RELATIONSHIP BETWEEN ACUTE PHASE PROTEINS AND SUBSEQUENT FERTILITY OF DAIRY COWS AFTER POSTPARTUM UTERINE INFLAMMATION AND HEALTHY COWS

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Abstract. The aim of this study was to evaluate the potential relationship between concentrations of serum amyloid A (SAA), haptoglobin (Hp), fibrinogen (Fb), and fertility parameters of treated and positive control animals with acute puerperal metritis (APM), clinical metritis (CM) and healthy cows.

Animals with CM and CE were assigned to treatment and control groups. A negative control group was composed of healthy cows. Measurements of acute phase proteins (APP) were done once a week for seven weeks postpartum (PP). Fertility data were recorded.

The first insemination after 90 days PP was correlated with increased APP levels during the first week PP. The failure of insemination until the 150th day PP and a lower first service conception rate were associated with an increase in APP concentrations at the end of the experimental period. Existence of APM or CM and treatment groups had no effect on the association between APP and fertility parameters.

The results indicate that the acute phase reaction reflected in concentrations of APP in plasma during the PP period could be related to the fertility of dairy cows with APM, CM and healthy cows.

Keywords: dairy cow, fertility, fibrinogen, haptoglobin, serum amyloid A

Introduction. Acute phase proteins (APP) are a group of blood proteins which assist in decreasing pathogen growth in the case of an acute phase response activated by trauma, stress, inflammation, parturition, neoplastic growth, surgery, or immunological disorders. They can be useful in monitoring the course of some postpartum (PP) reproductive diseases, such as endometritis and metritis (Hirvonen et al., 1999; Sheldon et al., 2001; Chan et al., 2004; Humblet et al., 2006; Tóthová et al., 2008; Huzzey et al., 2009; Galvão et al., 2010; Schneider et al., 2013). Uterine inflammations can result in poor fertility (Hirvonen et al., 1999; LeBlanc et al., 2008). Increased concentrations of an inflammatory marker interleukin-6 before parturition were found to be associated with the occurrence of endometritis in the PP period (Ishikawa et al., 2004). APP can also be used as inflammatory markers to help predict fertility in animals.

Some studies have shown that increased concentrations of Hp and SAA during acute puerperal metritis (APM) were associated with reduced fertility (Hirvonen et al., 1999; Chan et al., 2010). APP could serve as prognostic markers in cases of acute uterine infection and inflammation. However, APP have not been studied as prognostic markers of reproductive performance in dairy cows with less severe PP uterine inflammation and in animals treated using different schemes and healthy cows.

The aim of this study was to evaluate the potential relationship between serum haptoglobin (Hp), serum amyloid A (SAA), and fibrinogen (Fb) as inflammatory mediators, and the subsequent fertility in dairy cows suffering from APM, clinical metritis (CM), or clinical endometritis (CE), treated using different methods, in comparison with both untreated diseased and healthy control cows. The first hypothesis of the study was that one or several APP levels measured during the first seven weeks after parturition could be related to the reproductive performance of animals with postpartum (PP) uterine inflammation and/or in healthy animals. The second hypothesis was that different treatments could affect acute phase reaction reflected in concentrations of APP that, in turn, could affect the possible association between APP concentrations and fertility.

Materials and methods

Farms and animals

The study was performed from September 2007 to February 2008 (farm 1) and from February 2009 to August 2009 (farm 2) on two loose-housing commercial dairy farms both with 600 cows. All experimental cows were fed the same diet in the form of a total mixed ration (TMR) consisting of grass silage and a concentrate mix. The cows were milked three times a day in a 2×20 parallel milking parlour.

Multiparous late pregnant Estonian Holstein-Friesian cows (n = 98) with the mean milk production of 10.617 kg/ECM during the previous lactation, were included in the study. All experimental cows were expected to calve within two months from the start of the experimental period on the farm.

Diagnosis and experimental groups

Diagnosis of both APM and CM was made on the 3–5

PP using the definitions by Sheldon et al. (2006). Clinical metritis was defined if an atonic uterus with a purulent or mucopurulent vaginal discharge was found, without changes in general health condition.

A diagnosis of APM was confirmed when increased body temperature (≥39.5 °C), anorexia, an enlarged and atonic uterus and odoriferous vaginal discharge with the presence of pus were found. Animals with inflammation of the uterus (n = 76) were randomly matched by fibrinogen concentration into three treatment and positive control groups. A negative control groups (n = 22) were composed of healthy cows.

The treatment of animals began on the third–fifth day PP. Group A (n = 13) was treated by intramuscular injection of 1 mg/kg Ceftiofur (Exenell RTU®, Pharmacia Animal Health) for five days in combination with injections of 1.1 mg/kg Flunixin (Finadyne® vet., Schering-Plough Animal Health) for three days. Animals from group B (n = 22) were treated by intramuscular injection of 1 mg/kg Ceftiofur (Exenell RTU®, Pharmacia Animal Health) for five days followed by two injections of 25 mg PGF2α – dinoprost (Dinolytic®, Pfizer Animal Health) with an interval of 8 h on the eighth day PP. Animals from group C (n = 25) were re-examined on day 28 PP and (if clinical examination showed existence of CE) treated (group C1, n = 16) using the same treatment scheme as group B starting on the 30th day PP. Group D (n = 16) served as the positive control group without any treatment. Negative control group E (n = 22) composed of healthy cows.

Blood samples

Blood samples for APP (SAA and Hp) analyses were taken from coccygeal vein using heparinized Venoject® glass tubes (Terumo Europe N. V. Leuven, Belgium). Collection was started nine days before calving term, being performed once before the term and 5 to 7 days PP, and subsequently once a week for six weeks. Thus, the first blood sampling for the measurement of SAA and Hp of some animals was performed on the first day of the second week postpartum.

After immediate centrifugation, about 5 ml of plasma was removed and stored at –18 °C until the analyses were performed. Whole blood for Fb analysis was taken once a week starting from day 3 PP over the entire experimental period (seven weeks PP).

Methods of analysis of APP

The Fb concentration in plasma was measured by the heat precipitation method (Millar et al., 1971). Plasma Hp was determined using the haemoglobin binding assay described by Makimura and Suzuki (1982), with the modification of tetramethylbenzidine (0.06 mg/ml) used as a chromogen (Alsemgeest et al., 1994). Pooled and lyophilized aliquots of bovine acute phase serum were used to create standard curves. To calibrate the assay, a bovine plasma sample with a known Hp concentration, provided by the European Commission Concerted Action Project (QLK5-CT-1999-0153), was used. The range of the standard curve was 40–1160 mg/l. If the Hp concentration of a sample was higher, the sample was diluted with isotonic saline and re-assayed. The intra- and inter-assay coefficients of variation were <12% and <11 %, respectively. SAA concentrations in plasma were measured using a commercially available ELISA kit (Phase SAA kit, Tridelta Development Ltd.) according to the manufacturer's instructions for bovines. The detection limit of the assay for bovine samples was 0.3 mg/l. The intra- and inter-assay coefficients of variation were <7 % and <14 %, respectively.

Fertility analysis

To evaluate fertility performance, the date of the first insemination up to 90 days PP (days to first service 90, DFS 90), the first service conception rate (FSCR) and the date of successful insemination up to 150 days after calving (DO 150) were recorded for all cows.

Statistical analyses

Logistic regression models for DFS 90, FSCR and DO 150 were used to explore associations between PP APP levels and the listed fertility parameters. For the analysis, APP concentrations of each week PP were divided into 2-level categorical variable (high and low concentrations) using median values of PP week's samples and the associations between high APP concentrations and the fertility parameters by PP week were analysed. Treatment group and farm were included as the fixed factors in the models. Diagnosis (APM or CM) was not included in the final models for the reason of being non-significant. Interaction between treatment groups and APPs and diagnosis and APPs were checked. No significant interactions were found in any models. The fit of the model was evaluated with Hosmer-Lemeshow goodness-of-fit test.

The STATA 10.1 (StataCorp, Texas, USA) software was used for statistical analyses.

Results

Effects of treatment success on clinical cure, inflammatory response and fertility parameters are described by Jeremejeva et al. (2012).

Some of the animals, culled before the end of the sampling period due to the health problems not associated with uterus, were excluded from the analysis.

Ten cows showed signs of APM and 66 cows showed sings of CM. The raw data on APP concentrations and fertility parameters are presented in Table 1 and Fig. 1, respectively. Data of APP medians, their lower and upper quartile are presented in Fig. 2.

Cows with higher Hp levels (concentrations above the median) in the first week PP (≥331 mg/l) had a lower probability of the first insemination during the first 90 days PP (P = 0.034, OR = 0.23, CI 95 % = 0.06; 0.90) (Table 2).

Cows with higher Fb levels (above the median) in the seventh week PP (≥5.8 g/l) had a lower probability of conception after the first service than those with lower Fb concentrations (P = 0.034, OR = 0.39, CI 95 % = 0.16; 0.93) (Table 2).

Table 1. **Median** (lower quartile/upper quartile) **concentration of fibrinogen** (Fb), **serum amyloid A** (SAA) **and haptoglobin** (Hp) **in plasma of experimental cows at postpartum** (PP) **weeks**

Acute phase proteins	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7
Animals with acute puerperal metritis (n = 10)							
Fb (g/l)	8.4 (6.1/9.6)	5.9 (4.9/7.7)	4.7 (4.0/7.1)	5.9 (4.4/6.6)	6.1 (5.4/78.1)	5.1 (3.9/6.0)	5.8 (4.3/5.8)
SAA (mg/l)	99.9 (61.6/145.1)	12.6 (10.8/72.1)	15.5 (9.1/59.5)	12.9 (4.6/91.4)	40.2 (17.7/99.6)	43.9 (19.3/110.4)	16.2 (12.0/21.2)
Hp (mg/l)	762 (693/1175)	98 (47/280)	84 (35/119)	77 (49/526)	65 (51/249)	57 (38/169)	52 (37/78)
Animals with clinical metritis (n = 66)							
Fb (g/l)	6.7 (5.1/8.4)	6.0 (4.3/7.7)	6.0 (4.6/7.6)	5.9 (4.4/7.4)	5.8 (4.2/7.1)	5.9 (4.2/7.4)	5.8 (4.2/6.5)
SAA (mg/l)	53.7 (20.9/94.4)	23.4 (10.4/55.3)	15.5 (5.6/54.1)	24.2 (9./55.0)	35.8 (8.1/105.0)	28.4 (8.1/86.4)	16.6 (8.6/65.8
Hp (mg/l)	320 (195/830)	185 (134/512)	160 (100/233)	128 (81/197)	127 (56/191)	154 (85/229)	128 (96/178)
Healthy animals (n = 22)							
Fb (g/l)	5.8 (4.9/6.9)	5.6 (4.5/8.3)	6.3 (5.4/8.0)	5.9 (4.3/7.2)	4.9 (4.2/6.1)	4.8 (4.1/5.9)	5.2 (4.3/5.3)
SAA (mg/l)	41.6 (22.3/98.4)	42.8 (17.6/70.8)	12.3 (7.2/63.5)	16.9 (9.1/78.8)	9.0 (5.8/24.6)	11.5 (7.9/19.3)	7.8 (5.9/33.3
Hp (mg/l)	154 (120/231)	170 (71/336)	140 (40/173)	128 (48/175)	78 (35/136)	79 (36/128)	77 (50/134)

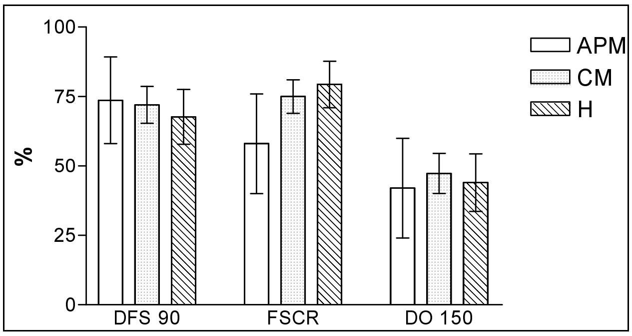
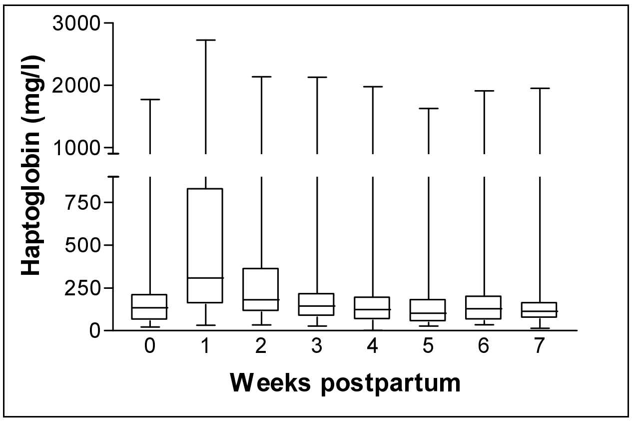
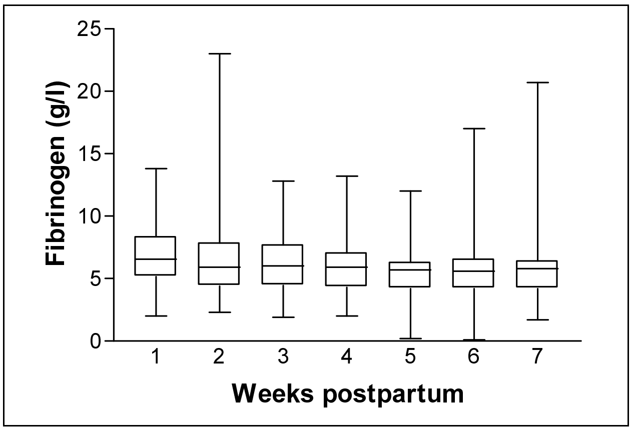


Fig. 1. **Mean fertility parameters** (± 95 % CI): **days to first service 90** (DFS 90 - first insemination up to 90 days), **the first service conception rate** (FSCR) **and successful insemination up to 150 days after calving** (DO 150) **of experimental cows with acute puerperal metritis** (APM), **clinical metritis** (CM) **and healthy animals** (H)**.**





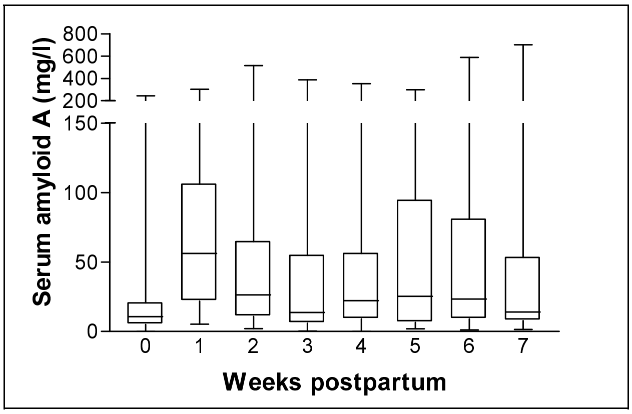


Fig. 2. **Median** (lower quartile/upper quartile and minimum/maximum) **concentration of serum amyloid A, haptoglobin and fibrinogen in experimental animals**

Table 2. **Associations between fertility parameters: first insemination up to 90 days postpartum** (PP; DFS 90), **the first service conception rate** (FSCR), **the date of successful insemination up to 150 days after calving** (DO 150) **and acute phase proteins** (serum amyloid A; SAA, haptoglobin; Hp and fibrinogen; Fb) **by PP weeks.**

Fertility parameter	APP (unit)	PP week	APP median (*n* of animals over and under the median)	Number of animals (*n* of animals in fertility parameter groups)	OR (P value)	CL 95% of OR
DFS 90 yes/no	Hp (mg/l)	1	331 (30; 30)	60 (42; 18)	0.23 (0.034)	0.06; 0.90
	Fb (g/l)	1	6.7 (49; 49)	98 (71; 27)	0.40 (0.065)	0.15; 1.06
FSCR preg./not preg.	Fb (g/l)	7	5.8 (48; 47)	95 (44; 51)	0.39 (0.034)	0.16; 0.93
DO 150 preg./not preg.	Hp (mg/l)	6	128 (48; 48)	96 (73; 23)	0.25 (0.030)	0.07; 0.87
	SAA (mg/l)	6	23.6 (48; 48)	96 (73; 23)	0.24 (0.016)	0.08; 0.77

Animals with higher concentrations (levels over the median) of SAA (≥23.6 mg/l) and Hp (≥128 mg/l) in the sixth week PP had a smaller likelihood of successful insemination up to 150 days after calving (P = 0.016, OR = 0.24, CI 95 % = 0.08; 0.76 for association between SAA and DO 150, and P = 0.030, OR = 0.26, CI 95 % = 0.07; 0.87 for association between Hp and DO 150) (Table 2).

The experimental groups, farm and diagnosis (APM, CM or healthy animals) showed no significant effect to the fertility parameters and no interactions effect between experimental groups and APP or diagnosis and APP were found.

Discussion and conclusions

Increased concentrations of APP are usually caused by inflammation processes. In this study only the animals with uterine inflammation and clinically healthy cows were used, so it is likely that enhanced concentrations of APP were associated with APM or CM. Sheldonet al. (Sheldon et al., 2006) have shown that uterine inflammation affects subsequent fertility. Therefore, increased concentrations of APP could be associated with the subsequent fertility of dairy cows.

In the present study, a relationship between APP levels and fertility parameters was found in both animals with uterine inflammation and clinically healthy cows. According to Chan and others (2010), in most healthy cows APP 'concentrations are typically not elevated and are associated with better fertility parameters. Increased concentrations of Hp can be detected not only in the clinically affected, but also in the subclinically affected dairy cows (Bertoni et al., 2008; Chan et al., 2010). Gilbert et al. (2005) have demonstrated the effects of subclinical uterine inflammations on fertility parameters; therefore it is proposed that some single increased APP values, which were associated with poorer fertility, could be explained by subclinical endometritis.

APP levels increase in all cows after parturition. The increase is associated with parturition, whereas it is more significant in diseased animals (Hirvonen et al., 1999). In the present study the higher levels of Hp during the first week after parturition were associated with delayed first insemination. All cows included in the study were inseminated during the first detected oestrus after the voluntary waiting period. So, all delayed first inseminations could be related to a prolonged anovulatory period due to a possible negative energy balance, or missed heats, which might be caused by silent heats. Galvão et al. (2010) detected greater NEFA concentrations at calving and at day 35 PP in cows with clinical and subclinical uterine inflammation. The negative energy balance could affect normal oestrous behaviour and recovery of ovarian activity. In the present study it seems that if these events that are related to calving take place earlier, they are more likely to be associated with increased APP levels in the first week after parturition.

In the period from the second to the fifth week PP, levels of APP remained elevated in most animals. The process of normalisation of uterine condition in most of the diseased animals had been finished by the sixth week PP (Hirvonen et al., 1999). However, in some animals an inflammation process could develop from the acute form to the chronic or subclinical form. Concentrations of APP in these animals persist at a high level and, related to possible uterine inflammation, are associated with poorer DO 150 and FSCR. It seems that these fertility parameters, which depend not only on resumption of ovarian activity but also on oocyte viability and uterine condition, were associated with increased APP concentrations at the end of the experimental period (weeks six and seven PP).

Hirvonen et al. (1999) and Chan et al. (2010) showed the relationship between APP levels and fertility in cases of severe puerperal uterine inflammation. The present study indicated this association in animals with APM, as well as in animals with CM and clinically healthy cows. The treatment groups had no effect on the relationship between SAA, Hp, and Fb and fertility parameters. Consequently, increased APP levels in animals with a more severe inflammation in the early PP period and treatments that appeared to be less effective by the end of the experimental period, were associated with poorer fertility, while lower APP concentrations in healthier animals were associated with better fertility.

In conclusion, the findings of this study indicate that plasma Hp, SAA, and Fb, measured during the PP period, may be associated with subsequent fertility in treated and untreated animals with APM and CM, and healthy dairy cows.

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References

1. Alsemgeest S. P., Kalsbeek H. C., Wensing T., Koeman J. P., Van Ederen A. M., Gruys E. Concentrations of serum amyloid-A (SAA) and haptoglobin (HP) as parameters of inflammatory diseases in cattle. The Veterinary quarterly. 1994. 16. P. 21–23.

2. Bertoni G., Trevisi E., Han X., Bionaz M. Effect of inflammatory conditions on liver activity in puerperium period and consequences for performance in dairy cows.

3. Journal of Dairy Science. 2008. 91. P. 3300–3310.

4. Chan J. P., Chang C. C., Hsu W. L., Liu W. B., Chen T. H. Association of increased serum acute-phase protein concentrations with reproductive performance in dairy cows with postpartum metritis. Veterinary Clinical Pathology. 2010. 39. P. 72–78.

5. Chan J. P. W., Chu C. C., Fung H. P., Chuang S. T., Lin Y. C., Chu R. M., Lee S. L. Serum haptoglobin concentration in cattle. Journal of Veterinary Medical Science. 2004. 66. P. 43–46.

6. Galväo K. N., Flaminio M. J. B. F., Brittin S. B., Sper R., Fraga M., Caixeta L., Ricci A., Guard C. L., Butler W. R., Gilbert R. O. Association between uterine disease and indicators of neutrophil and systemic energy status in lactating Holstein cows. Journal of Dairy Science. 2010. 93. P. 2926–2937.

7. Gilbert R. O., Shin S. T., Guard C. L., Erb H. N., Frajblat M. Prevalence of endometritis and its effects on reproductive performance of dairy cows. Theriogenology. 2005. 64. P. 1879–1888.

8. Hirvonen J., Huszenicza G., Kulcsár M., Pyörälä S. Acute-phase response in dairy cows with acute postpartum metritis. Theriogenology. 1999. 51 P. 1071–1083.

9. Humblet M. F., Guyot H., Boudry B., Mbayahi F., Hanzen C., Rollin F., Godeau J. M. Relationship between haptoglobin, serum amyloid A, and clinical status in a survey of dairy herds during a 6-month period. Veterinary Clinical Pathology. 2006. 35. P. 188–193.

10. Huzzey J. M., Duffield T. F., LeBlanc S. J., Veira D. M., Weary D. M., von Keyserlingk M. A. G. Short communication: Haptoglobin as an early indicator of metritis.

11. Journal of Dairy Science. 2009. 92. P. 621–625.

12. Ishikawa Y., Nakada K., Hagiwara K., Kirisawa R., Iwai H., Morioshi M., Sawamukai Y. Changes in interleukin-6 concentration in peripheral blood of pre- and post-partum dairy cattle and its relationship to postpartum reproductive diseases. Journal of Veterinary Medical Science. 2004. 66. P. 1403–1408.

13. Jeremejeva J., Orro T., Waldmann A., Kask K. Treatment of dairy cows with PGF2α or NSAID, in combination with antibiotics, in cases of postpartum uterine inflammation. Acta Veterinaria Scandinavica. 2012. 54. P. 45.

14. LeBlanc S. J. Postpartum uterine disease and dairy herd reproductive performance: A review. The Veterinary Journal. 2008. 176. P. 102–114.

15. Makimura S., Suzuki N. Quantitative determination of bovine serum haptoglobin and its elevation in some inflammatory diseases. The Japanese Journal of Veterinary Science. 1982. 44. P. 15–21.

16. Millar H. R., Simpson J. G., Stalker A. L. An evaluation of the heat precipitation method for plasma fibrinogen estimation. Journal of Clinical Pathology. 1971. 24. P. 827–830.

17. Sheldon I. M., Lewis G. S., LeBlanc S., Gilbert R. O. Defining postpartum uterine disease in cattle. Theriogenology. 2006. 65. P. 1516–1530.

18. Sheldon I. M., Noakes D. E., Rycroft A., Dobson H. Acute phase protein responses to uterine bacterial contamination in cattle after calving. Veterinary Research. 2001. 148. P. 172–175.

19. Schneider A., Corrêa M. N., Butler W. R. Short communication: Acute phase proteins in Holstein cows diagnosed with uterine infection. Research in Veterinary Science. 2013. 95(1). P. 269–271.

20. Tóthová C. S., Nagi O., Seidel H., Konvičná J., Farkašova Z., Kováč G. Acute phase proteins and variables of protein metabolism in dairy cows during the pre- and postpartal period. Acta Veterinaria Brno. 2008. 77. P. 51–57.

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