SIMULTANEOUS DETERMINATION OF LH AND PROGESTERONE IN PERIPHERAL BOVINE BLOOD DURING PREGNANCY, NORMAL AND CORTICOID-INDUCED PARTURITION AND THE POST-PARTUM PERIOD

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(Received 23rd March 1971, accepted 28th September 1971)

Summary. Levels of LH were determined by radioimmunoassay and those of progesterone by a competitive protein-binding technique. Blood samples from eight cows, representing two complete pregnancies (including post-partum periods), and from one cow in the first trimester and another around the time of parturition, were collected. The samples were withdrawn at intervals of 6 hr for LH and every 4 to 5 days for progesterone determination. Levels of LH were consistently low (1.0 to 1.6 ng/ml plasma) with a few single peaks occurring irregularly in individuals during the first 110 days. Distinct LH peaks were observed between Days 7 and 20 post partum. The decrease of progesterone from high levels during pregnancy (plateau between 5 and 9 ng/ml plasma with individual differences) before parturition and the low level following was not reflected in the LH concentration. In an experiment with one animal, an injection of 5 mg flumethasone near term caused a partial decrease in the progesterone concentration, and a larger dose of 10 mg induced parturition. This was preceded by a marked decrease of progesterone similar to that observed before a normal delivery.

INTRODUCTION

While progesterone has been determined in bovine peripheral blood during different periods of pregnancy and the post-partum period (Shemesh, Ayalon & Lindner, 1968; Erb, Estergreen, Gomes, Plotka & Frost, 1968; Pope, Gupta & Munro, 1969; Stabenfeldt, Osburn & Ewing 1970; Hoffman & Karg, 1970), LH has been assayed in comparatively few experiments during early pregnancy (Schams, 1969; Henricks, Dickey & Niswender, 1970). In order to draw conclusions about the relationship of LH and progesterone in vivo during pregnancy and the post-partum period, it would seem to be desirable to make frequent measurements of both hormones in identical blood samples. From incubation and perfusion experiments in connection with the exogenous administration of LH-active preparations, several authors (Wiltbank, Roth-
Radioimmunoassay determined, of Animals method used. from experiment the also.

These of peripheral luteotrophic anticoagulant, Blood enthal/Syntex). The progesterone specific found to they of antigens. It is probable that some other factors may have contributed to the observed increase in progesterone levels.

The results of the present study indicate that progesterone levels are significantly higher in the peripheral blood of pregnant cows than in those that are not pregnant. This finding supports the hypothesis that progesterone plays a role in maintaining pregnancy.

Materials and Methods

Animals

The animals used in the first series of the experiments were six pregnant cows (5 to 10 years old) with normal breeding histories, and two pregnant heifers; they were crossbreeds from Simmental/Holstein-Canadian races, which have an average pregnancy period of 279 days. The animals were in different periods of gestation during the collection of blood samples (January until April 1970). These samples represent two complete pregnancy periods. Progesterone was also analyzed in plasma samples from another cow (Holstein): LH had been previously assayed in an earlier experiment on the same cow carried out during the first trimester of gestation (Schams, 1969). One cow of the Brown Swiss breed, with a pregnancy of normal duration of 287 days, was included from an experiment involving thirty-nine animals (Karg, Böhm, Günzler & Müller, 1971) where parturition was induced by the injection of a glucocorticoid (6α-9α-difluor-16-methylprednisolone (flumethasone) by courtesy of Grünenthal/Syntex).

Blood sampling

Blood was collected daily at 6-hr intervals (05:00, 11:00, 17:00 and 23:00 hours) from the jugular vein by venipuncture into plastic centrifuge tubes. As an anticoagulant, the heparin preparation 'Liquemin' (Hoffmann-La Roche) was used. The blood was immediately cooled on ice, centrifuged and stored at −18° C until further use. All samples were evaluated for LH. Progesterone was determined, preferably in the 17.00-hour sample, at 4- to 5-day intervals and, in a few cases, once or twice a day.

Radioimmunoassay of luteinizing hormone

Luteinizing hormone was quantified by radioimmunoassay according to the method described by Schams & Karg (1969). For iodination, the LH preparations
LER-791-1 and III-17-BP were used. A highly specific antiserum to LH, obtained by the immunization of rabbits, was used. This antiserum showed no cross-reaction by immunodiffusion, immunoelectrophoresis and radioimmunoassay with serum proteins and pituitary hormones (bovine prolactin (NIH-P-B1), bovine growth hormone (NIH-GH-B10), synthetic adrenocorticotropic hormone 1-24 (Giba) and pure bovine thyroid-stimulating hormone) after the absorption with bull serum. Only in the high dose ranges were cross-reactions observed with HCG, PMSG or with highly purified sheep FSH (thirty-two times as active in bioassay as FSH NIH-S1). The antiserum was also able to neutralize the biological activity of LH estimated by the OAAD-test. Measuring serum values by radioimmunoassay and bioassay, the physiological variations were the same but the absolute values measured by bioassay were between three and ten times higher than those measured by radioimmunoassay (Karg, Schams & Böhm, 1969).

That the assay is suitable to measure LH in bovine blood is established by the parallel shape of the dilution curves of LH added to bovine plasma, and of endogenous LH compared with the standard curves. The results from LH recovery experiments (Schams & Karg, 1970) also confirm the suitability of the assay method. All results were expressed in terms of ng LH-LER-791-1/ml plasma, which is equivalent to 1-1 times LH-NIH-S1. The antibody-bound hormones were separated from the free labelled hormone by centrifugation. All plasma samples were run in duplicate with 0-2 or 0-3 ml plasma per test tube and each peak was re-examined. The minimum detectable value in this assay system was 0-5 ng/ml serum or plasma.

**Determination of progesterone**

Progesterone was determined in bovine peripheral plasma by the competitive protein-binding method (Hoffmann & Karg, 1970). For the exact calculation of recovery in individual samples, this method was further improved by applying internal standardization which was similar to that used in the method published by Reeves, de Souza, Thompson & Diczfalussy (1970). A small amount of \[^{3}H\] progesterone (2500 disintegrations/min) of high specific activity (progesterone 1a,2a-T(n), 41 Ci/mmol, the Radiochemical Centre, Amersham) was pipetted as internal standard into the extraction tube and dried down in a stream of nitrogen at 40°C before the addition of 1 ml plasma. Following the extraction procedure and thin-layer chromatography, the sample was taken up in 1 ml methanol and the recovery—generally between 60 and 70%—was determined from the disintegration/min rate measured in a 0-1-ml aliquot. To compensate for the remaining counts in the sample used for the protein-binding reaction, two-thirds of the amount of \[^{3}H\] progesterone used as internal standard were added to each point of the standard curve.

**RESULTS**

The results of the LH and progesterone determinations on the peripheral plasma from the eight animals, samples from which represented two complete pregnancy periods, are shown in Text-figs. 1 and 2. Each point for LH in these figures represents an average value of twenty plasma samples from 5 con-
Text-fig. 1. Progesterone and LH levels in blood plasma samples from four cows during pregnancy (○, cow Albi; ●, cow Zista; △, cow Alpiaka; □, cow Alante).
Text-fig. 2. Progesterone and LH levels in blood plasma samples from four cows during pregnancy (O, cow Zelline; ●, cow Ella; △, cow Zenzi; □, cow Pola, corticoid-induced parturition).
secutive days. In general, the LH levels range around 1 ng/ml plasma throughout the whole pregnancy period. The mean values for each cow, calculated from all samples measured, are shown in Table 1. Only in one cow (Zista) were small LH peaks observed on Day 94 and Day 103 (see Text-fig. 3). In this cow, progesterone was determined twice a day but the concentrations of both

**Table 1**

**MEAN VALUES OF LH IN PERIPHERAL BOVINE BLOOD-PLASMA FROM COWS IN DIFFERENT PERIODS OF PREGNANCY**

<table>
<thead>
<tr>
<th>Name of cow</th>
<th>Pregnancy period (days of gestation)</th>
<th>Mean LH values (ng/ml plasma)</th>
<th>No. of samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Albi</td>
<td>12 to 89</td>
<td>1·0 ± 0·34</td>
<td>357</td>
</tr>
<tr>
<td>Zista</td>
<td>82 to 180</td>
<td>0·96 ± 0·28</td>
<td>391</td>
</tr>
<tr>
<td>Alpiska</td>
<td>179 to 237</td>
<td>1·13 ± 0·20</td>
<td>233</td>
</tr>
<tr>
<td>Alante</td>
<td>220 to 276</td>
<td>1·30 ± 0·24</td>
<td>231</td>
</tr>
<tr>
<td>Zelline</td>
<td>7 to 105</td>
<td>1·24 ± 0·27</td>
<td>394</td>
</tr>
<tr>
<td>Ella</td>
<td>51 to 149</td>
<td>1·56 ± 0·25</td>
<td>394</td>
</tr>
<tr>
<td>Zenzi</td>
<td>111 to 209</td>
<td>1·30 ± 0·27</td>
<td>394</td>
</tr>
<tr>
<td>Pola</td>
<td>206 to 274</td>
<td>1·04 ± 0·20</td>
<td>275</td>
</tr>
</tbody>
</table>

Text-fig. 3. Progesterone–LH relationship in peripheral blood plasma of one cow (Zista) from Days 92 to 108 of gestation.

hormones did not seem to be correlated. In another cow, where an LH peak on Day 52 was observed (Schams, 1969), progesterone was consistently high—between 6·3 and 8·4 ng/ml plasma.

Progesterone values increased at the beginning of pregnancy with the development of the CL. After reaching maximal levels on Days 20 and 21, a slight drop was observed. The change from the CL of the normal cycle to the CL of pregnancy was characterized by progesterone values higher than 5 ng/ml plasma during Days 18 to 24. Allowing for individual variations, the concentration seems to
Text-FIG. 4. Progesterone and LH concentrations in peripheral blood plasma of one cow (Alante) around parturition.
TEXT-FIG. 5. Progesterone and LH concentrations in peripheral blood plasma of one cow (Pola) around parturition (corticoid-induced parturition).
LH and progesterone in cows during pregnancy

remain fairly constant after Day 40 but shows a tendency to decrease after Day 240. It then drops markedly within the 2 days preceding parturition. By the time that parturition occurs, minimum progesterone levels have already been reached (Text-figs. 4 and 5).

No change in the progesterone concentration was observed during the post-partum period up to 30 to 39 days following parturition. No significant alteration in LH occurred before, during and for a few days after parturition. In the data shown in Text-fig. 4, some small irregularly occurring LH peaks showed up around Day 20 post partum. No oestrus was observed during that time. In the data shown in Text-fig. 5, the first small LH peaks were observed on Days 7 and 28 following parturition. This cow was treated on Day 272 of pregnancy with 10 mg flumethasone for the initiation of parturition which occurred 2 days later.

In another animal with an expected duration of pregnancy of 287 days (Text-fig. 6), the first attempt to induce parturition was made on Day 274 with the low dose of 5 mg flumethasone and later on Day 280 with an effective dose of 10 mg of the same compound. The first injection was followed by a decrease in the progesterone level from around 6 ng/ml plasma to approximately 3 ng/ml. During the parturition induced with the second injection of flumethasone, no differences occurred in the decline of progesterone and in the basic levels of LH compared to the occurrence during a normal delivery. In this cow, the distinct first LH peak of 32 ng/ml plasma on Day 11 post partum was most obvious.
DISCUSSION

Our results in the cow clearly demonstrate the presence of low LH levels throughout the whole period of pregnancy. Progesterone values show the characteristic rise at the beginning and the decline at the end of the gestation period, with a plateau in between. The absolute values differ individually. The constant levels of progesterone through the gestation period until the approach of term, do not support the theory of a change in the source of progesterone during the last trimester of pregnancy (see Stabenfeldt et al., 1970). Since both hormones showed completely different secretion patterns, it seemed meaningless—at least during the pregnancy period and the post-partum phase—to apply statistical methods for correlation. It seems that progesterone secreted during pregnancy effectively prevents LH release in most individuals, though in our experiments, in the case of one cow (Zista), single LH peaks around Days 94 and 103 of gestation appeared (Text-fig. 3). Similar observations were described earlier by Schams (1969) and Karg & Schams (1970) for other cases during the first trimester. In this connection, it may be pointed out that, in the normal bovine oestrous cycle in addition to the distinct LH peak before ovulation, and extraordinary LH peak can occur during the early cl. phase in some individuals. This was first demonstrated by Schams & Karg (1969) and Karg & Schams (1970) during multiple blood-sampling periods of four to six times a day.

The extraordinary LH peaks observed in this study during pregnancy did not show an obvious correlation with the progesterone level preceding or following these peaks, probably because the progesterone level already had its 'pregnancy plateau'. Moreover if, according to the in-vitro perfusion studies of Mills & Morrissette (1970) with bovine ovaries, LH seems to be luteotrophic during the early and late phases of pregnancy, our endogenous LH data clearly showed that the decrease of progesterone levels near term was not accompanied by diminished LH values.

It may be concluded that, in the cow, the demonstration of a luteotrophic action of LH may occur in certain situations, for instance in the presence of active luteal tissue with a low progesterone synthesis rate, provided that there is no dominance of any possible luteolytic activity. The decline of progesterone near term is not due to a lack of LH, so other factors have to be considered to luteolysse and hence, to initiate parturition. The well-known rise of oestrogen secretion, particularly at the end of pregnancy (Erb, Randell, Mellin & Estergreen, 1968; Grunert & Ahlers, 1969; Robinson, Baker, Anastassiadis & Common, 1970), seems not to alter significantly the LH secretion rate. Further experimental evaluation is needed in order to explain why the basic level of LH in the peripheral blood of the cow also remains so constant around the time of parturition, when major changes in hormonal relationships are known to occur. This is especially remarkable since it was found earlier (Karg, 1966, 1967a, b) that pituitary glands of new-born calves showed depleted LH concentrations.

The interesting observation that progesterone levels decrease after the administration of a glucocorticoid, gives an indication of a possible mechanism...
involved in luteolytic action near term. The LH and progesterone secretion patterns following administration of the glucocorticoid show that this treatment simulates the naturally occurring events, at least those involving the function of these hormones around parturition. During the post-partum period, a distinct LH peak was first observed on Day 7 in one cow and a more distinct one on Day 11 in another cow. It seemed, at least in this early post-partum phase, that many individual differences are apparent in the re-establishment of the oestrous cycle, probably due to the irregular release pattern of LH. After these early peaks, progesterone levels still remained low and it was concluded that neither ovulation nor luteinization occurred.

The blood levels of LH and progesterone appear to reflect two rather different release patterns suggesting that the relationship of these hormones under different physiological conditions require very careful interpretation.

ACKNOWLEDGMENTS
This work was supported by the Deutsche Forschungsgemeinschaft. We also acknowledge gratefully the supply of LH standard preparations from the National Institutes of Health, Bethesda, from Dr. L. E. Reichert (LH-LER-791-1), Atlanta and Dr. H. Papkoff (LH-no. III-17-BP), San Francisco. The pure bovine tSH preparation was kindly supplied by Dr. Pierce, Los Angeles.

REFERENCES


