

Blood Serum Pepsinogen and Progesterone Concentrations During Pregnancy and Lactation in Sows

^{1,6}H. Banga-Mboko, ²W. Holtz, ³D. Maes, ²B. Sohnrey, ¹B. El Amiri,

⁴I. Youssao and ⁴P.T Sangild, ¹J.F. Beckers

¹Department of Physiology of Reproduction**, Faculty of Veterinary Medicine, University of Liege, Bd de Colonster n°20 B41, 4000, Sart Tilman Belgium; ²Institute of Animal Husbandry and Genetics, Albrecht - Thaier - Weg 3, D-3400 Gottingen, Germany; ³Department of Reproduction, Obstetrics and Herd Health, Faculty of Veterinary Medicine, Ghent University, Salisburylaan 133, 9820 Merelbeke, Belgium; ⁴Department of Biostatistics, Faculty of Veterinary Medicine, University of Liege, Bd de Colonster n°20 B 43, 4000, Sart Tilman Belgium; ⁵Division of Nutrition, Department of Animal Science and Health, Royal Veterinary and Agricultural University, 3 Grønnegårdsvej, DK-1870, Frederiksberg C, Denmark; ⁶University Marien Ngoubai BP 69, Brazzaville, Congo-Brazzaville

Abstract: The present study investigated a relationship between the gastro-intestinal system and pregnancy in sows. Blood samples were collected from three groups of sows: (1) pregnant sows (n=10) at day 21, 30, 40, 50, 60, 70, 80, 90, 100 after insemination, (2) lactating sows (n=10) at 10 and 20 days after parturition, and (3) non-pregnant and non-lactating sows as a control group; n=10. Serum pepsinogen was quantified using a specific RIA, while serum progesterone concentrations were determined using a direct solid phase ¹²⁵I RIA method. In the first experiment, the progesterone and pepsinogen concentrations were measured in the 3 groups. In individual sows, pepsinogen concentration decreased significantly during pregnancy and showed a tendency to increase during lactation but this difference was not statistically significant. There was a significant negative correlation between progesterone and pepsinogen concentrations ($r = -0.7$; $P = 0.002$). The pepsinogen concentrations (Mean \pm S.E) was significantly lower in pregnant sows than in non-pregnant (327.3 ± 11.8 ng/mL versus 449 ± 35.4 ng/mL; $P = 0.0016$) and in lactating sows (327.3 ± 11.8 ng/mL versus 397.2 ± 25.06 ng/mL; $P = 0.0135$). No significant difference in pepsinogen concentrations was observed between lactating and non-pregnant sows (449 ± 35.4 ng/mL versus 397 ± 25.06 ng/mL; $P = 0.2371$). In the second experiment, non-lactating sows were subjected to a dietary administration of Altrenogest, a synthetic progesterone like hormone in order to study the effect on pepsinogen concentration. The treatment did not influence pepsinogen concentrations ($P = 0.6452$). Taken together the two experiments, it seems unlikely that progesterone alone is involved in pepsinogen concentration changes observed in pregnant sows. However, the negative correlation observed between progesterone and pepsinogen during the reproducible cycle suggests that the endocrine system might play an important role.

Key words: Pepsinogen, progesterone, pregnancy, lactation and sows

Introduction

Pepsinogens, structurally related to aspartic proteinases, are pro enzymes of pepsin mainly involved in the digestion of proteins in the stomach. They are also detected in small amount in blood circulation in healthy subjects. The blood pepsinogen level is considered to reflect the morphologic and functional status of the gastric mucosa and, more especially, the chief cells that are the major source of pepsinogens (Heim *et al.*, 1997).

In swine, the ontogeny of blood pepsinogen concentrations indicated that pepsinogen concentrations increased with the age of the foetus or the pig (until 213 days of age). Before birth until 21 days of age pepsinogen concentrations increased rapidly (10ng/mL per day) whereas from day 21 till day 213 pepsinogen concentrations were characterized by a slow increase (0.4ng/mL per day) (Banga-

Mboko *et al.*, in press). Likewise, pepsinogen has been shown to increase during parasitic infection with *Hyostrogylus rubidus* in sows and young pigs (Enight *et al.*, 1972).

In 1980, Waldum *et al.*, investigated the serum pepsinogen in pregnant women in order to solve the question of many complaints during gestation. They concluded that serum pepsinogen, which previously has been found to be positively correlated with gastric acid secretion, was statistically similar in pregnant and non-pregnant women. Other investigations were carried out especially on pepsin activity in the gastric juice during pregnancy and lactation, but the results are not consistent (Takeuchi *et al.*, 1976; Jolicoeur *et al.*, 1981; Pelletier *et al.*, 1983).

On the another hand, many authors have reported the presence of receptors for progesterone in normal gastric mucus and

gastric cancer patients (Wu *et al.*, 1998; Kuru *et al.*, 2002). However, the results on the correlation between gastric carcinoma and progesterone which have been believed to be non-target for progesterone hormone are controversial. Indeed, Wu *et al.*, (1998) concluded that serum progesterone level reflects the presence or the absence of gastric carcinoma by some unknown factors. By contrast Kuru *et al.*, (2002) reported that serum pepsinogen does not correlate with their presence or the absence of gastric cancer or colorectal cancer.

Despite the evidence on physiological changes of blood pepsinogen during the gastrointestinal development and pathological cases, no study has been performed on the measurement of serum pepsinogen in relation with the reproductive cycle in sows. Therefore, considering that pepsinogen is produced in the gastric mucosa and progesterone receptors are present in gastric mucus, we prompted to investigate the relationship between the gastro intestinal tract and pregnancy in sows by measuring both pepsinogen and progesterone during pregnancy in sows.

Measuring blood pepsinogen in pregnant sows may increase our understanding on different adaptations related to biochemical and physiological changes that occur during gestation. In addition, it may provide information on of how some gastric diseases such as peptic ulcer remit during pregnancy and increase during lactation (Rubin and Janowitz, 1985; Singer *et al.*, 1991).

We reported here in the first experiment, serum pepsinogen and progesterone concentration in pregnant, non- pregnant and lactating sows. In the second experiment, the effect of dietary altrenogest as progesterone derivate on serum pepsinogen concentrations in non-lactating and non- pregnant sows was investigated.

Materials and Methods

Experiment 1. Serum pepsinogen and progesterone concentrations at different stages of the reproductive cycle in sows.: This experiment was carried out in the experimental farm of Göttingen University, Germany. Ten German hybrid sows (Large white x Landrace) of different parities, weighing 190 ± 21 kg) were selected and housed in individual boxes. Oestrus detection was performed at a daily basis and sows showing a standing reflex were artificially inseminated (Day 0). Gestation length was 116 ± 3 days and litter size was 11.7 ± 1.25). The daily mean food intake was 3.4 kg per sow and 5.7 kg during gestation and lactation, respectively. Diets during pregnancy contained 12.6 MJ/kg ME, 17% protein and 0.9% lysine while during lactation, diets covered 13.4 MJ/kg ME, 18% protein and 1% lysine.

Blood samples to determine pepsinogen and progesterone concentrations were taken from the jugular vein before the morning meal, at day 0 (mating), 21, 30, 40 50, 60, 70, 80, 90 and 100 days after mating and at day 10 and 20 after parturition. Sera were obtained by centrifugation and they were stored at -20° C until RIA analysis was performed.

Experiment 2. Effect of Altrenogest on serum pepsinogen concentration in sows: This experiment included 20 non-pregnant or non-lactating Piétrain sows and took place in the Faculty of Veterinary Medicine, University of Liège. Ten sows were submitted to oral administration of Altrenogest, synthetic progesterone-like hormone (Regumate®, Intervet, Belgium). Each sow received daily 20 mg of Regumate mixed in the food from day 0 to 7. This dose increased to achieve 40 mg from day 11 to 17. Ten sows were used as a control group. Blood samples were taken at day 0 before treatment and then at day 10, 20 and 25. Sera were obtained by centrifugation and they were stored at -20° C until RIA analysis was performed.

Measurement of pepsinogen and progesterone:

Serum samples from both experiments were analysed for pepsinogen concentrations by using a specific RIA as previously described (Banga-Mboko *et al.*, in press). The detection limit was 0.2 ng/mL. The intra-assay variation of the RIA was less than 8% while the inter-assay was 11.9%. Progesterone concentrations were determined using a direct solid phase 125 I method as described by Ranilla *et al.* (1994). In both RIAs, all standard and sample tubes were set up in duplicate.

Statistical analysis: Serum pepsinogen and progesterone concentrations were expressed as means \pm standard errors. The data obtained from both experiments were subjected to one-way analysis of variance (ANOVA) using the General Linear Model (GLM) procedure of SAS (1989). In order to investigate the differences between the control and treated groups, Student's t-test was applied. The correlation between pepsinogen and progesterone concentrations was assessed using the Spearman Rank Correlation test (PROC CORR, SAS, 1989).

Results

Experiment 1. Serum pepsinogen and progesterone concentrations in pregnant and non-pregnant sows: Serum pepsinogen and progesterone profiles during pregnancy and lactation are showed in Fig. 1. These profiles taken together can be separated in two crossed

Table 1: Serum Pepsinogen Concentration during altrenogest like pepsinogen (Regumate) treatment in non-pregnant or non-lactating sows (n=10)

Days	Control (ng/mL)	Treated (ng/mL)	Significance
0	152±46	167±57	NS
Day 10	149±34	153±58	NS
Day 20	156±28	158±66	NS
Day 30	153±31	150±42	NS

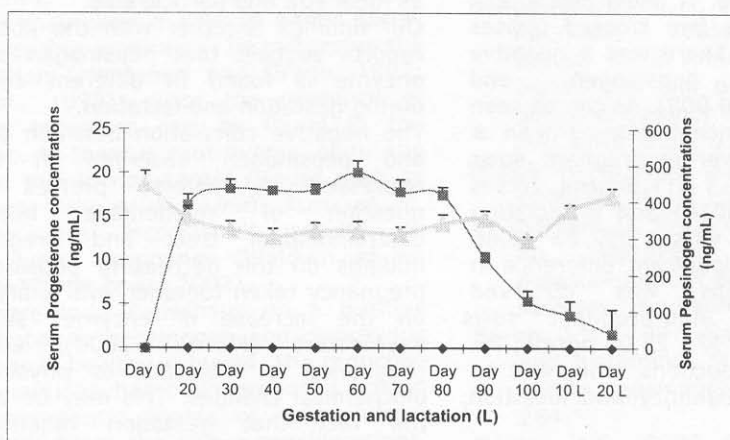


Fig. 1: Serum pepsinogen and serum progesterone profiles during the reproducible cycle of sows (n=10). Sows were inseminated at Day 0. Blood samples were taken before insemination, during gestation (from day 0 till day 100) and during lactation (from day 10L to day 20L). Values are expressed as Means \pm Standard Error.

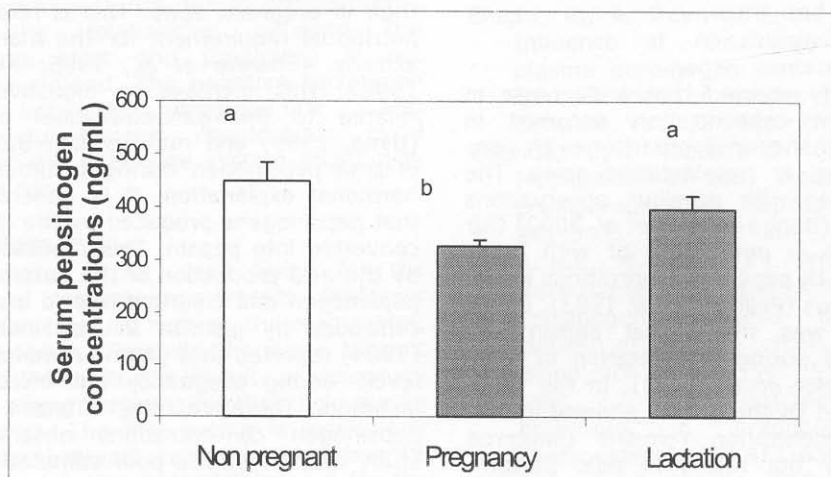


Fig. 2: Serum pepsinogen concentrations (Means \pm Standard Error) in non-pregnant sows (n=10), pregnant sows (n=10) and lactating sows (n=10). Two values that are not followed by the same superscript letter are different ($P < 0.05$).

phases and a linear phase. The first crossed profile took place from day 0 up day 20. During this phase, progesterone concentration increased whereas pepsinogen was decreasing. The second crossed phase from day 80 pregnancy till day 20 lactation, contrasted with the first crossed phase. Indeed, progesterone decreased continuously; on the other hand, pepsinogen was increasing. A linear phase can be observed between the two crossed phases from day 28 till day 70. There was a negative correlation between pepsinogen and progesterone ($r = -0.7$; $P < 0.002$). As can be seen in Fig. 2, pepsinogen concentrations (mean \pm S.E) was significantly lower in pregnant sows than in non-pregnant (327.3 ± 11.8 ng/mL versus 449 ± 35.4 ng/mL; $P = 0.0016$) and in lactating sows (327.3 ± 11.8 ng/mL versus 397.2 ± 25.06 ng/mL; $P = 0.0135$). No significant difference in pepsinogen concentrations was observed between lactating and non-pregnant sows (449 ± 35.4 ng/mL versus 397 ± 25.06 ng/mL; $P = 0.2371$). The average reductions amounted to 28% and 12% during pregnancy and lactation, respectively.

Experiment 2. Effect of Altrenogest on serum pepsinogen concentration in sows:

The administration of altrenogest did not significantly influence pepsinogen concentration ($P = 0.6452$) since all values in controlled sows were in the same magnitude as those of the treated animals (Table 1).

Discussion

The present study showed that a decrease in blood pepsinogen concentration occurred in sows during pregnancy in comparison with non-pregnant lactating or non-lactating sows. The results corroborate with previous observations in our laboratory (Banga-mboko *et al.*, 2002). Our findings are also in agreement of with other studies dealing with pepsin concentrations in the gastric juice of sows (Pelletier *et al.*, 1983). In the latter study, it was shown that pepsin was increased twofold during the lactation of first-litter sows (Pelletier *et al.*, 1983). In the same way, Takeuchi and Okabe (1976) showed in rats that pepsin concentration remains unaltered during pregnancy but raises in post partum. Jolicoeur *et al.* (1981) reported a significant decrease of pepsin concentration during pregnancy and a slight rise (+10%) during lactation in rats.

The decreased pepsinogen concentrations observed during pregnancy in our study contradict with earlier findings in humans

(Waldum *et al.*, 1980). This may be explained by the fact that in pigs, unlike the known factors involved in the gastric secretions, other factors act directly on parietal cells and chief cells in the secretion of pepsinogen and acid secretions (Low, 1990). These factors included nutritional factors such as feed of various chemical and physical compositions and physical factors such as meal size and particle size.

Our findings together with the above previous reports suggest that pepsinogen or its active enzyme is found in different concentrations during gestation and lactation.

The negative correlation between progesterone and pepsinogen observed in this study, expressed by inverse profiles raises the question of relationship between the gastrointestinal tract and pregnancy. Our findings on the decreasing pepsinogen during pregnancy taken together with a previous study on the increase of enzymes such as iso-oxytocinase (Klimek, 2000), confirm that pregnancy is subjected to physiological and biochemical changes. This may be explained by the fact that gestation requires selected metabolites to ensure all the need of the maternal organism and the growing of the fetuses (Baird, 1986).

Data recorded on food intake may be helpful to understand the pepsinogen concentration changes during pregnancy and lactation. We observed greater food intake in lactating sows than in pregnant sows. This is related with the nutritional requirement for the mammary gland activity. (Etienne *et al.*, 1985; Kusina *et al.*, 1998). This increase in digestive activity is related to the gastrointestinal endocrinology (Uvna, 1989) and may explain the increase in plasma pepsinogen during lactation. Based on hormonal explanation, it is generally accepted that pepsinogens produced by the chief cells are converted into pepsin. This process is mediated by the acid production of the parietal cells. Both pepsinogen and chlorhydric acid are hormonally controlled by gastrin. In his review, Hornnes (1984) reported that gastrin remains at baseline levels during pregnancy but increases during lactation. Therefore, the different changes in pepsinogen concentrations observed in this study may be due to poor stimulation of G cells during pregnancy and high gastrin activity during lactation.

In the second experiment, we noticed that dietary progesterone treatment did not modify pepsinogen concentration in non-pregnant sows and non-lactating sows. Such findings are in agreement with the results of previous reports

that treatment with oestrogen and progesterone separately or in combination had no significant effect on pepsinogen and acid concentrations (Baird, 1986; Montoneri and Drago, 1997). However, females given oestrogen or progesterone or both are relatively resistant to experimental ulceration, but again, there is no correlation with acid and pepsin changes (Aguwa, 1984; Baron, 1997). Therefore, progesterone may play a role in peptic ulcer, which remits during pregnancy, by increasing the mucus secretion (Montoneri and Drago, 1997; Drago *et al.*, 1999); since the presence of progesterone receptors can be found in the gastric mucus. As there is controversy, about the progesterone role in non-target tissues such as gastric mucus, (Wu *et al.*, 1998; Kuru *et al.*, 2002) there are inconsistent results on pepsinogen during pregnancy (Waldum *et al.*, 1980; Banga-Mboko *et al.*, 2002).

Likewise, there is a consensus on a positive correlation between pepsinogen and chlorhydric acid secretions in healthy subjects. The reduction of gastric secretions has been proposed among numerous therapeutic agents of peptic ulcers (Doster, 2000). From the above considerations, this study suggests that the lower food intake during pregnancy may allow fewer risks of ulcers further to a decrease of gastrin and consequently the gastric secretions. Taken together the two experiments in this study, data indicate that the lower pepsinogen concentration in pregnant sows was not due to progesterone. Therefore, the relationship between the gastrointestinal tract and pregnancy is not completely elucidated. The negative correlation observed between progesterone and pepsinogen during the reproducible cycle suggests that the endocrine system might play an important role.

Acknowledgments

We are grateful to Dr. José Sulon for the preparation of progesterone tracers, Professor Jean Bister for carefully reading the manuscript, Drs. Martine Laitat, Christelle Lhonneux, Vincent Verleyen and Marcel Bustin (Faculty of Veterinary Medicine of Liège, Belgium) for their help during blood collection. The first author is particularly grateful to the technical staff of the experimental farm of Gottingen University (Germany) for the excellent collaboration during the first experiment. This work was funded by grants from the Belgian National Fund of Scientific Research.

References

- Aguwa, C.N., 1984. Effects of exogenous administration of females sex hormones on gastric secretion and ulcer formation in the rat. *Eur. J. Pharmacol.*, 104: 79-84.
- Baird, J.D., 1986. Some aspects of the metabolic and hormonal adaptation to pregnancy. *Acta Endocrinol. Suppl.*, 277: 11-18.
- Banga-Mboko, H., P. Thilman, H. Desbulleux, N. Aoumeur, I. Youssao, Z. Perenyi, N. M. De Sousa, B. El Amiri and J.F. Beckers, 2002. Pepsinogen and progesterone concentration during pregnancy in sows. *Biotechnol. Agron. Soc. Environ.*, 6: 12-13 (Abstract).
- Banga-Mboko, H., J. Sulon, J. Closset, B. Remy, I. Youssao, N. M. Sousa, B. El Amiri, P. T. Sangild, D. MAES and J.F Beckers. (in press). An improved Radioimmunoassay for measurement of pepsinogen in porcine blood samples. *The Veterinary Journal*
- Baron, J.H., 1997. Sex, gonads, sex hormones and histamine- stimulated gastric acid and serum pepsinogen. *Inflamm.Res.*, 46: 260-264.
- Doster, A.R., 2000. Porcine gastric ulcer. *Vet. Clin. North. Am. Food Anim. Pract.*, 16: 163-174.
- Drago, F., C. Montoneri, C. Varga and F. Laszlo, 1999. Dual effect of sex steroids on drug-induced gastroduodenal ulcers in the rat. *Life Sci.*, 64: 2341-2350.
- Enight, K., A. Dey-Hazra and H. Feder, 1972. Influence of *Hyastromylus* infection on plasma pepsinogen content and electrolyte concentration of gastric juice in the pig. *Zentralb. Vet.*, 19: 416 -25.
- Etienne, M., J. Noblet and B. Desmoulin, 1985. Mobilization of body store in the lactation primiparous sows. *Reprod. Nutr. Dev.*, 25: 341-343.
- Heim, H.K., M. Piller, E. J. Schwed, P. Kilian, S. Netz-Piepenbrink and K.F Sewing, 1997. Pepsinogen synthesis during long-term culture of porcine chief cells. *Biochimica Biophysica Acta.*, 1359: 35-47.
- Hornnes, P.J., 1984. Gastroentero- pancreatic hormones in pregnancy. *Scand. J. Gastroenterol.*, 19: 451-456.
- Jolicoeur, L., J. Asselin and J. Morisset, 1981. Effect of gestation and lactation on rat gastric fundus. *Can. J. Physiol. Pharmacol.*, 59: 96-101.

- Klimek, R., 2000. Enzymes: the most important markers of pregnancy development. *Early Pregnancy.*, 4: 219-229.
- Kuru, B., C. Ozaslan, K. Yalman and M. Camlybel, 2002.. Serum progesterone levels in patients with gastric and colorectal cancers. *Acta Chir. Belg.*, 102: 125.
- Kusina, J., J. E. Pettigrew, A. F. Sower and M.R Hathaway, 1998. Effect of protein intake during gestation on mammary development of primiparous sows. *J. Anim. Sci.*, 77: 925-930.
- Low, A.G., 1990. Nutritional regulation of gastric secretion, digestion and emptying. *Nutr. Res. Rev.*, 3: 229-252.
- Montoneri, C. and F. Drago, 1997. Effect of pregnancy in rats on cysteamine- induced peptic ulcers. Role of progesterone. *Digest. Dis. Sci.*, 42: 2572-2575.
- Pellettier, G., P. Charbonneau and J. Morisset, 1983. Anatomical and biochemical changes of the stomach and duodenum during a reproductive cycle of first -litter sows. *Can. J Anim. Sci.*, 63 59-65.
- Ranilla, M.J., J. Sulon, M. D. Carro, A. R. Mantecon and J.F Beckers, 1994. Plasmatic profiles of pregnancy associated glycoproteins and progesterone levels during gestation in churra and merino sheep. *Theriogenology*, 42: 537-545.
- Rubin, P.H and H.D. Janowitz, 1985. The digestive tract and pregnancy. In: Cherry S.H Berkowitz R.L, Kase N.G., eds , 1985. Medical surgical and gynecology complications of pregnancy, 3rd ed. Baltimore: Williams and Wilkins, 196-206.
- SAS Institute. SAS user' guide.; 1989. Statistic - SAS Institute Inc, Cary N.C .
- Singer, A.J. and L.J Brandt, 1991. Pathophysiology of the gastrointestinal tract during pregnancy. *Am.J.Gastroenterol.*, 12: 1695-1712.
- Takeuchi, K., S. Okabe and K. Takagi, 1976. Influence of pregnancy on the development of various gastric lesions in rats. *Am. J. Dig. Dis.*, 21: 853-858.
- Uvnas- Moberg, K., 1989. The gastrointestinal tract in growth and reproduction. *Scientific American.*, 62-65.
- Waldum, H.L., B. K. Straume and R. Lundgren, 1980. Serum group I pepsinogens during pregnancy. *Scand J. Gastroenterol.*, 15: 61-63.
- Wu, C.H., Chi, C.W., Hisieh, M.C., Lo, S.S., Shen, K.H., Lui ,W.Y.and F.K, P'eng, 1998. Serum progesterone levels in patients with gastric adenocarcinoma before and after gastrectomy. *Cancer*, 83: 445-448.