. The values obtained, with a mean of 6.8% and a range of 3.8–9.2%, were similar to results for follicular oxygen concentration in other species [36,66]. In cows in the high estradiol-17β follicle class, which had a prototypical preovulatory follicle (high estradiol-17β concentration and low progesterone concentration), the follicular oxygen concentration tended to decrease as follicle diameter increased. This is not surprising because the complex matrix of follicular vasculature is confined to the theca layer [67] and becomes more distant from the center of the follicle as the follicle expands in diameter. An increase in follicular diameter has also been shown to be accompanied by decrease in follicular oxygen concentration in humans [68] and pigs [47].

The oocyte is surrounded by fluid with \( P_{O2} \) much lower than the atmospheric \( P_{O2} \) used in most in vitro maturation systems [69–72]. Indeed, under many conditions, oocyte maturation in vitro is more effective under atmospheric oxygen concentration than under 5% oxygen [69,73,74]. The greater requirement for a high oxygen pressure in vitro may reflect the static nature of the in vitro system. In addition, steroid synthesis is affected by oxygen concentration. In rat granulosa cell cultures, basal progesterone secretion was lower for cells in 5% oxygen than for cells in 20% oxygen, but LH or FSH stimulation of progesterone secretion was enhanced by culture in 5% oxygen [75].

In summary, acute heat stress did not cause a reduction in \( P_{O2} \) or concentrations of estradiol-17β and progesterone in fluid from follicles that had follicular fluid steroid concentrations typical of a preovulatory follicle. Therefore, acute reductions in blood flow and a resulting hypoxia are unlikely to be the primary cause of oocyte damage caused by heat stress [6,12,13,16].

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References


