



Second Belgian Workshop on Animal Endocrinology Leuven (Belgium), 15 November 2000

The second Belgian Workshop on Animal Endocrinology has been held in the University of Leuven. The special topics of the workshop focused on leptin mechanism in ruminant and poultry. One of the essential roles of this hormone is to inform the organism about the level of fat reserves. The leptin gene is expressed in ruminants and poultry adipose tissues. Recent results on variations in plasma leptin and/or levels of leptin mRNA in adipose tissues show positive effects of body fatness and feeding level, and an inhibitory β -adrenergic effect in cattle. In other respects, *in vitro* leptin production is stimulated by glucocorticoids and insulin, whose effects are inhibited by growth hormone. Progress in knowledge about leptin will allow to better understand and control the adaptations of energy metabolism and reproductive activity of ruminants to seasonal variations in day length and food supply, as well as variations in carcass fatness of growing animal (for more information see review by Chilliard *et al.* (1999). La leptine chez le ruminant. Facteurs de variation physiologiques et nutritionnels, *INRA Prod. Anim.*, 12 p. 225–237). The third Belgian Workshop on Animal Endocrinology will be organized by the University of Namur, Facultés universitaires Notre-Dame de la Paix, in October 2001.

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Abstracts

ISOLATION AND PARTIAL PURIFICATION OF PREGNANCY-ASSOCIATED-GLYCOPROTEINS FROM SHEEP PLACENTA

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Keywords. Purification, ewes, pregnancy-associated-glycoproteins.

The placenta is a source of a wide range of hormones and proteins, placental lactogen, steroids and pregnancy specific or associated proteins. During the last decade, pregnancy associated glycoproteins (PAGs) were purified from bovine and caprine placenta. The PAGs belong to the aspartic protease family, in which they coexist with cathepsin D and E, chymosin, pepsinogen and renin. In 1997, Xie *et al.* identified three different PAGs molecules from the culture medium of sheep placenta removed around day 100 of pregnancy. In a recent study by means of the Ouchterlony method we have shown that the extracts of ovine placenta removed at different stage of pregnancy revealed different precipitation lines (El Amiri *et al.*, 1999). These results suggest that PAGs molecules vary during pregnancy.

The aim of this study was to isolate and further to characterize the PAGs from placental extracts by monitoring the fractions using the Ouchterlony technique, the SDS-PAGE, Western blot and heterologous RIA. Our procedure was based on that initially described by Zoli *et al.* (1991) for boPAG-1.

PAGs were isolated from cotyledons removed from pregnant ewes at the third part of gestation. The tissue was extracted in phosphate buffer containing proteases inhibitors, then the proteins were submitted to acidic and ammonium sulfate precipitations, anion and cation exchange chromatographies (DEAE Sephadex and CM Ceramic columns, respectively). Two fractions (0.04 M NaCl and 0.08 M NaCl) from the DEAE (**Table 1**) were loaded separately onto the CM Ceramic column. In the optical density profile of the CM Ceramic column, peaks were pooled, dialyzed and lyophilized. The peaks exhibiting activity in RIA were used to immunize rabbits. The antisera were tested at different dilutions.

The electrophoresis analysis of immunoreactive peaks after the CM Ceramic column showed at least three proteins between 67 kDa and 30 kDa and justified further investigations before characterizing the ovine PAGs.

Tested in buffer and without preincubation with the bovine PAG tracer, the antisera gave the following titers three months after the first immunization (**Table 2**).

Further investigations are in progress to purify till homogeneity the most abundant ovine PAGs and to characterize by amino acid sequencing after two-dimensional electrophoresis.

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Table 1. Total protein (TP) and PAG:TP ratio of the fractions eluted from the DEAE-cellulose and CM Ceramic columns.

Ion exchange chromatographies					
DEAE column			CM Ceramic column		
NaCl concentration	TP (mg)	PAG:TP Ratio (%)	Immunoreactive peak used for immunization	TP (mg)	PAG:TP Ratio (%)
0 M	478.7	0.48	-	-	-
0.02 M	632.8	0.90	-	-	-
0.04 M	118.9	35.35	VIII (eluted at NaCl concentrations of 0.33 to 0.38 M)	6.8	78.68
0.08 M	317.2	17.58	VII (eluted at NaCl concentrations of 0.37 to 0.47 M)	8.9	60.41
0.16 M	820.6	0.80	-	-	-
0.32 M	186.6	1.64	-	-	-
1 M	35.1	2.26	-	-	-

Table 2. Antisera titer three months after the first immunization.

Antisera	Antigens	Total amount (mg)	Titer
778	0.04 M NaCl DEAE Peak VIII CM Ceramic column	1.30	1/100 000
779	0.08 M NaCl DEAE Peak VII CM Ceramic column	1.80	non determined
780	0.08 M NaCl DEAE Peak VII CM Ceramic column	1.35	1/100 000

References

- El Amiri B., Garbayo JM., Mecif K., Sousa NM., Banga-Mbonko H., Perenyi Z., Beckers JF. (1999). The pregnancy-associated glycoproteins in sheep: new investigations by using the Ouchterlony technique. *In 15th Sci. Meet. Eur. Embryo Transfer Assoc. (AETE)*, 10–11 September, Lyon, France, p. 150 (abstract).
- Xie RJ., Green J., Bao B., Beckers JF., Valdez KE., Kakami L., Roberts MR. (1997). Multiple pregnancy-associated glycoproteins are secreted by day 100 ovine placental tissue. *Biol. Reprod.* **57**, p. 1384–1393.
- Zoli AP., Beckers JF., Closset J., Ballman-Wouters P., Falmagne P., Ectors F. (1991). Purification and characterization of a bovine Pregnancy-Associated Glycoprotein. *Biol. Reprod.* **45**, p. 1–10.

RADIOIMMUNOASSAY OF PREGNANCY-ASSOCIATED GLYCO-PROTEIN 1 (PAG-1) ISOLATED FROM ZEBU (*BOS INDICUS*) PLACENTA: PRELIMINARY RESULTS

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Keywords. Pregnancy-associated glycoproteins, zebu cattle, radioimmunoassay.

The Pregnancy-Associated Glycoproteins (PAGs), also known under a variety of other names including pregnancy-specific protein B (PSPB) and pregnancy-specific protein 60 (PSP-60), were first described as placental antigens of cattle placenta that were also present in the blood serum of the mother soon after implantation. They constitute a large family of glycoproteins expressed specifically in the outer epithelial cell layer (chorion/trophoblast) of the placenta of ungulate species. Isolation of the cDNA coding for bovine and ovine PAG showed that they belonged to the aspartic proteinase gene family, having greatest sequence identity with pepsinogens (Xie *et al.*, 1991). By using biochemical procedures, some molecules of the PAG family were isolated from cotyledons of cow, ewe and goat. These molecules were used to immunize rabbits and the antisera obtained allowed the development of homologous and heterologous radioimmunoassay systems. In veterinary practice, the measurement of PAG concentrations in maternal blood is useful for both pregnancy confirmation and follow-up of the trophoblastic function. The first aspect can help breeders in the management of reproduction, while the second concerns more specifically clinicians and researchers in their investigations of the reproductive physiology during gestation, as well as in the establishment of differential diagnosis of pathologic conditions affecting pregnancy. Although there is abundant literature on characterization of placental proteins and their use for pregnancy diagnosis in temperate breeds of cattle, till now, there was only few reports on the investigation of the biochemical characteristics and secretory profiles of placental proteins in zebu cattle (Sousa *et al.*, 2000, 2001).

The aim of this study was to analyze the characteristics of a new preparation of zebu PAG-1 in the development of a new homologous PAG radioimmunoassay system.

Zebu PAG-1 (0.04 M NaCl fraction of DEAE Sephadex A25 column, CM Ceramic column peak XI) purified from foetal cotyledonary tissue (Sousa *et al.*,

2001) was used as tracer and antigen for antiserum production in rabbits. The purified zebu PAG-1 was radiolabelled by lactoperoxidase method. Unreacted iodine was discarded by gel filtration. The immunizing dose for antiserum production was 250 µg dissolved in 0.01 M phosphate buffer, pH 7.4. Dilution curves determined the optimal dilution of the antiserum (binding of 20–30% of tracer with nonspecific binding 2%). In the presence of antibody in excess, 73.6% of labeled zebu PAG-1 was bound. The optimal dilution of the antiserum issued from the first bleeding (1-week after the third injection of antigen) was 1:100,000. These first results indicate that a new homologous radioimmunoassay system is now available for physiological investigations on secretory profiles of PAG in zebu cattle.

Further investigations are in progress to produce new tracers and antisera for the development of a highly sensitive radioimmunoassay method for PAG detection in peripheral blood of pregnant females. This new system could be used in experimental and/or farm conditions in order to improve the knowledge about the endocrine physiology of zebu females.

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References

- Sousa NM., Remy B., El Amiri B., Banga-Mboko H., Figueiredo JR., Gonçalves PBD., Wattiez R., Dode MAN., Falmagne P., Beckers JF. (2000). Isolation of the pregnancy-associated glycoprotein 1 (PAG-1) from zebu (*Bos indicus*) placenta. *16th Sci. Meet. Eur. Embryo Transfer Assoc.* (AETE), 8–9 September, Santander, Spain, p. 212 (abstract).
- Sousa NM., Remy B., El Amiri B., Banga-Mboko H., Perényi ZS., Figueiredo JR., Gonçalves PBD., Wattiez R., Falmagne P., Beckers JF. (2001). Identification of pregnancy-associated glycoprotein 1 (PAG-1) in zebu (*Bos indicus*) placenta. *Theriogenology* **55** abstract n°327 (in press).
- Xie SC., Low BG., Nagel RJ., Kramer KK., Anthony RV., Zoli AP., Beckers JF., Roberts RM. (1991). Identification of the major pregnancy-specific antigens of cattle and sheep as inactive members of the aspartic proteinase family. *Proc. Natl. Acad. Sci. USA* **88**, p. 10247–10251.

RADIOIMMUNOASSAY OF PORCINE PEPSINOGEN

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Keywords. Pepsinogen, radioimmunoassay, sensitivity, specificity, swine.

Pepsinogens are the proenzymes of pepsins, they belong to the group of gastric acid aspartic endoproteinases. Exocrine secreted pepsinogens function as zymogens for the main proteolytic activity of gastric juice. There is also a small secretion in blood, the function of this process is not understood. However, exploiting this endocrine process, we can get information on the condition of the gastric mucosa by measurement of the plasmatic or seric concentration of pepsinogen (Biemond *et al.*, 1989). In this abstract we describe the development of a RIA of porcine pepsinogen. Using the Vaitukaitis (1971) method, antisera were raised in rabbit immunized with commercial porcine pepsinogen or with the same preparation treated by gossypol. The purpose of this treatment was to avoid the transformation of pepsinogen into pepsin (cleavage of the 41 amino acid residues from the NH₂ terminus) according to the method of Wong *et al.* (1972).

Tracer was prepared using ¹²⁵Iodine and two methods: the lactoperoxidase and chloramine T. Buffer for incubation was prepared with Tris-HCl (0.01 M, pH 7.5), BSA 0.1% additionned with Tween 20 0.5% v/v and an inhibitor of aspartic protease: the pepstatin 5 mg/L. Incubation was realized during 24 or 48 hours at 4°C. The separation of free and bound fractions was completed by using a solution containing an anti immunoglobulin antiserum, normal rabbit serum, and polyethylenglycol. The reaction was allowed for 30 minutes. After aspiration of the supernatant and washing of the pellet, the tubes were counted in a Gamma counter (Wallac LKB, Finland).

Rabbits immunized with native pepsinogen gave antisera with high titres, whereas the rabbits immunized after treatment of pepsinogen by gossypol gave lower titre in dilution test.

The lactoperoxidase method gave high non-specific binding and low incorporation of ¹²⁵Iodine. The chloramine T method produced a tracer allowing a highly sensitive RIA with very low non-specific binding and a high specific binding. The standard curve ranged from 0.2 ng to 100 ng/mL (**Figure 1**). There was no cross-reaction with others members of aspartic proteinases such as renin, cathepsin D and bovine PAG.

Preliminary comparison showed that the Tween and the pepstatin in buffer decrease the NSB and increase the sensitivity of RIA system. Our data show that the RIA is available for determination of pepsinogen circulating in blood and suggest that addition of Tween and pepstatin in buffer could improve the performance of the RIA of other members of proteinases aspartic family.

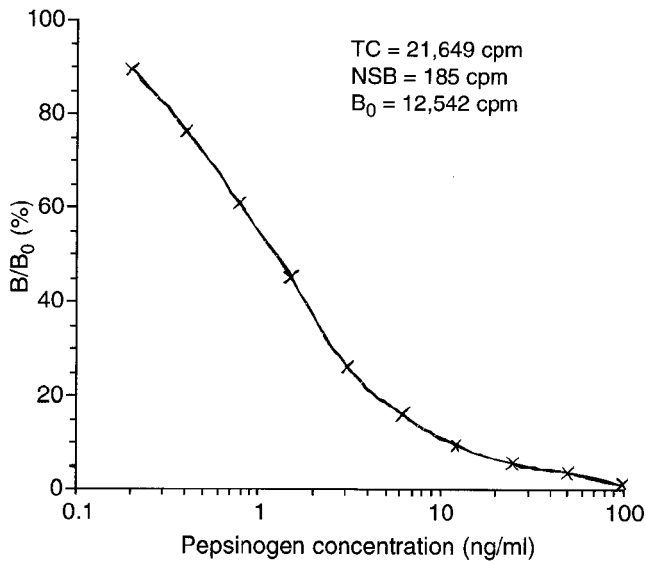


Figure 1. Standard curve for the radioimmunoassay of porcine pepsinogen.

References

- Biemond I., Jansen JB., Crobach LF., Kreuning J., Lamers CB. (1989). Radioimmunoassay of human pepsinogen A and pepsinogen C. *J. Clin. Chem. Clin. Biochem.* **27**, p. 19–25.
- Vaitukaitis J., Robbins JB., Nieschlag E., Ross GT. (1971). A method for producing specific antisera with small doses of immunogen. *J. Clin. Endocrinol. Metab.* **33**, p. 988–991.
- Wong CR., Nakagawa Y., Perlmann GE. (1972). Studies on the nature of inhibition by gossypol of the transformation of pepsinogen to pepsin. *J. Biol. Chem.* **247**, p. 1625–1631.

THE INACTIVE MEMBERS OF THE ASPARTIC PROTEINASE FAMILY IN THE RUMINANT PLACENTA: SPECIFICITY OF THREE DIFFERENT RADIOIMMUNOASSAY SYSTEMS

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Keywords. Aspartic proteinase, radioimmunoassay, specificity.

Pregnancy-associated glycoproteins (PAGs) have been isolated from the placenta of various ruminant species in the recent decade. Molecular biology studies showed that these glycoproteins are inactive members of the aspartic proteinase family (Xie *et al.*, 1991). Radioimmunoassay developed to detect PAGs in biological fluids (Zoli *et al.*, 1992) became important tools for

establishment of pregnancy diagnosis and pregnancy follow-up. As the PAGs share a high sequence homology with each other and with the other members of the aspartic proteinase family: cathepsin D, E chymosin pepsinogen and renin, in the present study the specificity of three commonly used RIA systems was tested.

In the three RIA systems 67 kDa PAG preparation was used as tracer (labelled with ¹²⁵Iodine according to the lactoperoxidase method) and as standard. In RIA 1, the antiserum was raised against 67 kDa PAG purified from bovine placenta. In RIA 2 and 3, antisera contained antibodies against cPAG 55+62 and cPAG 55+59 previously isolated from caprine placenta (Garbayo *et al.*, 1998). Serial dilutions ranging from 10 ng/ml to 1 mg/ml prepared from pepsin, pepsinogen, rennin and rennet in Tween Tris buffer were tested in the three systems in comparison with the PAG standard used for assays.

There was weak inhibition of binding caused by the four preparations examined in the concentration range of 10 ng/ml – 100 mg/ml. Pepsinogen caused a mild inhibition of binding in RIA 2 system at 500 mg/ml ($B/B_0=92.81\%$) and 1 mg/ml ($B/B_0=90.07\%$) concentrations. In the case of pepsin slightly

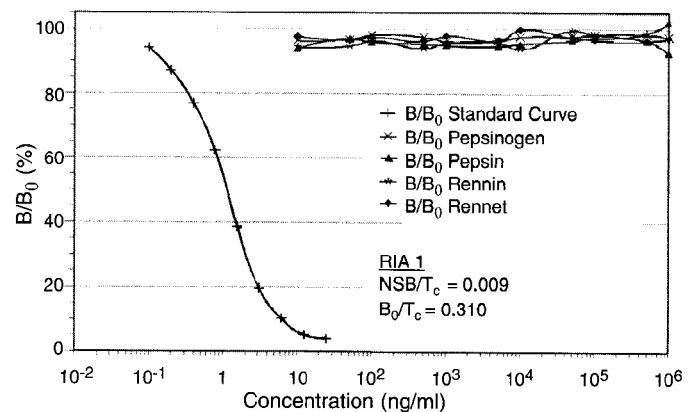


Figure 1. RIA 1: inhibition of binding by pepsinogen, pepsin, rennin and rennet.

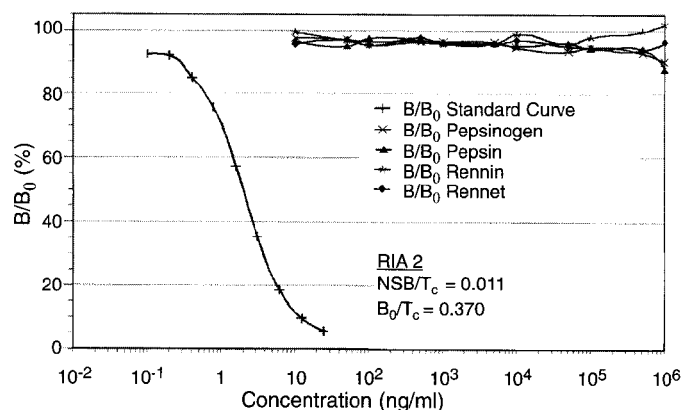


Figure 2. RIA 2: inhibition of binding by pepsinogen, pepsin, rennin and rennet.

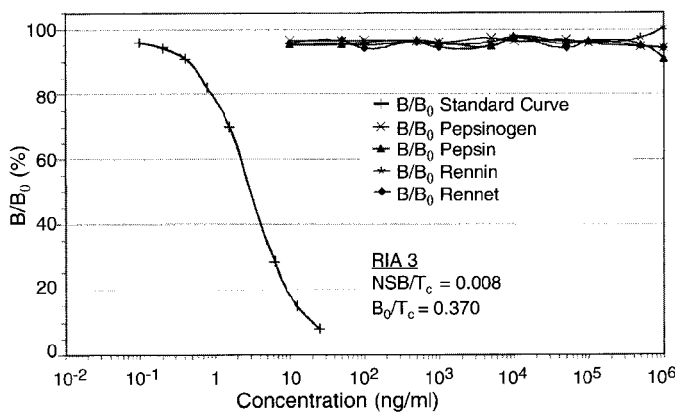


Figure 3. RIA 3: inhibition of binding by pepsinogen, pepsin, rennin and rennet.

stronger inhibition of tracer binding could be observed in RIA 1, 2 and 3 at 1 mg/ml concentration ($B/B_0 = 92.55\%$, 87.86% and 90.86% respectively).

In our experiment only pepsin and pepsinogen could decrease the binding of the radiolabelled PAG tracer to the antibodies, and were able to slightly crossreact with the antisera used. As the pepsin, pepsinogen, PAG are belonging to the aspartic proteinase family, the binding inhibition caused by the pepsin, pepsinogen can be explained by sequence identity between these products (by the ability of the antisera to recognize epitops having the same amino acid sequence). It is also possible that the enzymatically active pepsin (or the activated pepsinogen) attacks the radiolabelled PAG molecules in the solution during the incubation phase of the assay.

In physiological conditions the levels of pepsin and pepsinogen in biological fluids never reach the concentration range where these enzymes were able to crossreact with the antisera, so that RIA 1, 2 and 3 systems can be considered as specific for the detection of PAGs in the range of concentration in plasma or serum as reported in the literature.

References

- Garbayo JM., Remy B., Alabart JL., Folch J., Wattiez R., Falmagne P., Beckers JF. (1998). Isolation and partial characterization of a pregnancy-associated glycoprotein family from the goat placenta. *Biol. Reprod.* **58**, p. 109–115.
- Xie SC., Low BG., Nagel RJ., Kramer KK., Anthony RV., Zoli AP., Beckers JF., Roberts RM. (1991). Identification of the major pregnancy-specific antigens of cattle and sheep as inactive members of the aspartic proteinase family. *Proc. Natl. Acad. Sci. USA.* **88**, p. 10247–10251.
- Zoli AP., Guilbault LA., Delahaut P., Ortiz WB., Beckers JF. (1992). Radioimmunoassay of a bovine pregnancy-associated glycoprotein in serum: its application for pregnancy diagnosis. *Biol. Reprod.* **46**, p. 83–92.

RADIOIMMUNOASSAY MEASUREMENT OF INSULIN-LIKE GROWTH FACTOR-I IN CAMELS: EFFECTS OF ANTICOAGULANTS AND BODY CONDITION SCORE IN SUCKLING FEMALE

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Keywords. IGF-I, radioimmunoassay, dromedaries.

This communication reports a study of the effects of two anticoagulants and body condition score (BCS) on the blood concentration of insulin-like growth factor-I (IGF-I) in dromedary (*Camelus dromedarius*). Three blood tubes (serum, heparin, and EDTA anticoagulant) were taken from the jugular vein of twenty suckling dromedary females (Maghrebi breed) which were divided into two BCS groups (BCS was 5.2 ± 0.9 and 3.8 ± 0.6 in group 1 and group 2, respectively; $P < .0001$). Females were at 3 to 5 months postpartum. Bloods with anticoagulants were immediately centrifuged while serum samples were centrifuged after an incubation of 3 h at room temperature. Serum and plasma were conserved at -20°C until assays. The serum and plasma IGF-I concentrations were measured by a double-antibody RIA procedure. IGF-binding proteins were removed by acid-ethanol extraction. Concentration of IGF-I in samples with heparin was significantly higher ($P < .0001$) than IGF-I in serum and in EDTA samples (68.7 ± 42.6 ng/ml; 34.11 ± 12.5 ng/ml; 35.1 ± 6.9 ng/ml, respectively). IGF-I concentrations in serum and heparin plasma were significantly correlated ($r = 0.84$, $P < .0001$). Interaction between BCS level and anticoagulant was very significant ($P < .0001$). Heparin increased the detection of IGF-I in group 1 but did not affect the concentration of IGF-I in group 2 in which the concentration of IGF-I in serum was significantly lower (41.4 ± 14.2 ng/ml vs. 26.8 ± 2.9 ng/ml in group 1 and group 2 respectively). With EDTA, any difference between groups was noted (36.6 ± 7.5 ng/ml vs. 33.5 ± 6.0 ng/ml in group 1 and group 2, respectively). It was concluded that heparin increased the immunoreactivity of IGF-I probably by reducing its affinity to the binding proteins. This action was not observed with EDTA. Finally, the concentration of IGF-I is higher in animal with good body conditions score.

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A HOMOLOGOUS RADIOIMMUNOASSAY FOR QUANTIFICATION OF INSULIN-LIKE GROWTH FACTOR-BINDING PROTEIN-3 IN BLOOD FROM CATTLE

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Keywords. Cattle, insulin-like growth factor-binding-protein-3, radioimmunoassay.

Insulin-like growth factor-I and -II (IGF-I, IGF-II) circulate in biological fluids bound to at least six different IGF-binding proteins that regulate IGF bioactivity. The IGF-binding protein-3 is regulated by growth hormone, and its concentration depends on nutrition and physiological state. Unavailable from commercial societies, bovine IGFBP-3 has been previously purified from precolostrum collected 3–5 days before parturition. This preparation has been used to produce specific antibodies, and IGFBP-3 levels in bovine blood samples were quantified by radioimmunoassay. Parallel displacement curves showed strong cross-reactivity with bovine and ovine plasma and no cross-reactivity with porcine, rat, dromedary or chicken plasma. Addition of IGF-I to a control pool of bovine plasma did not significantly alter control IGFBP-3 values in a radioimmunoassay. Nycthemeral periods, determined for three young bulls bled on two occasions were stable throughout the day; two or three samples were sufficient to characterize the animal. Heifers treated with recombinant bovine somatotropin (bST) had significantly higher serum levels of IGF-binding protein-3 than did control cows. Likewise, plasma IGFBP-3 concentrations were decreased in growing bulls treated with clenbuterol (a β -agonist) while these concentrations increased after corticoid (dexamethasone) injection. Furthermore, IGFBP-3 levels were dramatically increased at the onset of puberty and decreased during the first postpartum weeks. This radioimmunoassay for bovine IGFBP-3, which enables quantitative assessment of IGFBP-3 concentration in cattle, confirmed the previous observations using the less precise Western ligand blotting method.

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DOES ANNUAL REPETITION OF OESTRUS INDUCTION INFLUENCE THE FERTILITY OF GOATS AFTER A.I. AT A FIXED TIME ?

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Keywords. Goat, fertility, oestrus, induction.

To verify the effects on various herd parameters of repeated gonadotropic treatments we followed throughout four consecutive years two herds of goats (H1=31; H2=67) annually subjected to AI after oestrus induction including progestagen administration for 11 days followed by prostaglandins and eCG. Various reproductive parameters such as occurrence of oestrus, LH surge outcoming, number of ovulations, pregnancy rates, kidding rate and prolificacy after AI at a predetermined time were recorded. Blood samples were collected before and after each treatment. These samples were screened for eCG-binding activity (BR). Results were analyzed taking the herd, number of treatment, female within the herd, eCG binding rate before treatment and eCG binding rate after treatment as independent variables. The binding rates were correlated with other parameters: herd, delay for LH surge, time of oestrus, number of ovulation, number of kids, prolificacy and fertility.

Table 1. Oestrus and repetition of treatments in goats.

Parameter	Herd	Treatment			
		1 st	2 nd	3 rd	4 th
Interval sponge	H1	24±3.9	26±2.0	22±6.5	26±8.8
Removal-oestrus (hours ± SD)	H2	17±2.0	21±3.9	20±6.7	21±7.3

Table 2. Kidding rate in goats, antibodies level and repetition of treatment.

Parameter	Herd	Treatment			
		1 st	2 nd	3 rd	4 th
Fertility (%)	H1	68.0	56.0	37.0	40.0
	H2	57.0	65.0	71.0	51.0
BR before treatment	H1	—	5.6	8.8	11.8
	H2	1.57	3.0	6.9	11.3
BR after treatment	H1	0	4.1	26.0	27.0
	H2	0	5.1	20.0	26.0

Statistical analysis of the results established a significant effect of female and treatments on eCG-binding but no effect of the herd neither of the age. We found that the antibodies significantly influenced the time of oestrus outcoming as well as the time of LH surge. No influence of the age on the time of oestrus or the time of LH surge was found. The antibodies after treatment significantly influenced the ovulation rate and the kidding rate whatever the age of the female. Finally, a significant effect of antibodies on fertility was found but no effect of antibodies on prolificacy.

The originality of this experiment resides in the fact that it was performed over 4 years on the same animals, which did not receive eCG injection before the experiment. We found a linear liaison between eCG antibodies and delay for oestrus outcoming what allowed us to confirm the variability of the onset of oestrus in dairy goats after synchronization with a progestagen/eCG treatment described by Baril *et al.* (1993) and Freitas (1997) and to reasonably postulate that the significant decrease in fertility induced by late oestrus, was to rely to the immune response against eCG. In herd 1, we observed that the repetition of treatments was associated with both increases in eCG binding and in the percentage of females that did not present LH surge. As dairy goats are inseminated at a predetermined time after the end of the progestagen administration, it is highly probable that the decrease in fertility observed after repeated treatments is due to the consequences of the reaction against eCG by increasing delay between sponge removal and oestrus/ovulation time. Baril *et al.* (1996) already suggested that the late oestrus be related to the appearance of anti-eCG antibodies with its frequency being dependent on the number of treatments the females had received previously. The same proposed a diminution of the biological activity of eCG with subsequent delayed oestrus and ovulation. We support this hypothesis by our experiment with a highly significant effect of the eCG binding on the time of apparition of oestrus (herds considered together).

References

- Baril G., Leboeuf B., Saumande J. (1993). Synchronization of estrus in goats: the relationship between time of occurrence of estrus and fertility following artificial insemination. *Theriogenology* **40**, p. 612–628.
- Baril G., Remy B., Leboeuf B., Beckers JF., Saumande J. (1996). Synchronization of oestrus in goats: the relationship between eCG binding in plasma, time occurrence of oestrus and fertility following artificial insemination. *Theriogenology* **45**, p. 1553–1559.
- Freitas VJ., Baril G., Martin GB., Saumande J. (1997). Physiological limits to further improvement in the efficiency of oestrous synchronization in goats. *Reprod. Fertil. Dev.* **9**, p. 551–556.

EFFECTS OF FSH, GH, GROWTH FACTORS AND FOLLICULAR FLUID ON OESTRADIOL-17 β PRODUCTION BY OVINE OOCYTES DURING *IN VITRO* MATURATION

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Keywords. Sheep, oocyte, maturation, Oestradiol-17 β (E2), oestrogens, steroids.

1. Introduction

The oestradiol production during *in vitro* maturation has been measured in order to correlate the cumulus-oocyte-complex (COC) secretory ability and the success of the maturation.

2. Materials and methods

During various experiments on the control of *in vitro* maturation of ovine oocytes, samples of the medium have been collected before and after the treatment and assayed for steroids (P4, Δ 4 and E2). The difference in steroid concentration was expressed in term of production by COC.

Some of these experiment's goal was to compare the effects of FSH (100 ng/ml) and/or GH (50 ng/ml) in TCM 199 with 10% follicular fluid (FF). Other's goal was to replace FF by various growth factors (IGF-1, IGF-2, FGF-2, EGF).

3. Results and discussion

The hormones and growth factors have no effect on P4 and D4 production. The results show that FF is necessary to maintain the COC ability of E2 production (**Figure 1**). FSH and GH significantly

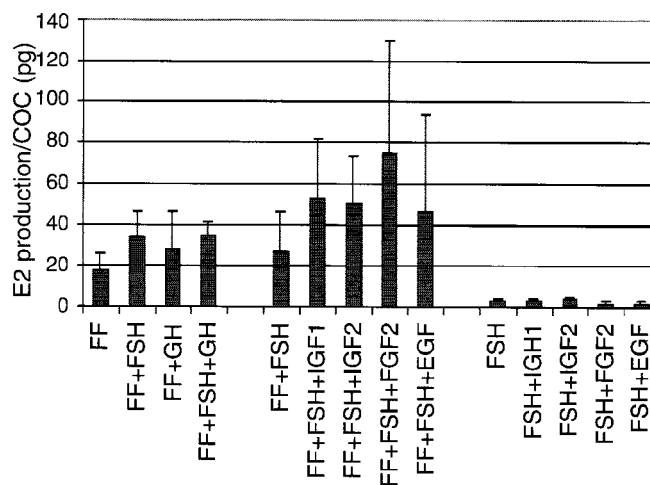


Figure 1. Composition of IVM medium.

increase the E2 production without synergistic activity. All the tested GF show a positive effect on E2 synthesis induced by FSH. These results are not strictly related to the maturation because for this phenomenon FF can be effectively substituted by GFs, especially IGF-1 and EGF (1).

References

Bister JL., Gaspard O., Paquay R. (1999). Effects of growth factors and follicular fluid on ovine oocyte maturation. *In Proc. 3rd Conference of the European Society for domestic Animal reproduction* (Anger, november 1999), p. 42.

DIRECT ACTION OF GROWTH HORMONE (GH) IN CHICKEN OVARY: EFFECTS OF STEROIDOGENESIS AND EVIDENCE OF GENE EXPRESSION OF MEDIATING GH RECEPTORS (GHR) IN THE GRANULOSA AND THECA CELLS

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Keywords. Chicken, growth hormone (GH), growth hormone receptor (GHR), ovary, quantitative RT-PCR.

There is a dearth of information concerning the effect of GH on avian ovarian function. GH injections were shown to augment reproductive activity such as increased number of growing follicles. This is an example of indirect evidence of GH action on the ovary. Direct evidence such as localization of GHR or effect of GH in *in vitro* experiments with follicular cells is scarce. Against this background, we have

examined the effect of human GH on chicken granulosa and theca cell steroidogenesis. Furthermore, we examined the gene expression of GHR in these cells and quantified the mRNA of GHR in developing follicles by competitive RT-PCR.

GH increased the progesterone production by granulosa cells or androstenedione by theca cells from F1, F2 and F4 follicles. The GH effect was significantly lower than the effects of IGF-I or LH. GH enhanced LH-stimulated progesterone and androstenedione production. The effect of GH in combination with LH was significantly lower than the effect of LH with IGF-I. The effect of GH in combination with IGF-I was far less than the effect of GH with LH or that of LH with IGF-I or GH with LH. The combined addition of GH and IGF-I resulted in a lower stimulation than GH and LH combined or LH together with LH.

The GHR/GHBP expression was studied by RT-PCR in both the granulosa and theca cell layers of the F1, F2, F3 and F4/5 follicles. The expression of the GHR gene was demonstrated using two specific RTPCR's, one for extracellular (GHRe) and another for the intracellular (GHRi) part of the GHR cDNA parts. Using a specific internal standard, that we developed for competitive RT-PCR, the quantification of the GHRe showed a decline in the granulosa cells with follicular development, whereas in the theca, GHRe increased with follicular development. Overall, GHRe mRNA was more abundant in granulosa cells than in the theca of all follicles studied. Surprisingly, the levels of expression of GHRe in the granulosa of developing follicles were inversely related to the levels of progesterone production whereas in the theca there was some positive correlation between GHRe levels and androstenedione production. It is speculated that GHRe levels in follicular cells may be more related to follicular cell proliferation rate, as this may involve the IGFs, than to steroidogenic activity. Of course it shouldn't be forgotten that GHRe mRNA can also give rise to GHBP instead of GHR through alternative splicing. We conclude that GH influences, directly, chicken granulosa and theca function through its receptors which are present in the cells.

List of institutions' abbreviations

FNRS	Fond National de la Recherche Scientifique
BTC/CBT Belgium	Coopération Technique Belge
CAPES/Brazil	Fondation de Coordination pour le Perfectionnement du Personnel de l'Enseignement Supérieur
IRA/Tunisie	Institut de Recherches Agronomiques
DGCI/Belgium	Direction Générale pour la Coopération Internationale