Hormonal and follicular relationships in ewes of high and low ovulation rates*

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Summary. Total follicular populations and peripheral plasma concentrations of LH, FSH, prolactin, oestradiol-17β and progesterone during the preceding cycle were studied in two breeds of sheep (Romanov and Ile-de-France) which differed widely in their ovulation rates (3.2 and 1.5 respectively).

No LH parameters could be correlated with the follicular details measured. The second peak of FSH occurring 20–30 h after the preovulatory surge of LH was significantly larger in the Romanov ewes and the area under this peak was correlated ($P < 0.01$) with the number of antral follicles present in the ovary 17 days later. This suggests that formation of the antrum during the follicular growth phase is under the control of FSH. The discharge of prolactin preceding the LH peak, although not significantly different between breeds, was correlated with several of the follicular classes measured, including the number of preantral follicles. The peak value of oestradiol-17β measured before the LH peak was significantly higher ($P < 0.05$) in the Romanov ewes and was correlated with the number of the largest follicles present. There was no significant difference between breeds in the concentration of oestradiol at the onset of oestrus. The progesterone concentration during the luteal phase was highly correlated with the number of preovulatory follicles.

Introduction

The mechanisms determining ovulation rate in the sheep remain unknown. It is evident that ovulation is the result of pituitary gonadotrophins acting at the ovarian level since hypophysectomy of the adult ewe results in a drastic reduction in the ovarian follicular population and follicular growth rate and a cessation in ovulation (Dufour, Cahill & Mauléon, 1979). Furthermore, injection of exogenous gonadotrophins such as PMSG have long been known to have a stimulatory effect on the ovary (Cole & Hart, 1930).

Attempts have been made to correlate circulating gonadotrophins in the adult ewe with the observed ovulation rate. The interval between the onset of oestrus and the discharge of LH has been correlated with ovulation rate (Thimonier & Pelletier, 1971; Land, Pelletier, Thimonier & Mauléon, 1973; Bindon, Blanc, Pelletier, Terqui & Thimonier, 1976), but this correlation is probably an association with and not a determinant of ovulation rate, because at the onset of oestrus the follicles to ovulate have already been differentiated (Cahill, Mariana & Mauléon, 1979). Other LH characteristics, such as the duration of the preovulatory LH discharge, the mean basal level and the maximum concentration reached, did not vary according to ovulation rate, although on Days 1 and 8 of the cycle there were significant differences in the basal level of LH with ovulation rate (Land et al., 1973).

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Studies of FSH concentrations between breeds (Bindon et al., 1976) and within a breed (Findlay & Cumming, 1976) did not show any difference in the level of FSH to explain the observed ovulation rate differences.

The concentration of oestrogens and the ovulation rate may also be an important relationship as it has been suggested that sensitivity to the feed-back effects of oestrogens on LH secretion may be related to ovulation rate (Land, 1976). Bindon et al. (1976) and Scaramuzzi & Land (1976) found no difference in the oestrogen concentration during pro-oestrus in ewes with different ovulation rates. Although Wheeler, Baird, Land & Scaramuzzi (1977) did find a genetic variation in the secretion rate of oestradiol-17ß in ewes with ovarian autotransplants it is difficult to reconcile these results with what occurs in the normal cycle.

The present study was carried out to investigate the concentrations of LH, FSH, prolactin, oestradiol-17ß and progesterone during the cycle in two breeds of sheep which differ widely in their ovulation rate, and to correlate the findings with differences in the follicular populations which have already been reported (Cahill et al., 1979).

Materials and Methods

During November 1975, oestrus was synchronized by using progestagen-impregnated sponges in 5 Romanov and 11 Ile-de-France ewes. All ewes were 14 months of age, primiparous, in good condition and housed together.

The experimental period extended from before the second until the third oestrus after sponge removal. Approximately 15 days after the first oestrus following sponge withdrawal a ram was placed with the ewes for 15 min every 2 h to detect oestrus. Blood sampling began when the ewes were observed in oestrus and continued every 2 h for 72 h; thereafter detection of oestrus and blood sampling were carried out twice daily (09:00 and 16:00 h) for 11-11.5 days. Checks for oestrus and blood samplings were then made every 2 h until 10 h after the onset of the LH discharge.

At each sampling a 10 ml blood sample was taken by puncture of the jugular vein and immediately centrifuged. The plasma was collected and stored at −15°C until assayed at a later date. However, in order to ovariectomize the ewes at a precise moment in relation to the onset of the LH discharge at the third oestrus following synchronization, the LH concentration was immediately determined at this time by using a modified rapid assay technique. The results of this assay were later verified by using the complete assay.

Assay procedures

The assay procedures were those defined in Table 1. All the plasma samples collected were assayed in a single assay for each hormone (with the exception of oestradiol-17ß) and a reference sample was included in quadruplicate every 100 tubes. For oestradiol-17ß measurements, plasma samples were pooled thus: in the period when blood sampling was carried out every 2 h, two consecutive samples were pooled (1:1) to give a mean concentration during the 4 h period, and when blood samples were taken twice daily the samples were similarly pooled to give a mean daily concentration. Oestradiol-17ß concentrations were measured in 5 assays and animals from both breeds were represented in each assay.

Follicular populations

All ewes were bilaterally ovariectomized at the third oestrus after sponge withdrawal at 2–4 h after the onset of oestrus or 10.5–16.5 h after the onset of the LH discharge. The total number of all small follicles (< 3 layers of granulosa cells) and all larger follicles (with 3 or more layers of
Table 1. The characteristics and procedures of the assays used to determine hormone concentrations in ewes

<table>
<thead>
<tr>
<th>Hormone</th>
<th>Technique</th>
<th>Results expressed as</th>
<th>Potency of standards used relative to NIH standards</th>
<th>Sensitivity of assay</th>
<th>No. of assays used</th>
<th>Reference samples mean-coeff. var.</th>
</tr>
</thead>
<tbody>
<tr>
<td>LH</td>
<td>Pelletier et al. (1968)</td>
<td>ng LH M&lt;sub&gt;4&lt;/sub&gt;/ml</td>
<td>1·8 x NIH-LH-S1</td>
<td>0·3 ng/ml</td>
<td>1</td>
<td>4·16 ng/ml, 26%</td>
</tr>
<tr>
<td>FSH</td>
<td>Blanc &amp; Poirier (1979)</td>
<td>ng FSH P&lt;sub&gt;40&lt;/sub&gt;/ml</td>
<td>0·1 x NIH-FSH-S6</td>
<td>2 ng/ml</td>
<td>1</td>
<td>16·58 ng/ml, 18%</td>
</tr>
<tr>
<td>Prolactin</td>
<td>Kann (1971)</td>
<td>ng NIH-P-S6/ml</td>
<td>1·0 x NIH-P-S6</td>
<td>0·3 ng/ml</td>
<td>1</td>
<td>32·58 ng/ml, 15%</td>
</tr>
<tr>
<td>Oestradiol-17β</td>
<td>Thibier &amp; Saumande (1975), modified by Testart et al. (1977)</td>
<td>pg/ml</td>
<td>—</td>
<td>0·6 pg/ml</td>
<td>5</td>
<td>6·61 pg/ml mean CV per assay 16%, mean overall CV 24%</td>
</tr>
<tr>
<td>Progesterone</td>
<td>Palmer &amp; Jousset (1975)</td>
<td>ng/ml</td>
<td>—</td>
<td>0·2 ng/ml</td>
<td>1</td>
<td>2·31 ng/ml; before extraction 1·7%, after extraction, 2·6%</td>
</tr>
</tbody>
</table>

granulosa cells) was determined in one ovary of each of the 5 Romanov and 9 of the 11 Ile-de-France ewes used in this study. The ovulation rate was determined from the number of preovulatory follicles which have been previously defined; the procedures used and results of this work have also been described (Cahill et al., 1979).

Statistical analysis

The onset of the preovulatory discharge of LH for each animal was defined as being the time when the level exceeded a value of 15 ng/ml and all subsequent hormonal data were aligned and chronologically expressed in relation to this point (i.e. 00:00 h of Day 0). Data from the beginning of blood sampling to Day 14-14·5 were aligned in relation to the LH discharge at the second oestrus after synchronization whilst the rest of the data were aligned in relation to the LH peak at the third oestrus after sponge removal.

The onset and end of the peaks of FSH and oestradiol-17β were defined as when the level exceeded the mean + 1·5 times the s.d. of the mean daily concentration. Due to the extreme variations between successive prolactin values, the discharges of prolactin were determined on a chronological basis from 0 to 12 h after the LH discharge at the second synchronized oestrus and the pro-oestrous rise from −48 to 0 h before the LH discharge at the third oestrus after synchronization. The area under the curve of the peaks is expressed as ng (or pg) hormone/ml per h as if all the hormone secretion occurred at a constant rate during 1 h. Luteolysis was identified when the concentration of progesterone fell to < 1 ng/ml.

Comparisons between breeds (5 Romanov and 11 Ile-de-France ewes) for the hormone data were carried out using analysis of variance. Linear regressions and factor analysis (Harmon, 1967) of 32 hormonal and follicular parameters from the 5 Romanov plus 9 Ile-de-France ewes were calculated.

The hormonal characteristics considered were (1) for LH, the area under the curve of the preovulatory discharge and mean concentrations on Days 1, 4–13 and 9–11; (2) for FSH, the area under the curve of the discharge coincident with the LH peak and of the second peak 20–30 h after the first peak; mean concentrations on Days 4–13 and 9–11; (3) for prolactin, the area under the curves of the discharges 0 to 12 h after the LH peak at the second synchronized oestrus −48 to 0 h before the LH peak at the third synchronized oestrus; mean concentration on Days 4–13; (4) for oestradiol-17β, the area under the curve of the pro-oestrous rise and discharges −48 to 0 and −24 to 0 h before the LH peak at the third synchronized oestrus; the highest concentration measured and the mean concentration on Days 4–13; and (5) for
progesterone, the highest concentration measured and the mean concentration on Days 4–13 and 9–11.

The time intervals considered were those between the onset of oestrus and onset of LH discharge; the highest oestradiol-17β concentration measured and the onset of oestrus; luteolysis and the onset of oestrus.

The follicular populations were considered under the following categories: (i) the number of small follicles (<3 layers of granulosa cells); (ii) the number of non-atretic large follicles (3 or more layers of granulosa cells); (iii) the numbers of non-atretic large follicles with diameters >1.13 mm (area 10^6 μm^2), >0.36 mm (area 10^5 μm^2), >0.11 mm (area 10^4 μm^2) or <0.11 mm with an antrum, without an antrum (preantral), preovulatory follicles; and (iv) the numbers of atretic large follicles. These follicle dimensions refer to fixed ovaries and the antrum developed at a diameter of 0.17 and 0.24 mm in the Romanov and Ile-de-France ewes respectively.

**Results**

The mean ± s.e.m. ovulation rates deduced from the numbers of preovulatory follicles for the Romanov and Ile-de-France ewes were 3.2 ± 0.2 and 1.5 ± 0.2 respectively (P < 0.01) and the mean lengths of the second oestrous cycle after synchronization 17.5 ± 0.41 and 16.7 ± 0.13 days respectively (P < 0.01).

**Hormone data**

**LH.** There appeared to be little difference between breeds (Text-fig. 1). There were no significant differences (P < 0.05) between the mean daily LH levels of the Romanov and Ile-de-France ewes (2.74 ± 0.42 and 3.24 ± 0.34 ng/ml, respectively), the highest value measured (54.4 ± 14.1 and 78.5 ± 10.5 ng/ml, respectively) or area under the preovulatory peak (302.8 ± 64.7 and 392 ± 57.6 ng/ml/h respectively). However, the mean concentration on each of Days 9, 10 and 11 of the cycle was significantly higher for the Romanov ewes (1.17 ± 0.2 and 0.73 ± 0.07 ng/ml respectively).

There were significant breed differences in the time intervals between some hormonal events and the onset of the LH peak (Text-fig. 2). Whereas there was no significant difference between breeds in the interval from luteolysis and the onset of the LH peak (mean 42.8 ± 2.5 h) or between the highest oestradiol-17β value measured and the LH peak (mean 7.8 ± 2.2 h), there was a difference between breeds (P < 0.01) in the interval from the onset of oestrus to the LH peak at the second and third synchronized oestrous.

**FSH.** There were breed differences in the characteristics of the FSH peak although the overall profiles were the same (Text-fig. 1). There was a fall in FSH concentrations approximately 24 h before the LH peak and two distinct FSH peaks at oestrus; the first peak coincided with the preovulatory LH discharge whilst the second began approximately 20–30 h after the LH peak depending on the breed. The first FSH peak was slightly higher, but not significantly so, in the Ile-de-France ewes but the second FSH peak was larger (P < 0.05) in the Romanov ewes (193 ± 84 and 57 ± 15 ng/ml/h respectively). The second FSH peak also began earlier (P < 0.05) in the Romanov than the Ile-de-France ewes (19.6 ± 2.8 and 27 ± 1.9 h after the LH peak respectively).

Despite the differences in the characteristics of the FSH peaks between breeds, the mean daily concentration (8.85 ± 1.53 and 8.07 ± 0.74 ng/ml), the mean concentration per day of the cycle and the highest value measured (18.4 ± 3.6 and 17.4 ± 1.6 ng/ml) did not differ between the Romanov and Ile-de-France ewes respectively.

**Prolactin.** Concentrations were similar in both breeds with a pro-oestrous rise commencing approximately 48 h before the LH peak, followed by a decrease immediately before the LH peak and then a small peak immediately after the LH peak (Text-fig. 1). There were no significant
Text-fig. 1. The mean concentrations of (a) LH, (b) FSH and (c) prolactin for 5 Romanov (○—○) and 11 Ile-de-France (△—△) ewes. Levels for Days 0 to 14 are chronologically aligned in relation to the first LH peak studied and for Days −3 to 0 in relation to the following LH peak.

differences in the areas under the curve 0–12 h after the LH peak (674 ± 268 and 424 ± 229 ng/ml/h), or the pro-oestrous rise 48 h before the LH peak (1290 ± 257 and 778 ± 248 ng/ml/h) in the Romanov and Ile-de-France ewes respectively.

Prolactin and oestrogen levels were correlated: the area under the curve of prolactin concentration 0–12 h after the LH peak was positively correlated ($P < 0.05$) with the area under the curve of oestradiol-17β concentrations −48 to 0 h and −24 to 0 h before the LH peak and also with the highest value of oestradiol-17β measured.
Text-fig. 2. The hormonal events around oestrus for 5 Romanov and 11 Ile-de-France ewes.

(a) Not significantly different: luteolysis–LH; oestradiol-17β–LH; LH–FSH (first peak);
(b) Significantly different (P < 0.05): luteolysis–oestrus; luteolysis–oestradiol-17β; LH–FSH (second peak);
(c) Significantly different (P < 0.01): oestrus–LH; oestradiol-17β–oestrus; FSH (first peak) –FSH (second peak).

Oestradiol-17β. The pro-oestrous discharge -24 to 0 h before the LH peak was significantly larger in the Romanov than the Ile-de-France ewes (436.6 ± 30.2 and 314.6 ± 32.4 pg/ml per h respectively). There was no significant difference between breeds in the interval between the highest oestradiol-17β value measured and the LH peak, but oestradiol concentrations fell slightly before the LH peak and then dropped markedly following the LH peak. There was no significant difference in oestradiol concentrations between Romanov and Ile-de-France ewes at the onset of oestrus (16.1 ± 0.5 and 14.5 ± 2.0 pg/ml respectively), but the level then fell in the Ile-de-France ewes while continuing to rise in the Romanovs, giving a significantly higher (P < 0.05) concentration at the time of the onset of the LH peak. Between Days 6 and 12 the mean oestradiol concentrations were higher in Romanov than Ile-de-France ewes, although the difference was not significant (P > 0.05).

Progesterone. The overall profiles of progesterone were a direct reflection of the differences in ovulation rate and cycle length between breeds (Text-fig. 3); the mean highest concentration measured and the mean concentration on Days 4–13 were higher in the Romanov than the Ile-de-France ewes (P < 0.05).

Correlations between hormone data and follicular characteristics (linear regression)

Due to the small number of animals involved per breed (5 Romanov and 9 Ile-de-France) correlations on a per breed basis between hormone data and follicular characteristics could not be considered reliable, thus the breed difference was not considered.

LH. There were no significant correlations between the area under the curve of the LH peak nor the mean level of LH during Days 1, 4–13 or 9–11 of the cycle and the follicular characteristics considered.

FSH. There was a highly significant (P < 0.01) correlation between the area under the curve of the second FSH peak and the number of follicles with an antrum: the regression equation is y = 0.182x + 30.17 (r = 0.71), where y = the number of antral follicles and x = the area under the second FSH peak (ng/ml/h). The correlation between the second FSH peak and the number of follicles greater than 0.36 mm was also significant (r = 0.62, P < 0.05), but not with the number of follicles greater than 1.13 mm diameter nor with the number of pre-antral follicles. There were no significant correlations between the FSH peak coincident with the LH peak and the follicular characteristics measured.
Text-fig. 3. Concentrations of (a) oestradiol-17β and (b) progesterone for 5 Romanov (●—●) and 11 Ile-de-France (△—△) ewes. Values for Days 0 to 14 are chronologically aligned in relation to the first LH peak studied and for Days —3 to 0 in relation to the following LH peak.

Prolactin. The area under the curve of the prolactin peak 0–12 h after the LH peak was not significantly correlated with any of the follicular parameters considered. However, the area under the curve of the pro-oestrous rise —48 to 0 h before the LH peak was significantly correlated with several of the classes of antral follicles, including the largest follicles in the growth phase and also the number of preantral follicles.

Oestradiol-17β. There were significant correlations (P < 0.05) between the highest oestradiol value measured around the time of oestrus, the area under the defined oestradiol peak with the number of follicles greater than 0.36 mm diameter.

The area under the curve —24 to 0 h before the LH peak was significantly correlated with the number of preovulatory follicles (P < 0.05). There was no correlation between the area under the curve —48 to 0 h before the LH peak and the follicular characteristics considered.

Progesterone. There were significant correlations between the highest progesterone level measured and the mean concentration on Days 4–13 with the number of preovulatory follicles (P < 0.05).
Factor analysis of hormonal and follicular parameters

The factor analysis divided all the 32 parameters into categories such that parameters within one category are more closely related than those in other categories. Five categories were obtained (Table 2).

<table>
<thead>
<tr>
<th>Table 2. The distribution of 32 hormonal and follicular parameters in 5 classificatory categories by factor analysis</th>
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</thead>
<tbody>
<tr>
<td><strong>Category</strong></td>
</tr>
<tr>
<td>1 FSH Prolactin</td>
</tr>
<tr>
<td>Time Follicles</td>
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<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>2 LH Progesterone</td>
</tr>
<tr>
<td>Follicles</td>
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<tr>
<td></td>
</tr>
<tr>
<td>3 FSH Oestradiol-17β</td>
</tr>
<tr>
<td>Follicles</td>
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<td></td>
</tr>
<tr>
<td>4 FSH LH</td>
</tr>
<tr>
<td>Time Follicles</td>
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<td></td>
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<tr>
<td></td>
</tr>
<tr>
<td>5 Prolactin Oestradiol-17β</td>
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<td></td>
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</tbody>
</table>

Discussion

The principal features of this study concern the associations between hormonal data, particularly FSH, and follicular characteristics. The general pattern of FSH secretion previously described by L'Hermitte, Niswender, Reichert & Midgley (1972), Salamonsen et al. (1973) and Pant, Hopkinson & Fitzpatrick (1977) has also been found in this present study, i.e. a depression in FSH values before the LH discharge followed by two peaks of FSH, the first coincident with the LH peak and the second 20–30 h afterwards. The factor analysis shows this second peak of FSH to be related to practically all the parameters concerning large or growing non-atretic follicles. The direct correlation of the area under this second peak with the number of antral follicles present some 17 days later suggests that the rate follicles enter the antral phase may be another mechanism determining the subsequent ovulation rate in the sheep and that this mechanism may be under the control of FSH. These results closely resemble the model suggested by Greenwald (1973) who concluded that, in the hamster, the peri-ovulatory levels of gonadotrophins recruit the next set of follicles for development during the new cycle. As in many other species there also appears to be a high incidence of atresia in the sheep following the discharge of LH; Turnbull, Braden & Mattner (1977) found that the very large follicles (>3.5 mm in diam.) underwent luteinization after hCG treatment while two thirds of the follicles 1–3.5 mm became atretic. Our factor analysis also showed a relationship between LH parameters and the number of atretic follicles. It is possible that the second peak of FSH serves initially, but perhaps not solely, to replenish the stock of antral follicles after the 'wave' of atresia following the LH peak. This stock of antral follicles is apparently more rapidly replenished in ewes with a higher ovulation rate.
LH showed only one significant difference between breeds, i.e. on Days 9–11 after the LH peak the level was significantly higher in the Romanov than the Ile-de-France ewes. However, the level of LH at this time was not directly correlated with any of the follicular characteristics measured. The elevated level of LH about this time of the cycle in ewes with a higher ovulation rate has also been noted in two other studies (Land et al., 1973; Findlay & Cumming, 1977). How this effect is involved in the mechanism determining ovulation rate remains unexplained. The factor analysis did show a relationship between LH, atresia and ovulation rate, suggesting that the final selection of follicles to ovulate or become atretic is LH dependent. However, the relationship between the number of atretic follicles and ovulation rate in these normally cyclic animals is a positive relationship and not negative as may be expected. As found in other studies (Land et al., 1973; Bindon et al., 1976) characteristics of LH release at oestrus could not be related to breed or the number of follicles present.

There were significant differences in oestradiol concentrations between breeds. Bindon et al. (1976) and Scaramuzzi & Land (1976) found no difference about the time of oestrus between breeds which differed in their ovulation rates. However, the factor analysis and linear regression analyses showed relationships between oestradiol values in the period before oestrus with the number of larger and preovulatory follicles which agrees with the relationship found in the calf between the level of oestradiol-17β before oestrus and the ovulation rate after PMSG stimulation (Saumande, 1980).

The present study supports the suggestion of Land (1976) that a breed difference exists in the sensitivity of LH secretion to oestrogens because during the pro-oestrous period the oestradiol concentration was higher in the Romanov breed but the LH concentrations were similar in both breeds. Given that there are similar LH levels in both breeds although ovulation rate varies markedly, it is difficult to see how the sensitivity of LH secretion to oestrogens can determine ovulation rate as suggested by Land (1976) and Scaramuzzi & Land (1976). Nevertheless, the factor analysis of the present study indicated that there might be a link via the relationship between LH and the number of atretic follicles.

The correlations of the discharge of prolactin with the follicular characteristics in both the linear regressions and factor analysis were an unexpected result. The lack of correlation between the FSH and prolactin suggests that the correlations of each of these hormones with follicular data are independent. Also the correlation of prolactin with the number of preantral follicles suggests that prolactin is involved with this early phase of follicular growth, which agrees with the work of de Reviers (1974) who showed that an injection of prolactin increased the number of preantral follicles in the hypophysectomized rat. It is known that the basal level of prolactin varies according to season in the sheep (Pelletier, 1973; Ravault & Ortavant, 1977) and such variation may be related directly to seasonal ovarian variation. However, the action of prolactin at the follicular level is not clearly defined although prolactin-specific receptors are present in granulosa cells of the pig (Rolland & Hammond, 1975).

The concentrations of progesterone were a direct reflection of the ovulation rate, with Romanov ewes having the higher mid-cycle progesterone values. However, the increase in the progesterone level at about Day 4 occurred at the same time and the same rate in both breeds. The fall in progesterone at luteolysis occurred 1 day later in the Romanov ewes, thus causing a longer cycle length according to the mechanism proposed by Hauger, Karsch & Foster (1978). However, the actual fall in progesterone was similar in both breeds, as also found by Bindon et al. (1976).

References


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