

## CIRCULATING MONOCYTE AND RED CELL COUNTS AS PRECALVING PREDICTORS FOR RETAINED PLACENTA OCCURRENCE IN DAIRY COWS UNDER FIELD CONDITIONS IN FRANCE

Barnouin J.<sup>1</sup>, Chassagne M., Chacornac J.P.

*Une enquête écopathologique prospective centrée sur la pathologie du peripartum a été conduite en France dans des élevages privés volontaires avec la collaboration d'enquêteurs spécialisés. Des variables de production, de reproduction, de santé et des indicateurs biologiques circulants ont été utilisés afin de mettre en évidence des prédicteurs de l'occurrence de la rétention du placenta (RP). Les vaches sélectionnées étaient de race Holstein, avaient mis bas un seul veau après un mois de tarissement au moins et avaient eu un prélèvement de sang dans les 14 jours précédant le part. Deux groupes de vaches avec (RP+, n=45) et sans (RP-, n=184) RP ont été comparés. Des régressions logistiques multiples ont été mises en œuvre incluant l'élevage en tant qu'effet fixe. Finalement un nombre élevé de globules rouges et un nombre faible de monocytes circulants ont été mis en évidence en tant que variables prédictives de l'occurrence de RP chez la vache laitière. Par ailleurs, le risque de RP est apparu plus faible chez les femelles en 3<sup>ème</sup> lactation. Les résultats ont été notamment discutés au travers des relations entre l'équilibre métabolique en acides gras polyinsaturés, les fonctions du monocyte et du globule rouge et la motilité et la microcirculation utérines.*

### INTRODUCTION

In large scale surveys, individual blood indicators were rather rarely used as predictive markers in the late gestation period for disease occurrence after calving (Kweon et al., 1985). Concerning retained placenta (RP), overconditioned cows were more sensitive to RP and subsequent infertility than cows with normal body scores, but no blood predictors underlined such conditions (Badinand and Sensenbrenner, 1984). Some studies investigated the relationship between RP and blood markers (carotens, vitamin A, selenium, vitamin E, urea, gamma-glutamyl transferase) before calving (Inaba et al., 1986; Brzezinska-Slebodzinska et al., 1994; Lotthammer, 1983) or at calving (glucose and PGFM, main PgF2alpha metabolite) (Chassagne and Barnouin, 1992). The present work aims to display individual blood and other biological predictors for RP in late gestation Holstein cows under field conditions using a multiple logistic regression model.

### MATERIAL AND METHODS

An ecopathological survey was carried out by specialized surveyors in France to study herd risk factors and individual predictors for peripartum diseases in the dairy cow (Faye and Barnouin, 1987; Barnouin et al., 1995; Lescourret et al., 1993). RP was defined as the failure of placenta to be expelled within 24 h after calving. The study population of cows was standardized with respect to the following criteria : 1) Holstein breed ; 2) calving after a dry period of at least 30 days ; 3) calving of a single calf ; 4) calving followed by a lactation period of at least 30 days ; 5) a blood sample collected within the two last weeks before calving ; 6) a complete data set available. For any cow which would have been selected on two successive lactations a randomized process of elimination of one of the two lactations was performed to ensure a satisfactory independence between data. Cows from herds without any RP case were removed from the study to allow the statistical adjustment for the effect of herd. Finally two sets of cows were selected in 23 herds, including 184 females with a normal placental expulsion (RP- group) and 45 without (RP+ group). Statistical analysis were performed using SPlus 3.2 (Statistical Science, Inc., Seattle, USA). Individual cow was the study unit. Univariate tests were performed between RP+ and RP- groups. Then the effect of potential predictive variables on RP occurrence was analyzed using the multiple logistic regression. Criteria to enter the model based on likelihood ratio Chi<sup>2</sup> statistic were set for each variable at p=0.25 and p=0.05 to leave it, except for calving range, calving year, calving season and calf perinatal mortality which were entered in the final regression as key-variables (Bouyer, 1991) to increase biological validity of the model. As animals within a herd are more similar with regards to genetics, production, management and environment than animals in different herds, herd was forced into each model as a fixed effect.

### RESULTS

19.7% of the selected cows experienced RP. Average calving number was  $3.85 \pm 1.62$  in RP- cows vs.  $3.76 \pm 1.77$  in RP+ ones, average 305-d milk yield during the previous lactation was  $6927 \pm 1275$  kg vs.  $6771 \pm 1338$  kg and average gestation length  $279.3 \pm 4.7$  days vs.  $277.8 \pm 7.0$  days (variables not significant). Three variables differed significantly between groups from univariate analysis : glucose concentration ( $62.1 \pm 7.1$  mg/100ml in the RP- group vs.  $59.6 \pm 7.6$  mg/100ml in the RP+ one,  $p < 0.05$ ), circulating monocyte count ( $285.5 \pm 188.2$  per  $\text{mm}^3$  vs.  $196.6 \pm 138.1$  per  $\text{mm}^3$ ,  $p < 0.01$ ) and red blood cell count ( $6.07 \pm 0.77 \cdot 10^6$  per  $\text{mm}^3$  vs.  $6.40 \pm 0.78 \cdot 10^6$  per  $\text{mm}^3$ ,  $p < 0.05$ ). In the final logistic regression model adjusted for the effect of herd (Table I) antepartal high red cell ( $p = 0.02$ ) and low monocyte counts ( $p = 0.001$ ) were associated with RP occurrence while third calving ( $P = 0.04$ ) involved a lower RP risk.

<sup>1</sup> INRA, Unité de recherche d'Ecopathologie, Centre de Clermont Ferrand-Theix, 63122 Saint Genès-Champanelle (France)

**Table I**  
**Final logistic regression model for estimating RP occurrence in the cow.**

Variable	Coefficient	Odd ratio (OR)	OR95%CI
<b>Parity</b>			
2	-	1.0	-
3	- 1.344	0.3	0.07-0.9
4	0.076	1.1	0.3-3.5
5	- 1.470	0.2	0.04-1.2
>5	- 0.258	0.8	0.2-2.5
<b>Calving year</b>			
1	-	1.0	-
2	0.040	1.0	0.3-3.4
3	0.674	2.1	0.6-8.0
<b>Calving season</b>			
Winter	-	1.0	-
Spring	0.846	2.3	0.8-7.1
Summer	- 0.268	0.8	0.2-3.3
Autumn	0.719	2.1	0.6-7.5
<b>Calf at birth</b>			
Alive	-	1.0	-
Dead	1.107	3.0	0.4-24.6
<b>Red blood cells (10<sup>6</sup>/mm<sup>3</sup>)</b>			
<6	-	1.0	-
6-7	- 0.182	0.8	0.3-2.5
>7	1.970	7.2	1.4-36.1
<b>Blood monocytes (/mm<sup>3</sup>)</b>			
<180	-	1.0	-
180-280	- 0.813	0.4	0.2-1.3
>280	- 2.616	0.07	0.02-0.3**

\*p<0.05 ; \*\*p<0.01 ; effect of herd was adjusted for in all analyses, but the results are not shown.

## DISCUSSION

In the study population, RP incidence (19.7%) is higher than in EEPB general survey (10.1%) (Chassagne et al., 1996) due to exclusion of first lactating animals (8.2% RP in EEPB) and cows from herds without any RP case. The lower risk at third calving is in accord with a study in which twins and herd effects were controlled (Markusfeld, 1986) and RP risk increased with parity except for tripara. Relationships between placenta hormones and milk secretion (Delouis, 1984) maximum in lactation 3 (Keown et al., 1986) could involve a lower RP risk in tripara via progesterone (Chew et al., 1977).

Monocyte and red cell counts could be connected with RP via a high dietary n-3/n-6 polyunsaturated fatty acid (PUFA) ratio (Barnouin and Chassagne, 1991 ; Chassagne and Barnouin, 1992). An unbalanced ratio would involve reductions in synthesis of cyclooxygenase products (particularly PgF<sub>2</sub>alpha) , impair uterine contractions, vascular tone and platelet aggregation and lead to RP (Oliw et al., 1983). Lipid composition of the red cell membrane is a good indicator of dietary intake of PUFAs (Brown and Roberts, 1992). An increased membrane content of n-3 PUFAs causes changes in its viscoelasticity, fluidity (Hayam et al., 1993 ; Bellary et al., 1994), aggregation behaviour (Hessel et al., 1990), lysis and haemolytic process (Van den Berg et al., 1991) mainly stress oxidative-dependent (Caprari et al., 1995). A high red cell count before calving would be an indicator of high intakes of n-3 PUFAs and increase the RP risk via low PgF<sub>2</sub>alpha at calving in subsequent RP cows. A decrease in blood monocyte counts of cows with a subsequent RP was observed from 10 days before calving to parturition, while it increased in control females (Cai et al., 1994). A reduced leucocytic activity against the chemotactic stimulus of cotyledons was observed in the last week before calving in RP cows and RP occurred in 100 per cent of cases in the absence of leucocytic activity against the cotyledons (Gunnink, 1984). Few other studies concern the bovine mononuclear cell, but monocytes could play a key role in hemolytic reactions (Davenport et al., 1994) and production of prostaglandins and thromboxanes (Pastoret et al., 1990). A diet rich in n-3 PUFAs decreases the release of arachidonic acid from monocytes and monocyte inflammatory activity (Fisher et al., 1990). Fatty acid balance plays a central role in the activation (Osterud and Hansen, 1989) and proliferation of monocytes (Molvig et al., 1991), which are major cellular components in the uterus and placenta throughout pregnancy (Takahashi et al., 1991).

Finally a decreased circulating number of monocytes could be related to an impairment of monocyte functions via PUFAs balance and explain high erythrocyte counts in RP females. But such an hypothesis is partially derived from studies concerning the human and the rat. Consequently further experimental studies should be conducted specifically in the dairy cow to precise PUFAs metabolic conditions leading to abnormal calving and subsequent RP occurrence.

## ACKNOWLEDGEMENTS

We sincerely thank all the farmers, technicians, practitioners, researchers and colleagues who collaborated with proficiency and friendship to the present work.

## REFERENCES

- Badinand F., Sensenbrenner G., 1984. Non-délivrance chez la vache: données nouvelles à propos d'une enquête épidémiologique. *Point Vét*, 16, 483-496.
- Barnouin J., Chassagne M., 1991. An etiopathological hypothesis for the nutrition-induced association between retained placenta and milk fever in the dairy cow. *Ann Rech Vét*, 22, 331-343.
- Barnouin J., Chassagne M., Aimo I., 1995. Dietary factors associated with milk somatic cell counts in dairy cows in Brittany, France. *Prev Vet Med*, 21, 299-311.
- Bellary S.S., Anderson K.W., Arden W.A., Butterfield D.A. 1994. Effect of lipopolysaccharide on the physical conformation of the erythrocyte cytoskeletal proteins. *Life Sci*, 56, 91-98.
- Bouyer J., 1991. La régression logistique en épidémiologie. Partie II. *Rev Epidém et Santé Publ*, 39, 183-196.
- Brown A.J., Roberts D.C.K., 1992. Red blood cell fatty acids as markers for dietary n-3 PUFA intake. *Proc Nut Soc Austr*, 14, 87-89.
- Brzezinska-Slebodzinska E., Miller J.K., Quigley J.D., Moore J.R., Madsen F.C., 1994. Antioxidant status of dairy cows supplemented prepartum with vitamin E and selenium. *J Dairy Sci*, 77, 3087-3095.
- Cai T.C., Weston P.G., Lund L.A., Brodie B., Mac Kenna D.J., Wagner W.C., 1994. Association between neutrophil function and periparturient disorders in cows. *Am J Vet Res*, 55, 934-943.
- Caprari P., Bozzi A., Malorni W., Iosi F., Santini M.T., Salvati A.M., 1995. Junctional sites of erythrocyte skeletal proteins are specific targets of ter-butylhydroperoxide damage. *Chem Biol Interact*, 94, 243-258.
- Chassagne M., Barnouin J., 1992. Circulating PgF<sub>2</sub>α and nutritional parameters at parturition in dairy cows with and without retained placenta: relation to prepartum diet. *Theriogenology*, 38, 407-418.
- Chassagne M., Barnouin J., Faye B., 1996. Epidémiologie descriptive de la rétention placentaire en système intensif laitier en Bretagne. *Vet Res*, 27, 491-502.
- Chew B.P., Keller H.F., Erb R.E., Lalven P.V., 1977. Periparturient concentrations of prolactin, progesterone and the estrogens in blood plasma of cows retaining and not retaining fetal membranes. *J Anim Sci*, 120, 1055-1060.
- Davenport R.D., Burdick M., Strieter R.M., Kunkel, S.L., 1994. Monocyte chemoattractant protein production in red cell incompatibility. *Transfusion*, 34, 16-19.
- Delouis C., 1984. La mamelle ; croissance mammaire et montée laiteuse en relation avec le développement foeto-placentaire. In : *Physiologie et pathologie périnatales*. Jarrige ed, INRA Paris, pp 49-60.
- Faye B., Barnouin J., 1987. Motivations et objectifs de l'enquête éco-pathologique Bretagne. *Bull. Tech. CRZV Theix*, INRA, 59, 61-67.
- Fisher M., Levine P.H., Weiner B.H., Johnson M.H., Doyle E.M., Ellis P.A., Hoogasian J.J., 1990. Dietary n-3 fatty acid supplementation reduces superoxide production and chemiluminescence in a monocyte-enriched preparation of leukocytes. *Am J Clin Nutr*, 51, 804-808.
- Gunnink J.W., 1984. Prepartum leukocytic activity and retained placenta. *Vet Quat*, 61, 512-514.
- Hayam I., Cogan U., Mokady S., 1993. Dietary oxidized oil enhances the activity of (NA+K+) ATPase and acetylcholinesterase and lowers the fluidity of rat erythrocyte membrane. *J Nutr Biochem*, 4, 563-568.
- Hessel E., Agren J.J., Paulitschke M., Hanninen O., Hanninen A., Lerche D., 1990. Freshwater fish diet affects lipid composition, deformability and aggregation properties of erythrocytes. *Atherosclerosis*, 82, 37-42.
- Inaba T., Inoue A., Shimizu R., Nakano Y., Nori J., 1986. Plasma concentrations of progesterone, estrogens, vitamin A and β-carotene in cows retaining fetal membranes. *Jap J Vet Sci*, 48, 505-508.
- Keown J.F., Everett R.W., Empet N.B., Wadell L.H., 1986. Lactation curves. *J Dairy Sci*, 69, 769-781.
- Kweon K., Ono H., Seta T., Onda M., Oboshi K., Kanagawa H., 1985. Relationship between serum total cholesterol levels before calving and occurrence of diseases after calving in Holstein heifers and cows. *Jpn J Vet Res*, 33, 11-17.
- Lescouret F., Genest M., Barnouin J., Chassagne M., Faye B., 1993. Data modeling for database design in production and health monitoring systems for dairy herds. *J Dairy Sci*, 76, 1053-1062.
- Lotthammer K.H., 1983. Comparative investigations on the course of the levels of minerals, metabolites enzymes and hormones in the blood serum antepartum in dairy cows with and without later retained placenta. *Deutsche Tierarz Wochen*, 90, 427-433.
- Markusfeld O., 1986. Periparturient traits in seven high yielding dairy herds. Incidence rates, association with parity and interrelationships among traits. *J Dairy Sci*, 70, 158-166.
- Molvig G., Pociot F., Worsaae H., Wogensen L.D., Baek L., Christensen P., Mandrup-Poulsen T., Andersen K., Madsen P., Dyerberg J., Nerup J., 1991. Dietary supplementation with omega-3 polyunsaturated fatty acids decreases mononuclear cell proliferation and interleukin-1β content but not monokine secretion in healthy and insulin-dependent diabetic individuals. *Scand J Immunol*, 34, 399-410.
- Oliw E., Granström E., Angard E., 1983. In: Pace-Asciak, C. (ed), *Prostaglandins and related substances*. Elsevier, Amsterdam, 1-44.
- Osterud B., Hansen J.B., 1989. Fatty acids, platelets and monocytes. Something to do with atherogenesis. *Ann Med*, 21, 47-51.
- Pastoret J.P., Govaerts A., Bazin H., 1990. In: *Immunologie animale*, Flammarion, Paris, 35-49.
- Takahashi K., Naito M., Katabuchi H., Higashi K., 1991. Development, differentiation, and maturation of macrophages in the chorionic villi of mouse placenta with special reference to the origin of Hofbauer cells. *J Leukoc Biol*, 50, 57-68.
- Van Den Berg J.J.M., Fouw N.J., Kuypers F.A., Roelofsen B., Houtsmuller U.M.T., Op Den Kamp J.A.F., 1991. Increased n-3 polyunsaturated fatty acid content of red blood cells from fish oil-fed rabbits increases in vitro lipid peroxidation, but decreases hemolysis. *Free Rad Biol Med*, 11, 393-399.