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PROSTAGLANDIN F2 ALPHA AND PROSTACYCLIN IMBALANCE IN COWS WITH PLACENTAL RETENTION: NEW FINDINGS

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Résumé

DÉSÉQUILIBRE RELATIF DE PgF2 ALPHA ET Pgl2 CHEZ LES VACHES A RÉTENTION PLACENTAIRE. NOUVELLES DONNÉES. — Le but de ce travail était de détecter d’éventuelles différences des concentrations plasmatiques au niveau périphérique de la 13,14 dihydro 15 céto PgF alpha (PGFM) et de la 6 céto PgF1 alpha (PGIM) entre vaches à rétention (RP) et vaches à vêlage sans rétention (NRP) du placenta (vêlage normal). Des prélèvements de sang ont été effectués 5, 30, 60 minutes postpartum sur 8 vaches à rétention et 17 vaches à vêlage normal. Les teneurs plasmatiques en PGFM étaient plus faibles chez les vaches à non-délivrance que chez les vaches à vêlage normal 60 minutes postpartum (8156 pg/ml vs 12016 pg/ml, P < 0,05). Une augmentation significative du taux de PGFM a eu lieu entre 30 et 60 minutes postpartum chez les vaches du groupe NRP (6225 pg/ml vs 12016 pg/ml, P < 0,01) tandis que dans le groupe RP, le niveau de PGFM est resté constant au cours de la période étudiée. Aucune différence significative n’est apparue en ce qui concerne le métabolite de Pgl2 malgré des valeurs légèrement supérieures pour le groupe RP. Le rapport PGFM/PGIM était significativement plus haut 60 minutes après le vêlage dans le groupe NRP que dans le groupe RP (15,5 vs 8,8 ; P < 0,01). Ce rapport a augmenté entre 30 et 60 minutes (7,2 vs 15,5 ; P < 0,01) chez les vaches à vêlage normal, résultat non observé chez les vaches à non-délivrance. Il n’est pas apparu de corrélation significative entre les niveaux de PGFM et PGIM au cours des soixante premières minutes du postpartum pour les vaches du groupe NRP. Cependant, une telle corrélation était significativement positive pour les vaches du groupe RP (r = 0,75, P <0,01). Les résultats suggèrent que la synthèse de PgF2 alpha entre 30 et 60 minutes postpartum est significativement moindre chez les vaches à non-délivrance comme le reflète le niveau de PGFM. Ils suggèrent en outre que la synthèse de prostacycline semble stable chez les vaches à vêlage normal au cours des 60 premières minutes postpartum, mais non chez les vaches à rétention dont la concentration en PGIM augmente en même temps que celle de PGFM. Il est proposé qu’un déséquilibre de synthèse de PgF2 alpha et Pgl2 au cours des 60 premières minutes suivant le part, conduisant à un défaut de PgF2 alpha et une augmentation relative de Pgl2, est associé à la rétention annexielle chez la vache.

The role of prostaglandins upon the physiological mechanism of placental separation in the cow has been stated in an earlier study (Horta, 1981), where it was shown that cyclooxygenase inhibition early after calving leads to a retention of the afterbirth for a period longer than 24 hours.

Later, further experiments confirmed and added new knowledge to the previous finding. In one of these experiments, it was studied the pharmacological effects of prostaglandins E2 and F2 alpha in relation to the time needed for placental separation and expulsion, and to the strength of uterine contractions (Horta, 1984). The results obtained suggested that PGE2 inhibits the normal placental
Materials and Methods

1 - Animals

Twenty five Holstein Friesian cows were observed during the peripartum so as to register the moment of expelling within 12 hours after calving. None of these animals received any treatment with drugs known to affect the biosynthesis of prostaglandins, at least during the last month of gestation.

During the dry period, the cows were fed ad libitum with chopped fresh sorghum (75 % of the total), corn silage (20 %) and lucerne hay (5%).

2 - Blood collection and plasma conservation

All animals were bled by venipuncture strictly five, thirty and sixty minutes after the expulsion of the fetus. Blood samples were taken from the jugular vein into a syringe tube containing EDTA K (anticoagulant) and lysine acetyl-salycilate (cyclooxygenase inhibitor, 18 µg/ml of blood). After collection, blood samples were immediately reconstituted at + 4 °C and centrifuged 15 minutes at 3500 rpm. Then, plasma was decanted and frozen at − 20 °C until analysis were performed.

3 - Analytical steps

The main metabolites of PGF2 alpha and PG12, respectively the 13,14 dihydro 15 keto PGF alpha (PGFM) and 6 keto PGF1 alpha (PGIM), were measured by radioimmunoassay (RIA) according to the following procedures:

3-1 Extraction

- 1000 µl of each plasma sample, acidified to pH 3 with 100 µl of citric acid (1M), were twice treated with 3 ml of ethyl acetate in an assay tube. After a 5 minutes stirring, the tubes were centrifuged during 10 minutes at 3500 rpm. The upper organic phases were transferred into conic tubes and the solvent evaporated under a nitrogen flow at room temperature.

- Each dry lipid extract, containing prostaglandins, was reconstituted in 1 ml of phosphate buffer at pH 7.4; the solution was stirred for 1 minute and maintained overnight at 4 °C for complete dissolution.

3-2 Radioimmunoassay

- 100 µl of solution were used for each RIA determination after (or without any) dilution of the reconstituted prostaglandin extract with buffer according to the prostaglandin measured (1/10 for dhk PgF, no dilution for 6k PgF). The radioimmunological reactions were developed in presence of the specific antiserum (Institut Pasteur) and the titrated metabolites (about 5000 dpm), during overnight incubation at + 4 °C. After that time, 1 ml of a dextran-coated charcoal suspension (T70) was added and the tubes were centrifuged at 3500 rpm 10 minutes at + 4 ºC. An aliquot of 0.5 ml of the supernatant (bound fraction) was counted in the presence of 5 ml of scintillation fluid (Aquasol 2) in a Packard counter.

3-3 Concentrations determination

The concentrations of the metabolites were calculated in relation to a standard curve ranging from 0 to 400 pg/100 µl (400, 200, 100, 50, 25, 12.5, 6.25 pg treated respectively the 13,14 dihydro 15 keto PGF alpha (PGFM) and 6 keto PGF1 alpha (PGIM) with each group were compared by ANOVA.

3-4 Statistical analysis

Statistical differences in variations, for RP and NRP cows, of the plasma concentrations of PGFM and PGIM found for each of the sample collections were tested using analysis of variance (ANOVA) and PGFM/PGIM ratio were compared in the same way. Differences for mean concentrations of each group (RP and NRP) were compared for each sampling session by the «t» Student’s test after or no logarithmic transformation.

Regression analysis were performed between the levels of PGFM and PGIM during the first 60 minutes postpartum. The linear regression equations found for each group were compared by ANOVA.

Results

Eight out of the total 25 cows studied retained placenta.
their placenta for a period longer than 12 hours after calving (all, except one, longer than 48 h) and were included in the RP group. The others 17 cows delivered their placenta 5.5 ± 2.2 hours postpartum. They had identical mean lactation numbers and gestation lengths, and gave birth to calves of similar mean weights; the number of twins was 1 pair in the RP group and none in the NRP one (table 1).

The mean level of PGFM in peripheral blood plasma from the RP cows was significantly lower than that from the NRP cows 60 minutes postpartum (8156 ± 6965 vs 12016 ± 6811 pg/ml; P < 0.05, table 2). One of the cows belonging to the RP group presented very high values of PGFM in relation to the mean found for the group (22257, 11285 and 17255 pg/ml, respectively at 5, 30 and 60 minutes). The individual discrepancy in the PGFM level of the RP group, which does not appear in the NRP one, is reflected by the F value (6.78; P < 0.01) of the analysis of variance.

The interesting feature is the significant increase of PGFM 60 minutes postpartum in the NRP cows (table 2). This pattern was not observed in the RP cows where the levels of PGFM remained fairly constant along the period studied. Although the mean values of PGIM found in RP cows tended to be higher than those from NRP cows, the differences were not statistically signifi-
cant (P > 0.05, table 2) and remained constant during the same period. The individual variance is significant too in this group (F = 11.338; P < 0.01).

The mean value of the PGFM/PGIM ratio in samples taken 60 minutes postpartum was significantly lower in RP cows than that from NRP cows (8.8 ± 6.7 vs 15.5 ± 8.9; P < 0.01, table 2). In RP cows the PGFM/PGIM ratio remained constant, while in NRP cows it rose significantly between 30 and 60 minutes postpartum (F = 11.100 P < 0.01 ; table 2).

In order to investigate whether the differences found in PGFM/PGIM ratio were due to fluctuations of PGFM, PGIM or both, figures 1 and 2 present the regression equations between the levels of these prostanoids during the first 60 minutes, respectively in RP and NRP cows. In RP cows, a positive correlation was found between the levels of PGIM and PGFM during this time (r = 0.75 ; P < 0.01, fig. 1). On the other hand, no correlation could be found between PGIM and PGFM levels in the NRP group (r = 0.23 ; P > 0.05, fig. 2). The linear regression equations found for each group were statistically different, as well as their interception and regression coefficient values (F = 20.87 ; P < 0.01).

Discussion

The identical calving conditions of the cows of both groups indicate that retention of the membranes was not due to four of the main known causes of this pathology in our work (table 1).

The results presented (table 2) strongly suggest that at least, during the first 60 minutes postpartum, the capacity to synthesize PGF2 alpha is lower in RP than in NRP cows. Differences in peripheral plasma PGF levels between RP and NRP are evident but only significant 60 minutes postpartum, traducing a rise of the PGFM levels between 30 and 60 minutes postpartum in NRP cows, and relative stability of the metabolite in the RP cows.

Bosu et al. (1984) found lower levels for PGFM than us ; that may have been due to differences in timing of blood samples since these authors analysed daily samples.

Although cyclooxygenase inhibition early in the postpartum period can induce placental retention (Horta, 1981), the lower production of PGF2 alpha in RP cows at this time is suggested to be due to causes others than a lack of the cyclooxygenase activity. This assumption arises from the fact that instead of being depressed, PG12 synthesis seems to be stimulated in RP cows, as reflected by the tendency of PGIM to be higher in this group and by the positive correlation between the synthesis of PGFM and PGIM in RP cows. On the other hand, the higher PGFM/PGIM ratio in NRP cows, when compared to RP cows, strongly suggests that the metabolism of the PGG2 and PGH2 endoperoxides is altered in RP cows, leading to a lack of PGF2 alpha and an increase in PG12 synthesis.

Some unknown factor seems to maintain PG12 synthesis constant during the first 60 minutes after birth of the calf in NRP cows since when PGFM, increases, there is no related increase in PGIM, as opposed to what occurs in RP cows, where the correlation between the synthesis of both prostanoids is significantly positive (fig. 1 and 2).

Furthermore, the positive correlation between PGFM and PGIM production in RP cows, suggests...
that PG12 and PGF2 alpha seem to have antago-
nist effects regarding to the ultimate stage of
parturition: PG12 would have an anti-placental
separation activity, a similar effect to that which
was previously demonstrated for PGE2 (Horta,
1984), while PGF2 alpha would stimulate placent-
al separation and expulsion.

The results here obtained give support to the
proposed action for PGF2 alpha of stimulating
placental separation in the cow (Horta, 1984).
This effect was also observed by Herschler and
Lawrence (1984) in a clinical trial using fenprosta-
lene, a long-acting PGF2 alpha analogue.

Although the key step of physiological mecha-
nisms leading to the normal separation of fetal
membranes are not yet perfectly understood, it is
thought that changes in connective tissue toge-
ther with vascular changes in microcirculation
of placentomes, play an important role in the physi-
ology of the third stage of labour (Grunert, 1984).
Otherwise, it seems that the maintenance of the
number of the giant epithelial cells of placentomes
from prepartum by 1 h postpartum is related with
placental retention (Grunert, 1980; Margolis et
al., 1983; Williams et al., 1984).

PG12 and PGE2 are both implicated in break-
down of collagen (Ellwood, 1980) and vasodilata-
tion in several microcirculations (Bell et al., 1980).
Congestion of the vascular bed of the placentomes
was reported to be associated with placental
retention in cows (Dzuvic et al., 1976; Grunert,
1980). Sclerosis of the maternal placenta, which
is achieved through a progressive collagenization
of the caruncles, is thought to be an important
prerequisite for uncomplicated delivery of fetal
membranes (Grunert, 1980, 1984).

One of the roles we propose for the imbalance
in prostaglandin synthesis towards PG12 in RP
cows and for the pharmacological action of PGE2
in placental retention induction, is their ability to
induce collagen breakdown and consequently to
inhibit the collagenization process of the caruncles
near term.

However, it will be necessary to undertake
further studies to explain the linkage between the
imbalance found in this work concerning prosta-
glandin synthesis and the changes found by others
at the placentome level regarding placental
retention.

Immunological disturbance, which was recently
found in RP cows by Gunnink (1984 a, b, c, d),
and traduced by a lack of chemotaxis of leuko-
cytes, may be related with our results. Recently, it
was found that leukotrienes, lipoxygenase-
pathway metabolites of arachidonic acid, are the
main regulators of chemotaxis and chemokinesis
of the leukocytes (Samuelsson, 1981). On the
other hand, 5-hydroxyeicosatetraenoic acid
(HETE) and other products of the lipoxygenase
pathway, can selectively inhibit prostacyclin
synthetase activity as it was recently found in
bovine luteal cells (Aliia et al., 1983; Milvae and
Hansel, 1984). So, it is possible that the factors
regulating PG12 synthesis in NRP cows may be
the lipoxygenase products, and that these factors
do not act in RP cows as a consequence of reduc-
tion of the number of leukocytes, and/or a depres-
sed synthesis of leukotrienes in these cows.

Other mediating mechanisms leading to imba-
lanced prostaglandin synthesis are known to exist,
such as the 9 - ketoreductase, an enzyme propo-
sed by Williams and Gross (1981) to be respon-
sible for the conversion of PGF2 alpha into PGE2
in placentomes of bovine after calving.

The results presented here strongly suggest
that cows with placental retention synthesize
significantly less PGF2 alpha in the very early
postpartum, which seems to be primarily due to a
change in the metabolism of the prostaglandin
endoperoxides towards PG12 and probably PGE2
(earlier postulated by Horta, 1984), rather than
PGF2 alpha synthesis. They also suggest that in
cows delivering their placenta in due time, prosta-
cyclin synthetase activity seems to be maintained
at a basal level, compared to what happens in
cows with retention of placenta.

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animals.

Summary

The aim of this work was to investigate possible differences in peripheral blood concentrations of 13,14
dihydro 15 keto PGF alpha (PGFM) and 6 keto PGF1 alpha (PGIM), between dairy cows retaining (RP)
and not retaining (NRP) their placenta. Blood samples were collected 5, 30, and 60 minutes postpartum
from 8 RP and 17 NRP cows. PGFM concentrations were significantly lower in RP cows than in NRP
cows 60 minutes after birth (8156 pg/ml vs 12016 pg/ml ; \( P < 0.05 \)). There was a significant rise of
PGFM levels between 30 and 60 minutes in NRP cows (6225 pg/ml vs 12016 pg/ml ; \( P < 0.01 \)), while
in RP cows PGFM levels remained fairly constant along the period studied. No significant differences
were found between RP and NRP cows regarding PGIM levels during this period although absolute
values were slightly higher in RP cows. The PGFM/PGIM ratio was significantly higher 60 minutes post-partum in NRP cows than in RP cows (15.5 vs 8.8; P < 0.01). This ratio increased significantly in NRP cows between 30 and 60 minutes (7.2 vs 15.5; P < 0.01). The above results suggest that the synthesis of PGF2 alpha between 30 and 60 minutes postpartum is significantly decreased in cows with placental retention as reflected by the levels of PGFM. They further suggest that prostacyclin synthesis in cows not retaining their placenta does not increase during the first 60 minutes postpartum, as it does in cows with placental retention as reflected by the positive correlation between the levels of PGIM and PGFM found in these animals during the same period. It is proposed that an imbalance in the synthesis of PGF2 alpha and PG12 during the first 60 minutes conducting to a lack of PGF2 alpha and a relative increase in PG12 is associated with placental retention in bovine.

References


