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Pregnancy-associated glycoprotein (PAG) pattern and pregnancy detection in Boer goats using an ELISA with different antisera

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ABSTRACT

Pregnancy-associated glycoproteins (PAGs) are macromolecules produced by placental tissue and released into the maternal circulation where they allow pregnancy diagnosis and follow-up. The present study addresses the question to what extent plasma PAG determination may serve as a means of early pregnancy detection in goats in a similar way it is practiced in cows, and whether an ovine or bovine PAG-ELISA may be utilized to this end. Blood samples were collected from eight pregnant pluriparous Boer goat does twice weekly during the first seven weeks and the last four weeks of pregnancy and weekly in-between and during four weeks following parturition. Plasma PAG concentrations (mean ± SEM) were determined using a competitive enzyme-linked immunosorbent assay. Assays were conducted with polyclonal antisera raised in rabbits against purified preparations of caprine (AS#706), ovine (AS#780) and bovine PAG (AS#726). In the assay systems purified bovine PAG served as standard and tracer and goat anti-rabbit IgG served as coating antibody. With the antibody raised against caprine PAG (AS#706) a steep increase to a climax of 69 ± 9 ng/ml on day 56 of pregnancy was followed by a gradual decline to 16 ± 3 ng/ml at parturition and 0.3 ± 0.07 ng/ml four weeks postpartum. The results achieved with the anti-ovine PAG (AS#780) showed close similarity, a maximum of 92 ± 14 ng/ml being reached at 56 days of pregnancy. With anti-bovine PAG (AS#726), the PAG level increased to a maximum of 3.1 ± 0.2 ng/ml on day 105 of pregnancy and fluctuated around 3 ng/ml until the end of pregnancy. The difference between pregnant and non-pregnant does reached a significant level 21 days after conception, one week earlier than with caprine and ovine antisera.

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1. Introduction

The availability of a means of early pregnancy diagnosis is of practical relevance in the goat business. In cows the most common way of pregnancy detection is by rectal palpation. For morphological reasons this method is not applicable in goats. Apart from observing the return to estrus, the most common means of diagnosing pregnancy in goats are transrectal or transabdominal ultrasonic scanning (Martınez et al., 1998; Padilla-Rivas et al., 2005), progesterone measurement in blood or milk (Agwu and Holtz, 1986), estrogens in blood (Dhindsa et al., 1981; McArthur and Geary, 1986; Sindermann et al., 1992) or feces (Holtz, 1992; Sindermann et al., 1992; Ledezma-Torres, 2002) and, more recently, the determination of pregnancy associated glycoprotein (PAG) in blood (Sousa et al., 1999; Gonzalez et al., 1999; Batalha et al., 2001) or milk (Gonzalez et al., 2001). PAG may be measured by radioimmunoassay (RIA) (Sasser et al., 1986; Zoli et al., 1992) or enzyme-linked immunosorbent assay (ELISA)

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using monoclonal (Green et al., 2005) or polyclonal antibodies (Friedrich and Holtz, 2004, 2010). The aim of the present study was to (a) establish a PAG pregnancy profile for Boer goats, (b) determine from what stage of gestation onward plasma PAG may serve as a reliable diagnostic tool and (c) establish whether PAG in goat serum may be detected by an assay based on antibodies raised against ovine or bovine PAG.

2. Materials and methods

The investigation was conducted on Boer goats of the departmental flock of Goettingen University. The animals were group housed in open barns with straw bedding and outdoor concrete runs. Does were fed a daily ration of 600 g concentrate, consisting of equal parts of a pelleted diet for breeding ewes (16% crude protein, 12.2 MJ metabolizable energy/kg, supplemented with Se, I and Zn), oats and dried sugar beet pulp and had free access to straw, salt lick and water.

From eight pregnant does blood samples of 4 ml were drawn by jugular venipuncture twice weekly during the first seven and the last four weeks of pregnancy and weekly in-between and during four weeks following parturition. By way of comparison, blood samples were drawn from nine non-inseminated does twice weekly for seven weeks after estrus. Collecting tubes contained three drops of sodium citrate to prevent clotting. After centrifugation at $2000 \times g$ for 10 min at 4 °C, plasma was stored at −20 °C until being assayed.

Three ELISA systems were used to measure PAG concentration in Boer goats. Plasma concentrations of PAG were first determined by homologous competitive enzyme-linked immunosorbent assay (ELISA) in the way described in Friedrich and Holtz (2010). Briefly, PAG antiserum AS#706 raised against a purified caprine PAG preparation ($caPAG_{55+62\,kDa}$; Garbayo et al., 1998) served as specific antibody, whereas purified bovine PAG ($boPAG_{67\,kDa}$, Zoli et al., 1991) was used as standard and tracer. Two additional heterologous polyclonal antibodies (named AS#780 and AS#726), raised against ovine PAG ($ovPAG_{57+59\,kDa}$; El Amiri et al., 2003) (AS#780) and bovine PAG ($boPAG_{67\,kDa}$) (AS#726), respectively, were used. The respective antisera were diluted in assay buffer (0.1% casein, 0.005 M NaOH, 0.12 M NaCl, 0.02 M Na_2HPO_4, 0.01 M EDTA, 0.002% phenol red, 0.005% chlorhexidine digluconate (20%), pH 7.3) at a ratio of 1:200,000 (AS#726), 1:320,000 (AS#706) and 1:80,000 (AS#780), respectively. Volumes of 100 μl/well were added to goat anti-rabbit coated microtiter plates (Nunc Maxisorp®, Thermo fisher, Germany). The plates were incubated overnight at 4 °C. Standard curves were prepared from purified bovine PAG diluted in PAG-free serum at concentrations of 0.0, 0.39, 0.78, 1.56, 3.125, 6.25 and 12.5 ng/ml, respectively. Of the tracer (biotinylated $boPAG_{67\,kDa}$, diluted 1:1000 in assay buffer), 50 μl was added to each well, followed by 90 min of incubation at room temperature. After two washings (washer: Columbus Plus, Tecan, Germany) 100 μl/well streptavidin-peroxidase (50 μg/ml) and, after four more washings, 150 μl/well 3,3′,5,5′-tetramethylbenzidine (12.5 mg/ml DMSO, Sigma) were added, followed by 30 min incubation at room temperature in the dark. Optical density was measured by Tecan Sunrise® photometer with software MAGELLAN 4.0 (Tecan) at wave length 450 nm. Concentrations were calculated using a logit-log transformation according to Rodbard (1974).

Means and SEM concentrations were calculated using Proc means in SAS 9.1 software (SAS institute Inc., Cray, NC). Using software JMP IN (6.0.0), PAG concentration of pregnant and non-pregnant animals was compared using Dunnet's *t*-test, whereas the difference in PAG concentration of various antisera at different stages of pregnancy and post partum period was tested for significance by Student's *t*-test.

3. Results

The PAG profile of eight pregnant goats (two bearing singletons, five bearing twins and one bearing a triplet) established with an ELISA based on an antiserum raised against caprine PAG was characterized by a rapid increase to a climax of 69 ± 9 ng/ml arrived at 56 days after conception, followed by a gradual decline to 16 ± 3 ng/ml at parturition and 0.3 ± 0.07 ng/ml four weeks postpartum (Fig. 1). The plasma PAG concentration of the singleton-bearing does was between 25% and 40% (at the climax of the curve) below that of does bearing multiple fetuses. The PAG profile established when using antiserum raised against ovine PAG closely resembled that obtained with antiserum raised against caprine PAG, though at a slightly higher level (Fig. 2). A peak value of 92 ± 14 ng/ml was reached on day 56. The curves only differed significantly on days 49, 56 and 84 of pregnancy ($P < 0.05$). When using an assay system based on antiserum raised against bovine PAG, levels resembled those of the other tests until the second week of pregnancy. The subsequent increase, however, was rather modest; a maximum of 3.1 ± 0.2 ng/ml was reached on day 105 (Fig. 2). When changing the scale of the ordinate (Fig. 3) it became evident that, with antiserum raised against bovine PAG, the pattern differed from that observed when using caprine or ovine antisera. After an initial increase between days 14 and 28 of pregnancy, the PAG concentration fluctuated around a value of 3 ng/ml until parturition without a marked increase and declined gradually thereafter. Table 1 describes parameters characterizing the different PAG patterns. When using antiserum raised against caprine and ovine PAG the difference between pregnant and non-pregnant does reached significance levels on day 28. When using antiserum raised against bovine PAG, much lower levels were recorded, but the difference

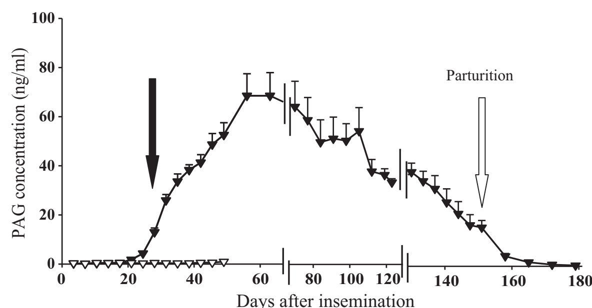

Fig. 1. PAG profile (mean ± SEM) of 8 pregnant (closed triangles) and 9 non-pregnant (open triangles) Boer goat does assessed by an ELISA based on caprine antiserum raised against caprine PAG (AS#706). Data are arranged around the times of mating and parturition. The black arrow signifies the point at which pregnant and non-pregnant does differed significantly ($P < 0.05$).

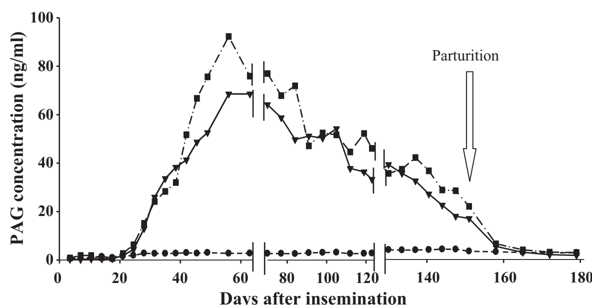

Fig. 2. Mean PAG profiles of 8 pregnant Boer goat does assessed by ELISAs based on antisera raised against caprine (AS#706, triangles), and ovine (AS#780, squares) and bovine PAG (AS#726, circles). Data are arranged around the times of mating and parturition.

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