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Third Belgian Workshop on Animal Endocrinology
Namur (Belgium), October 2001

The third Belgian Workshop on Animal Endocrinology has been held in the University of Namur, FUNDP, in October 2001. The special topics of the workshop has been focused on reproduction. Optimizing reproductive performance in breeding populations of economically important animals, including aquaculture species, is of major importance for more efficient animal production. In species managed for the production of food, suppression of reproductive cycles, sterilization or production of monosex populations may be desirable. New knowledge is needed to facilitate implementation of optimum integrated animal production systems that will contribute to sustainability of the animal production unit. This information will control or reduce animal production costs, provide product cost benefits to consumers, and may ultimately lead to increased productivity from fewer animals, thereby conserving natural resources and enhancing the environment. Research techniques developed to foster and manage animal reproductive phenomena are key to future application of biotechnologies. In the past decade, there have been many impressive advances in a number of scientific disciplines that have led to the discovery and development of exciting new approaches that offer the potential to improve reproduction efficiency of animal farming. The objective of this workshop has been to present some of these new developments.

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Abstracts

ABSENCE OF ESTRADIOL PRODUCTION IN FSH STIMULATED PREPUBERTAL CALVES

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In previous experiments, we stimulated prepubertal calves with purified FSH (0% LH). Despite obvious follicular development, peak production of estradiol on the time of FSH stimulation was absent. This was surprising and interesting enough to try to find the possible cause. One hypothesis was the use of the purified FSH preparation.

To test this hypothesis, we treated 2×5 calves (7 months old) alternatively with purified FSH (0% LH) *vs* FSH containing 20% LH (protocol in **table 1**). Ovum Pick Up (OPU) was used to remove all follicles from the ovaries. Daily throughout d14–20 and d28–34, ovaries were observed by ultrasound to measure follicular diameters. FSH treatment consisted of injecting a total of 230 mg FSH (evening and morning, decreasing doses). Jugular blood was sampled daily (d1->42), centrifuged within one hour and serum was frozen until analysis for estradiol.

For interpretation, results were grouped for both kinds of FSH. **Tables 2** and **3** show the results of follicular observations and estradiol levels. During the second part of the experiment, 3 animals were lost for various reasons.

Table 1. Experimental protocol (F0 = FSH + 0% LH and F20 = FSH + 20% LH).

	d1	d14	d15	d16	d17	d18	d19	d20	d28	d29	d30	d31	d32	d33	d34	d42
1	OPU	OPU		F0	F0	F0		OPU	OPU		F20	F20	F20		OPU	end
2	OPU	OPU		F20	F20	F20		OPU	OPU		F0	F0	F0		OPU	end

Table 2. Results of follicular observations (mean±sem) before (d OPU +1 = d15 and d29) and after (d OPU + 5 = d19 and d33) FSH administration (7 animals).

	FSH + 0% LH		**FSH + 20% LH**	
	(d15, d29)	**(d19, d33)**	**(d15, d29)**	**(d19, d33)**
n follicles	10.0±2.1[a]	18.7±2.1[b]	9.7±2.0[a]	18.3±2.0[b]
follicular diameter (mm)	3.5±2.7[a]	7.0±1.2[b]	3.6±2.8[a]	6.8±2.0[b]
n follicles > 10 mm	0.1±0.4[a]	2.7±0.4[b]	0±0.4[a]	1.4±0.4[b]
n follicles > 8 mm	0.1±1.0[a]	6.6±1.0[b]	0±0.9[a]	5.0±0.9[b]

ANOVA 1: $p<0.05$. Within FSH group: different superscripts indicate significant differences (horizontally).

Table 3. Estradiol blood levels (mean ± sem) of days 4 to 7, 17 to 20 and 31 to 34 (7 animals).

	(d4 to 7)	**FSH + 0% LH (d17 to 20 d31 to 34)**	**FSH + 20% LH (d17 to 20 d31 to 34)**
estradiol (pg/ml)	5.1±0.8	5.7±0.8	5.9±0.8

ANOVA 1: $p>0.05$

Conclusion

Both FSH treatments were effective in stimulating follicular development in these prepubertal animals. Nevertheless, estradiol peak production was again not observed after FSH treatment. Therefore we conclude that our initial hypothesis (no estradiol peak production because of lack of LH in the FSH preparation) has to be rejected.

PREGNANCY-ASSOCIATEDGLYCOPROTEIN PROFILES OF 5 HEIFERS MEASURED BY THREE RADIOIMMUNOASSAY SYSTEMS

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Pregnancy-associated glycoproteins (PAGs) are expressed in the superficial layer of the ungulate trophectoderm. Molecular biology investigations showed that several PAG genes are present in the bovine genome and they share high sequence identity with each other. These studies also showed that the expression pattern of the PAGs is temporal and spatial. Till now PAG I_{67} was isolated and characterized from the bovine placentomes (1), PAG_{55}, PAG_{59}, and PAG_{62} were purified from caprine placental extracts (2). Radioimmunoassay developed to detect PAGs in biological fluids (3) became efficient tools for establishment of pregnancy diagnosis and pregnancy follow-up in goat and cattle.

This study was undertaken to characterize the PAG profiles using 4 RIA systems of 5 Holstein Friesian heifers during the first part of their pregnancy.

Blood samples were collected frequently from the jugular and the coccygeal veins of the animals. After clotting the samples were centrifuged (at 1500g for 15 min), the serum was removed and was stored at –20°C till the RIA measurements. The four PAG RIA measurements were performed according to a protocol described by Zoli *et al.* (1992) and by Perényi *et al.* (2001). The PAG profiles are presented in **figures 1**–**5**. From the PAG concentrations determined by the four RIAsystems the following ratios were calculated: RIA 2/RIA1 and RIA 3/RIA 1. Analyzing the PAG profiles it can be seen that PAG molecules first appeared in the maternal blood in detectable concentrations at 19–25 days after AI. Intensive increase could be observed till day 33–37. After day 35–40 the PAG profile of RIA 1

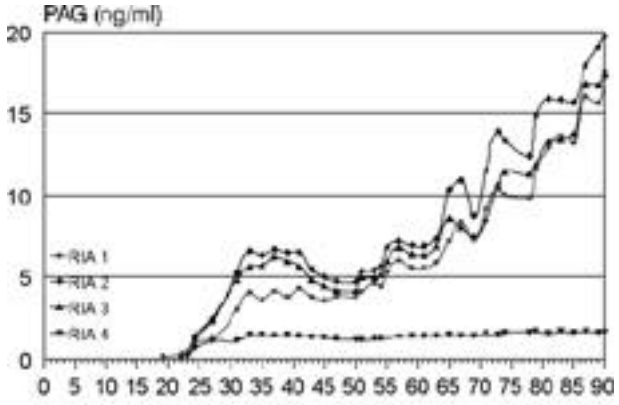

Figure 1.

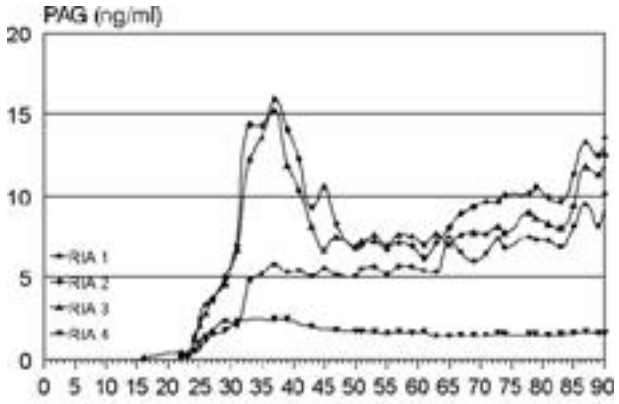

Figure 2.

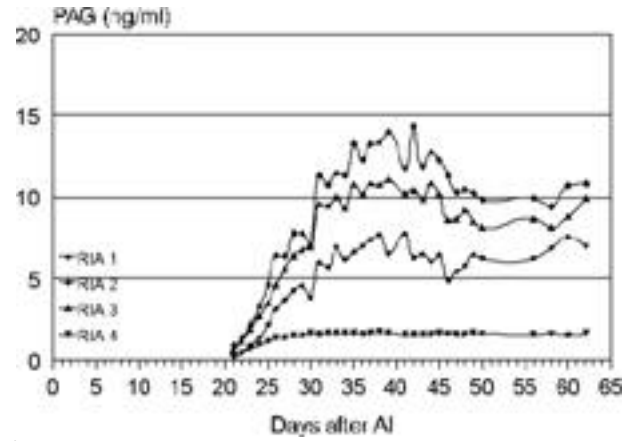

Figure 3.

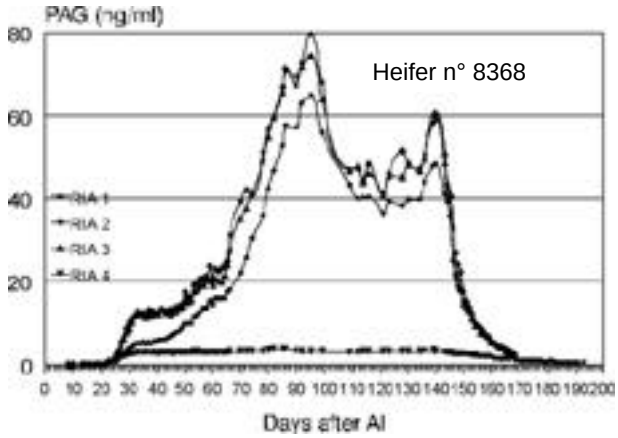

Figure 4.

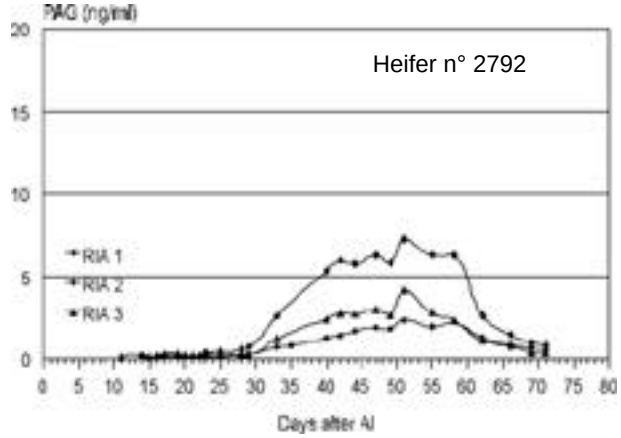

Figure 5.

showed a plateau with concentration of PAG remaining constant, in the first three figures in RIA 2 and RIA3 profiles decrease could be observed. Except some points at the detection limit of the tests, the PAG concentrations measured by RIA 2 and 3 were higher than those by RIA1. The mean RIA 2/RIA 1 and RIA 3/RIA 1 ratios determined for the first 90 days of pregnancy are shown in **figure 6**. From day 21–25 till day 41–50 these ratios were higher than 1.5, after they started to decrease and remained higher in the examined period for RIA2/RIA1.

The heifer N° 8368 presented increasing PAG levels in all the three RIA systems till day 90–100, after a decrease could be observed. The profile of this animal shows a second increase at around day 135–140. At day 144 abortion occurred and the expulsion of two fetuses was observed. After day 144 PAG concentrations were decreasing sharply. The heifer n° 2792 presented a late embryonic mortality. The embryonic death occurred probably between 51 and 62 days after AI, after PAG levels decreased sharply.

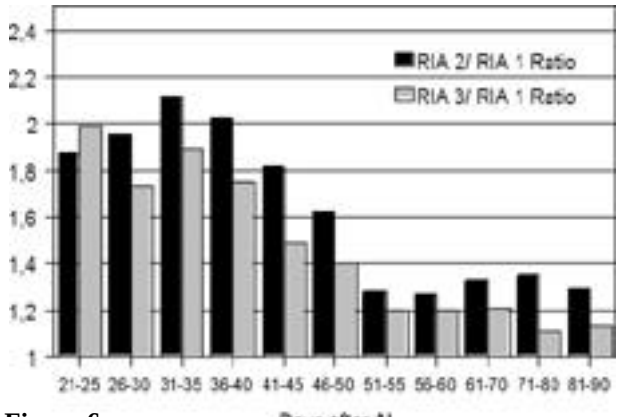

Figure 6.

Our results showed that between 20 and 50 days after AI, PAGs better recognized by RIA 2 and RIA 3 are secreted into the maternal blood. The higher RIA 2/ RIA 1 and RIA 3/RIA 1 ratios determined between day 21 and 50 suggest that RIA 2 and RIA 3 also detected additional PAG molecules non identical with PAG I67. It was also shown, that RIA2 and RIA 3 can be used as a sensitive tool to follow up pregnancy.

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EARLY PREGNANCY DIAGNOSIS IN SHEEP BY PROGESTERONE AND PREGNANCY-ASSOCIATED GLYCOPROTEIN TESTS

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Ovine pregnancy-associated glycoproteins (ovPAGs) and/or ovine pregnancy-specific protein B (oPSPB) are secreted by the trophoblastic binucleate cells (Green *et al.*, 2000). They are detectable in the maternal blood around the time of definitive attachment of the fetal placenta when the trophoblastic binucleate cells start to migrate and fuse to the endometrial cells forming the feto-maternal syncytium. Therefore, these glycoproteins are good indicator of both pregnancy and feto-placental well being.

The aim of this study was to compare the accuracy of the progesterone (P4) and the pregnancy-associated glycoprotein (PAG) tests for determination of early pregnancy in sheep.

Estrus was synchronized in 182 Awassi x Merino ewes and blood samples were collected at Days 0 (Day of the insemination), 18 and 22 after artificial insemination (AI). Plasma P4 concentrations at Days 0 and 18 were determined by double antibody radioimmunoassay (Ranilla *et al.*, 1994), while PAG concentrations at Day 22 were detected by a heterologous double-antibody RIA using bovine PAG 67 kDa as tracer and standard, and rabbit antiserum raised against caprine PAGs 55 and 59 kDa (R708) as the first antibody. The discriminatory value to diagnose pregnancy by the P4 and the PAG-RIA tests was ≥ 1 ng/ mL.

The sensitivity, the specificity, the positive and the negative predictive values for P4 and PAG tests were 100%, 95.4%, 81.5%, and 100% at Day 18 (P4) and 93.5%, 100%, 100% and 98.7% at Day 22 (PAG), respectively. For diagnosis of non-pregnant ewes the PAG test had significantly higher specificity than the P4 test ($P<0.01$).

In conclusion, the PAG test is highly valuable to diagnose pregnancy status as early as 22 days after A.I.

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ISOLATION AND RADIOIMMUNOASSAY FORMATION OF EQUINE OSTEOCALCIN – PRELIMINARY RESULTS

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Osteocalcin, also named bone gamma-carboxyglutamic acid containing protein, is one of the most abundant non collagenous proteins of bone. It is accepted as indicator of osteoblast activity (Risteli, Risteli, 1993) and different assays are used in the evaluation of bone metabolism in various species (Hauschka *et al.*, 1989; Fanti *et al.*, 1993). Aim of this study was to purify equine osteocalcin and for the first time to develop an equine specific osteocalcin radioimmunoassay system.

Equine osteocalcin was purified from equine bones by acid extraction, reverse phase chromatography, gel filtration and ion exchange chromatography (Carstanjen *et al.*, 2001). The protein's purity was proven by SDS-polyacrylamide electrophoresis and sequencing. Equine osteocalcin obtained had a purity of 99.5%. Purified osteocalcin was radiolabeled using $Na^{-125}I$ and Iodogen® as an iodinating reagent. The radio-labeled osteocalcin was separated from free $Na^{-125}I$ by gel filtration. Rabbits were immunized with equine osteocalcin and tested after six weeks for antibodies titration. The assays incubation was performed during 18 hours at room temperature. Free and bound fractions were separated using Tris-BSA(0.4%) buffer containing PEG (4%) and an anti rabbit IgG serum. The reaction was allowed for 60 minutes. After centrifugation the supernatant was discarded and total and antibody bound ^{125}I radiolabeled osteocalcin were determined with a gamma counter. The Iodogen® method produced a tracer allowing a highly sensitive radioimmunoassay with low non-specific binding

(<4%) and high specific binding. The ^{125}I radiolabeled osteocalcin bound at an antiserum final dilution of 1: 25,000. The lower detection limit of the standard curve was estimated to 0.2 ng/ml. The assay's precision was determined by the intra and inter assay coefficient of variation (CV). The intra assay (n=10) and inter assay (n=5) obtained by measuring a pool of plasma were respectively 4.2% and 7.3%.

Mean serum OC values and standard deviations (SD) for clinically normal yearlings were 63.6 ± 24.2 ng/ml (n=4) for two to five years old horses, 32.2 ± 8.9 ng/ml (n=8) and for six years old and older horses was 15.7 ± 12.6 ng/ml (n=10). Serum creatinine concentrations were in the normal range (between 90 µmol/L and 140 µmol/L) for all horses.

Our results show that, nowadays, a specific and sensitive equine osteocalcin radio-immunoassay is available. This assay allows to quantify immunoreactive osteocalcin circulating in blood in horses. The equine specific osteocalcin assay will therefore allow to monitor equine osteoblast activity associated with diet, exercise, therapy or bone diseases.

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ENDOCRINE PARAMETERS DURING GROWTH OF TIPALIA TRIPLOIDS WITH A REFERENCE TO THE EFFECT OF FEEDING SCHEDULE MODULATION

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Triploidy induction in tilapia species yields predominantly females and since males grow faster, the expected economical benefit of triploidisation is therefore virtually reduced. However, in an experimental trial performed in Congo (Pisciculture Research Center, UCB-AK), it was shown that triploid *Oreochromis aureus* females may display compensatory growth response when periods of normal feed delivery are alternated with periods of feed deprivation. A complete growth recovery in triploid, but not in diploid females was observed within 4 months under the restricted feeding regime (5 days/week of feeding). This finding is of economical importance.

Only little information is available concerning hormonal growth regulation in tilapia. High circulating 3,3',5-triiodothyronine (T_3) levels may be implicated in the growth dimorphism in favour of the males while gonadal steroids are suppressed in triploids. In intensive cultures of *O. aureus* (CERER-Tihange, Belgium), we showed that testosterone, 17-ß estradiol and 11-ketotestosterone were deeply depressed in comparison with diploid individuals. Blood levels of gonadotrophin (GTH) were constantly higher in triploids, as a result of a castration effect, while blood levels of growth hormone (GH), thyroxine (T_4) and T_3 exhibited variations not related to chromosomal status. Moreover, high levels of plasma T_3 and 11-ketotestosterone in males are likely the most permanent hormonal differences between males and females. However, the role of steroids in growth stimulation remains questionable. The delayed sexual maturation in triploids may explain growth capacities displayed in feed manipulation conditions. It is suggested that GH and thyroid hormone plasma levels and their metabolism in target tissues may be pointed as a central key of the regulation of available energy resources following the nutritional stress induced by food-deprivation.

FUNCTIONAL CHARACTERIZATION OF A GROWTH HORMONE-RELEASING PEPTIDE, GHRELIN IN THE CHICKEN

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Recently, ghrelin, a new growth hormone (GH)-releasing peptide was isolated from rat and human stomach as an endogenous ligand for the GH secretagogue receptor (GHS-R). In rat, peripheral and central administration of ghrelin stimulates the GH release from the pituitary.

In the first part of this study, the effects of ghrelin on the GH and adrenal axis were investigated in chickens. Several doses of chicken and human ghrelin were administered by intravenous injection to seven day old chickens (Cobb). The plasma was assayed for GH and corticosterone with a radioimmunoasssay (RIA). The results of these tests showed a ghrelin stimulated GH release 15 minutes after injection. On the other hand, a dose-dependent increase in plasma corticosterone levels was detected. To investigate the cause of this increasing corticosterone release, corticotropine-releasing hormone (CRH) levels were measured in the hypothalamus by RIA, but no increase could be found in the ghrelin treated chickens. It is concluded that the effect of ghrelin on the corticosterone secretion is not regulated at hypothalamic level. Further research has to be done to investigate whether ghrelin acts on the pituitary or directly on the adrenal to stimulate corticosterone release.

Using RT-PCR with degenerated primers two cDNA fragments could be amplified which showed strong homology with mammalian GHS-R cDNA's (73 %). One cDNA corresponded to GHS-R1a, which has also been identified in mammals. The second cDNA, GHS-R1c, is a new isoform. This GHS-R lacks 48 nucleotides in transmembane 6, which means that this variant probably lacks the ability to activate a successful signal transduction.

The central and peripheral distribution of GHS-R1a and GHS-R1c mRNA were studied by means of RT-PCR. Both pituitary and hypothalamus showed the highest GHS-R1a mRNA expression, whereas the expression in testis, heart, skin and stomach was somewhat lower. In contrast, lung, liver and kidney revealed a very low expression level. Comparison of GHS-R1a mRNA and GHS-R1c mRNA expression revealed that the latter mRNA is always present in lower amounts. The localization of both GHS-R isoforms in chicken hypothalamus and pituitary is consistent with their role in regulating GH and corticosterone release. The expression of the receptor in other peripheral regions may implicate its role in additional functions such as feeding.

EFFECT OF DIFFERENT FEEDING CONDITIONS OF THE GROWTH HORMONE BINDING PROTEIN IN NORMALAND SEX-LINKED DWARF CHICKENS

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A Western blot was developed according to the method of Laemlli to detect growth hormone binding proteins (GHBP) in plasma of normal and sex-linked dwarf (SLD) chickens. Four isoforms of the GHBP were found by means of a polyclonal antiserum raised in rabbits against the chicken growth hormone receptor (Prof. Dr. Baumbach, American Cyanamid, NJ, USA). The estimated molecular weights (M_r) were 115, 100, 82 and 59 kilodaltons. The variation in the M_r might be due to glycosylations, since N-linked glycosylation sites were detected in the protein sequence. On the other hand, dimerization or aggregation of the smaller growth hormone binding proteins could occur. Associations of the GHBP with non-GH binding proteins can most likely be excluded, since the samples were heated under denaturating conditions during the Western blot protocol.

The influence of fasting and administration of a low protein diet on the relative concentrations of the four GHBP was investigated. No significant differences could be found between dwarf and non-dwarf animals, nor between the different feeding conditions.

PREGNANCYASSOCIATED GLYCOPROTEIN CONCENTRATIONS DURING PREGNANCY AND POSTPARTUM PERIODS IN ZEBU CATTLE

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In the last two decades, a highly polymorphic family of placenta-expressed proteins has been discovered in ruminant species. These pregnancy-associated glycoproteins (PAGs) constitute a large family of aspartic proteinases, showing a greatest sequence identity with pepsinogens (Xie *et al.*, 1991). PAGs are synthesized by mono and/or binucleate trophoblastic cells, some of them being released in maternal circulation during almost the whole pregnancy period. By using biochemical procedures, some molecules of the PAG family were isolated from cotyledons of cows, ewes and goats. These molecules were used to immunize rabbits and the antisera obtained allowed the development of homologous and heterologous RIA systems for PAG measurement in plasma, serum (Zoli *et al.*, 1992) or milk samples (González *et al.*, 2001).

In the present work a specific RIA for zebu pregnancy-associated glycoprotein (PAG) was used to measure PAG concentrations during gestation and postpartum periods in 12 Azawak zebu cows. A pure preparation of zebu PAG (0.08 M NaCl fraction of DEAE Sephadex A25 column, CM Ceramic column peak IX) was used as standard and tracer. A rabbit antiserum (R782) raised against the same preparation was used as the first antibody at a final dilution of 1:150000. Heparinized blood samples (5 mL) were taken weekly from the jugular vein during gestation and postpartum periods. Plasma was removed by centrifugation (1500 g for 15 min) immediately after collection and stored at –20°C until assayed for PAG.

Eleven Azawak zebu cows were diagnosed as pregnant by rectal palpation of the uterus. In the non-pregnant cow, the PAG concentrations remained lower than the assay sensitivity (<1.0 ng/ml). One of the 11 pregnant females showed abnormally high (Dagnelie, 1975) PAG concentrations during gestation, being excluded from the general PAG profile.

During pregnancy, mean weekly PAG concentration varied significantly among the animals ($P<0.0001$) and the periods of pregnancy ($P<0.0005$). However, the time-related variation on PAG concentration was significant only at the end of gestation.

As shown in **figure 1**, the mean PAG concentration increased progressively from the week 6 to the 35 week of gestation (from 6.0 ± 4.2 ng/ml to 196.0 ± 34.8 ng/ml). Thereafter, PAG concentrations remained relatively constant until week 39 (210.8 ± 74.8 ng/ml), then they increase significantly reaching its highest level (1 095.6 ± 607.2 ng/ml) at parturition (week 40). After delivery, plasma PAG concentrations declined significantly ($P<0.05$) till the week 2 postpartum (348.4 ± 85.6 ng/ml). Thereafter, PAG concentrations decreased slowly reaching undetectable levels at the week 13 postpartum.

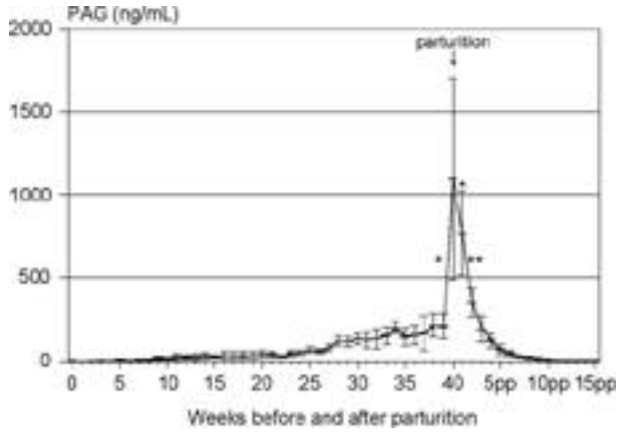

Figure 1. PAG profile during pregnancy and pospartum periods (pp) in 10 Azawak zebu cows. Significant differences between weeks of gestation are indicated by asterisks (*P < 0.0001, **P < 0.05).

To conclude, our findings showed that peripheral PAG concentrations in *Bos indicus* cattle were very similar to those previously described in *Bos taurus* (Zoli *et al.*, 1992), with significant higher concentrations being observed at the end of gestation followed by a slow decline in the postpartum period.

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PURIFICATION AND CHARACTERIZATION OF A PREGNANCY-ASSOCIATED GLYCOPROTEIN (OVPAG-6) FROM SHEEP PLACENTA REMOVED BETWEEN 66 TO 100 DAYS OF GESTATION

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The identification of antigens immunologically related to the bovine pregnancy-associated glycoprotein

(boPAG-1) or bovine pregnancy specific protein (bPSP-B) in the peripheral circulation of pregnant ewes encouraged the isolation and partial purification of pregnancy-associated glycoproteins in the ovine species. Recently, molecular biology studies identified 9 different molecules of pregnancy-associated glycoproteins in the sheep placenta (Xie *et al.*, 1997b). However, biochemical studies characterized only four different molecules (Xie *et al.*, 1997a) and only one molecule gave the same N terminal sequence in the two approaches.

The aim of this study was to isolate and to characterize PAGs from ewe placenta. Here we describe for the first time the purification of the protein identified as ovPAG-6 in molecular biology studies (Accession number O02726). The procedure was realized on placenta removed at 66 to 100 day of gestational stage. The tissue was washed with NaCl 0.9% and stored at -20°C. After thawing the tissue was extracted in phosphate buffer, then the proteins were submitted to acidic and ammonium sulfate precipitations, anion exchange chromatography (DEAE), gel filtration, cation exchange (CM ceramic), and chromatofocusing (mono P). The immunoreactivity was monitored by heterologous RIA that used caprine PAG_{55+62} and caprine PAG_{55+59} (Conzalez *et al.*, 1999) and the ovine PAG (Ranilla *et al.*, 1994).

In the crude extract, the PAGs represent 3.2% of the total proteins. After DEAE, gel filtration and CM columns the active fractions were tested in SDS-PAGE, Western blotting, 2D-electrophoresis and transferred onto PVDF membranes for sequencing. One of the CM immunoreactive peaks from the fractions eluted with 0.04 M NaCl DEAE gave single major stained bands on SDS-PAGE with apparent molecular masse of 58 kDa. Two dimensional gel electrophoresis showed that this major band was composed of several proteins (at least four), with acidic pIs ranging from 4.0 to 5.8 (5.8, 5.6, 5.0 and 4.0). The microsequencing of the spot corresponding to MM of 58 kDa and and pI 5.6 revealed the **RGSNLTIHPLRNTKS** sequence, characteristic of the ovPAG-6 (Xie *et al.*, 1997b). This report is the first to describe the biochemical purification and the characterization of ovPAG-6.

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PEPSINOGEN AND PROGESTERONE CONCENTRATION DURING PREGNANCY IN SOWS

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During pregnancy progesterone stimulates the secretion of proteins and other molecules that support the developing conceptus. In this way, many proteins or enzymes appear or increase in maternal blood circulation during pregnancy. Pregnancy is also claimed to be associated with a wide variety of physiological and biochemical changes in virtually all the organ systems and particularly the entire length of the gastrointestinal tract (Singer, Brandt, 1991). Among the enzymes secreted into the lumen of digestive tract, pepsinogen can be also found in blood in small but measurable quantity, which is relatively constant in a given individual. The aim of this study was to investigate the relationships between pregnancy and gastrointestinal enzyme by analysing progesterone and pepsinogen levels during pregnancy.

The experiment was carried out in the experimental farm of the faculty of Veterinary Medicine of Liege (Belgium). Belgian pietrain (n=3) from 12 to 16 months old and about 130 kg body weight were controlled daily for oestrus behaviour and were artificially inseminated. The day of the insemination was called day 0. Blood samples were collected in the same time in jugular vein into heparinized vacutainer tubes at day 0, 28, 42, 56, 70, 86, and 100. Plasma obtained by centrifugation (15

min at 1500g) were stored at –20°C until radioimmunoassay procedures. Progesterone concentrations were estimated by direct solid phase I125 RIAmethod as described by Ranilla *et al.* (1994). Whereas, pepsinogen concentration was measured by using a specific radioimmunoassay as previously described (Banga-Mboko *et al.*, 2000). The results showed a negative correlation ($r = -0.7$; $p<0.001$) between pepsinogen and progesterone showing inverse profiles. Pepsinogen and progesterone profiles presented in **figure 1**, can be separated in 3 parts:

– the first cross phase: it took place from day 0 up day 28. During this phase progesterone concentration was increasing whereas pepsinogen level is decreasing.
– the equilibrium phase (or a linear phase): from day 28 up day 70. During this stage there was no rise of concentration, although the pepsinogen concentration showed a small peak between day 70 and day 100.
– the second cross phase: from day 86 or day 100 up mid lactation. This phase contrasts with the first phase. Indeed, progesterone concentration decreased continuously, on the other hand pepsinogen increased and reached initial concentration.

This result shows for the first time the profile of porcine pepsinogen during pregnancy and the negative relationship with the progesterone concentration. Further studies are in progress in order to establish the profiles of other gastric aspartic proteases during pregnancy.

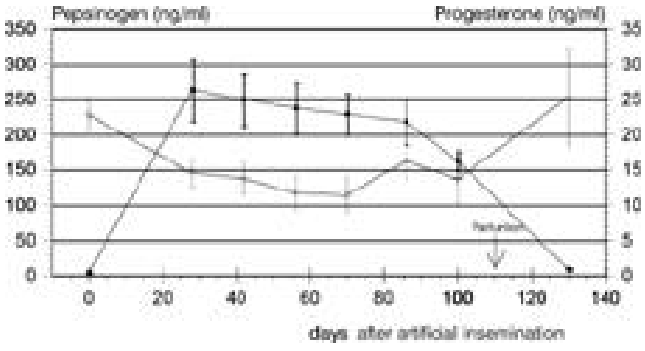

Figure 1. Pepsinogen and progesterone concentration during pregnancy in sows.

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EFFET DE LA PARITÉ SUR LES PARAMÈTRES MÉTABOLIQUES ET HORMONAUX DE LA FEMELLE DE RÉFORME BLANC-BLEU-BELGE CULARDE

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Introduction

Les paramètres endocriniens de la femelle de réforme Blanc-Bleu Belge sont actuellement très peu connus. Il a donc paru intéressant d'étudier les teneurs plasmatiques en quelques hormones impliquées dans les phénomènes d'engraissement chez des génisses et des vaches de réforme BBB.

Matériel et méthodes

Au cours de deux années d'expérience, un total de vingt-quatre femelles Blanc-Bleu Belge a été divisé en trois lots en fonction de la parité : génisses (G), vaches réformées après deux vêlages (V2) et vaches réformées après quatre vêlages (V4). Les animaux ont reçu une ration d'engraissement à base d'ensilage de maïs, distribuée *ad libitum*. Le glucose, les hormones thyroïdiennes (T3 et T4) et l'IGF-1 plasmatiques ont été mesurés sur des prélèvements sanguins bimensuels. Les teneurs en insuline et en hormone de croissance (GH) ont été déterminées, au début et en fin d'engraissement, sur des prises de sang réalisées pendant 12 h à 20 minutes d'intervalle.

Résultats

Il n'y a pas eu de différence significative au niveau de la glycémie. Par contre, les concentrations plasmatiques en T3 et en IGF-1 ont diminué significativement avec l'âge. Une tendance a

	Paramètres			
	G	**V2**	**V4**	**P**
Nombre d'animaux	8	8	8	
Glucose (mg/l)	762,9	770,3	723,7	0,282
T3 (nmol/l)	1,46	1,46	1,07	0,017
T4 (nmol/l)	63,3	64,3	52,7	0,153
IGF-1 (ng/ml)	130,5	100,7	97,6	0,035
Insuline (mU/l)				
(1)	21,3	24,4	22,2	0,771
(2)	15,0	17,6	21,7	0,287
GH (ng/ml)				
(1)	7,8	7,7	8,8	0,460
(2)	11,8	9,0	10,8	0,384

G = génisses Blanc-Bleu Belge culardes ; V2 = vaches Blanc-Bleu Belge culardes réformées après deux vêlages ; V4 = vaches Blanc-Bleu Belge culardes réformées après quatre vêlages ; P = degré de signification ; (1) = première phase de l'engraissement ; (2) = deuxième phase de l'engraissement.

également été observée avec T4. L'insuline et la GH n'ont pas été influencées par l'âge. Néanmoins, il est apparu que les concentrations en insuline ont diminué au cours de l'engraissement, tandis que celles en GH ont augmenté.

Ces résultats sont en contradiction avec des observations précédentes obtenues chez des taurillons Blanc-Bleu Belge culards à l'engrais.

Conclusions

Chez la femelle de réforme Blanc-Bleu Belge, l'augmentation de l'âge et de la parité se caractérise par une diminution des concentrations plasmatiques en plusieurs hormones impliquées dans les phénomènes de croissance. L'engraissement ne semble pas influencer le rapport insuline/GH de la même façon que chez les taurillons à l'engrais.

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CANNULATION OF THE DUCTUS DEFERENS IN RAM AND BULL

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The cauda epididymidis is a peculiar organ where mammalian spermatozoa can be stored for several weeks, without reduction of their fertilizing ability. To determine the composition of the cauda epididymal plasma (CEP), at least 1.5 ml of pure epididymal plasma is needed. The aim of this study was to collect epididymal semen of ram and bull *in vivo* during several days.

Six mature bulls and rams with average sperm quality were used for epididymal cannulation. Both rams and bulls were cannulated using a surgical technique. After making a small incision in the tunica vaginalis, the spermatic cord was isolated by blunt dissection and the ductus deferens was isolated from the adjacent blood vessels and nerves.

The ductus deferens was cut in a cross section and a silicone cannula (K-JITS-303520, Cook®, Strombeek-Bever, Belgium) consisting of an inner and an outer part was inserted as far as possible into the lumen of the ductus deferens (± 5 cm). To maximize the internal diameter of the cannula, the inner part was removed. The outer part of the silicone cannula was secured to the ductus deferens and the tunica vaginalis by 4–0 silk sutures (Ethicon®, Neuilly, France). To prevent cracking an extra cannula (Tygon®, R3603, Fisher Bioblock Scientific,Tournai, Belgium) was put over the silicone cannula. The epididymal sperm was collected in a cryotube (Nunc®, Denmark), which was replaced every 24 h. Patency of the cannula was assessed after ejaculation of the male into an artificial vagina. The volume of the epididymal semen was determined with a graduated 1 ml syringe. Sperm concentration was determined in a Bürker counting chamber by means of light microscopy (x 200). Membrane integrity was determined by means of Live/Dead, Sperm Viability Kit (Molecular probes) and fluorescence microscopy (x 400). Morphology was determined after eosin-nigrosin staining by means of light microscopy.

Epididymal cannulation of rams is easier to perform than cannulation of bulls, due to the less curved ductus deferens in rams. In rams, the collection period of uncontaminated epididymal semen lasts 5.5 days longer than in bulls. The concentration of the epididymal spermatozoa is larger in rams than in bulls, while the average number of spermatozoa per collection and the total production of epididymal semen per testicle is similar in rams and bulls. The average volume of cauda epididymal plasma, the percentage of membrane intact spermatozoa and the percentage of normal spermatozoa is higher in bulls than in rams.

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Table 1. Results (average ± st. dev.) of collected epididymal semen after cannulation of ram (n=6) and bull (n=6).

Parameters of collected epididymal semen	ram average ± st dev	bull average ± st dev
Patency	14.3 ± 8.1	9.9 ± 5.4
Average production (µl/day)	274.4 ± 166.4	486.7 ± 182.9
Total production of epididymal fluid (µl)	3284.2 ± 1849.6	3187.5 ± 2019.5
Average sperm concentration ($\times 10^9$/ml)	3.0 ± 1.6	1.8 ± 0.9
Average number of sp./collection ($\times 10^9$/ml)	1.1 ± 0.7	1.0 ± 0.6
Membrane intact spermatozoa (%)	57.3 ± 10.6	77.4 ± 13.4
Normal spermatozoa (%)	57.9 ± 32.2	61.4 ± 15.4

EFFECT OF BOVINE SOMATOTROPIN (BST) ON BACTERICIDALACTIVITY OF BOVINE NEUTROPHIL

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As a consequence of respiratory burst activation, neutrophils produce reactive oxygen intermediates (ROI) which play a central role in the killing of endocytosed microorganisms.

Substantial evidence shows that bovine somatotropin (bST) acts as an endo- or para-crine priming agent for the immunophysiological network in dairy cows, suggesting that bST application could be a promising alternative immunomodulator to stimulate host resistance against infectious diseases in immunocompromised dairy cows. The relative contribution of bST to the decreased ROI production of neutrophils during early lactation has been poorly investigated.

In this study, neutrophils were isolated from eight healthy high yielding dairy cows during mid-lactation. To investigate the influence of bST on neutrophil bactericidal activity, the effect of bST concentrations of 0, 2.5, 5, 7.5 and 10 ng/ml on neutrophil ROI production was measured both by a phorbol 12-myristate 13-acetate (PMA)-induced luminol enhanced chemiluminescence (CL) and by a superoxide dismutase-inhibitale reduction of ferric cytochrome c assay.

The CL response of neutrophils pre-incubated at 2.5, 5, 7.5 and 10 ng/ml increased by 8.1%, 13.4%, 13.1% and 13.8%, respectively, above the control value. Neutrophil superoxide anion production was 12.0, 12.9, 13.7, 13.8 and 14.1 nM / 106 neutrophils / 30 min after 20 min incubation at bST concentrations of 0, 2.5, 5, 7.5 and 10 ng/ml, respectively. The increase PMA-induced luminol-dependent CL and superoxide anion production of neutrophils pre-exposed to bST might explain an increased bactericidal efficiency of bovine neutrophil by bST treatment *in vivo*. Further studies are in progress to explain this finding.

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SENSITIVITY OF FOLLICLES FROM PREPUBERTAL CALVES OVARIES TO *IN VITRO* STIMULATION WITH LH AND FSH

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Ovaries from prepubertal calves were harvested in an abattoir and quickly brought to the laboratory for the dissection of the follicles. The intact entire follicles were put in individual chambers and continuously

perifused with TCM199 at the rate of 1ml/30 min. The medium was added LH at the time 60–90 min and FSH at the time 120–180 min. The perifusion lasted 5 hours and the perifused medium was collected every 30 min. The samples were assayed for Progesterone, Δ4-Androstenedione and Estradiol.

Four different stimulations were assayed: LH + FSHc (FSH commercial with an estimated 20% LH contamination), LH + FSHp (pure FSH, with 0% LH activity), FSHc alone, and FSHp alone.

No difference were observed between the treatments; this confirms the results of De Roover *et al.* (this workshop) that the absence of LH is not the reason for the lack of Estradiol peak after FSH stimulation of calves ovaries *in vivo*.

Only large follicles (diameter ≥ 13 mm) were able to produce Estradiol *in vitro*. According to the follicular size, no difference can be measured in Δ4 production, but medium sized follicles produced significantly more Progesterone than small or large ones. It can be interpreted as a growing ability to produce Progesterone and the late acquisition of ability to produce Estradiol.

The observation of the small proportion of follicles with diameter ≥ 13 mm may be the explanation of the lack of E2 peak production after *in vivo* stimulation.

High levels of Estradiol are suspected to be necessary for the acquisition of the oocyte competency to develop into viable embryos. The incapability of most of the follicles present in ovaries of prepubertal calves to produce significant level of E2 might be the reason of the poor results in embryo development after ovum pick-up in this kind of animal.