PLASMATIC PROFILES OF PREGNANCY-ASSOCIATED GLYCOPROTEIN AND PROGESTERONE LEVELS DURING EARLY PREGNANCY IN CARPATHIAN GOAT

Stela Zamfirescu, Andreea Anghel, Dorina Nadolu, N. Dobrin

FACULTY OF NATURAL AND AGRICULTURAL SCIENCES, “OVIDIUS” UNIVERSITY, CONSTANTA, ROMANIA

Summary

The choice of an adequate manner of breeding and exploiting small ruminants which will lead to the increased reproduction efficiency and also to the increased number and quality of these animals depends on the determination of an early diagnosis of gestation. This study was carried out to determine caprine pregnancy-associated glycoprotein (cPAG) and progesterone (P4) levels in the plasma of Carpathian goats throughout gestation. The cPAG levels were determined with a heterologous RIA. The P4 levels were measured by RIA. The statistical analysis of the PAG concentration values in goats with known reproduction grouped females into 4 groups, namely: pregnant RIA-clinically pregnant (positive diagnosis), non-pregnant RIA-clinically non-pregnant (positive diagnosis), non-pregnant RIA-clinically pregnant (incorrect diagnosis) and embryonic mortality. The results obtained demonstrated that the precision of the correct diagnosis for pregnancy and non-pregnancy on days 14-35 from mating was 92.85%, while the RIA-PAG error was 7.15%. Embryonic mortality was highlighted in 8.11% of the females and occurred in the interval of 25-35 days. On days 14-25 of pregnancy, the values of the serum progesterone were close (12.18-13.33 ng/ml), which confirms that the unique source of progesterone is the gestation corpus luteum. The results obtained led to the conclusion that the quantitative assessment of proteins associated to gestation (PAG) by the RIA technique can be used as a sure method to establish early gestation in goats. The presence of a large quantity of gestation proteins in Romanian goat breed confirms gestation on day 14 after mating, while the sudden drop of PAG values on day 30 after mating confirms the occurrence of embryonic mortality. In what regards the progesterone concentration, the precision of the gestation diagnosis is at its maximum on days 14-35 of pregnancy.

Keywords: goat, PAG, progesterone, pregnancy

zamfirescustela@yahoo.com

Introduction

An early and accurate diagnosis of pregnancy is of considerable value in improving efficiency of production in dairy goatherds. Progesterone (P4) and pregnancy-associated glycoproteins (PAG) were proposed as laboratory tools to establish or to confirm pregnancy diagnosis. In last years, PAG assay also provided useful information for researchers working in programs focused on the follow up of trophoblastic function. Three types of PAG were isolated in goats. They were named according to their molecular weight: PAG 55, PAG 59 and PAG 62 (Beckers et al., 1994). These reach a maximum concentration 8 weeks after mating and remain high till parturition, similarly to sheep (Folch et al., 1993). Humblot et al. (1988) demonstrated that the PAG level in goats is double in the case of twin or triple gestation. Also, embryonic mortality was correlated to low PAG levels, under the levels of positive gestation. Thus, the application of this test on goats with uncontrolled reproduction would indicate embryonic mortality (Batalha et al., 2001).

The purpose of the research was to identify the presence of PAG in an early phase of the gestation and to correlate it with progesterone levels in the plasma of local goat breeds.
Material and methods
The studies were conducted at the experimental farm of the ANCC Caprirom and the biochemical determinations were realized on the Ovidius University - Laboratory of Cell and Molecular Biology. The researches were accomplished on 84 goats which were monitored from the beginning of estrus and till parturition. The plasma samples were collected starting with day 0 (insemination day) and then on days 7, 14, 20, 30 and 35 after mating. Blood samples (5 mL) were withdrawn from the jugular vein into heparinized vacutainer tubes. The plasma was separated within 1 hours after collection by centrifugation at 1500 x g for 20 min, and then stored at -40°C until assayed for progesterone and PAG concentrations.

1. PAG Radioimmunoanalysis. The radioimmunologic analysis for PAG detection was accomplished in the Laboratory of Reproduction Physiology – The Faculty of Veterinary Medicine in Liege, Belgium. The PAG concentrations were determined by the radioimmunoanalysis of the double heterological antibodies (Zoli et al., 1992). The sediment radioactivity was measured with the help of a Gamma counter (PerkinElmer 2470, USA) with 75% efficiency.

2. Progesterone analysis. The determination of plasmatic progesterone was accomplished with DRG-progesterone kits (Germany) by the double-anitbody radioimmunoassay according to Ranilla et al. (1994).

Results
The gestation diagnosis can be considered early when it is established 14-20 days after mating, it is correct for the determined moment, but its value may decrease because 21-30 days after mating there are frequent embryonic deaths.

After kidding on time, the goats were separated into 2 groups, pregnant (including all the females that became pregnant after the mating that occurred on estrus day) and non-pregnant (including the females that did not kid after the mating that occurred on day 0).

On estrus day, the PAG value was between 0.036±0.016 ng/ml and 0.58±0.37 ng/ml. On day 7, the PAG value increased to 1.13±0.5 ng/ml in the pregnant goats, and to 1.52±0.57 ng/ml in the non pregnant ones. Very rarely, values over 3-5 ng/ml occurred on day 7 after fertilization. One of the causes for this could be the presence of several preimplantational embryos in the uterus. Embryonic death can occur immediately after implantation (day 14) or later, 20-30 days after mating (Fig. 1).

Concentrations increase faster from week 3 to 4, reaching higher levels during the first month of pregnancy (up to 20ng/mL). Reliable techniques for early detection of pregnancy are valuable aids in culling or rebreeding of barren does, and are valuable tools for control breeding programs. The method of choice to diagnose pregnancy in goats will depend upon the availability of equipment, number of days post-breeding if known, and the desired accuracy. Our results were comparable to with those obtained by other authors. On pregnant sheep and goats, PAG concentrations are detectable from days 17 to 18 after mating, reaching 3 to 5 ng / ml around day 21-22 (Folch et al., 1993; Sousa et al., 1999). PAG concentrations increased until the 8th week.
of gestation (30-50 ng / ml), then to fall between the 12th and 14th week (16-32 ng / ml) and remain relatively constant before calving (Gonzalez et al., 1999).

Regarding determination of plasma proteins of gestation for the diagnosis of pregnancy in goats, Gonzalez et al. (2004) described a sensitivity of 94.9% and a specificity of 100% for a dose conducted on samples in the 22 th day of gestation.

The progesterone levels were 12.181ng/ml on day 14 and 16.62 ng/ml on day 35, with minimums of 6.907 ng/ml and maximums of 19.052 ng/ml (fig. 2). On days 14-25 of pregnancy, the values of serum progesterone were close (12.18-13.33 ng/ml), which confirms that corpus luteum is the only source of progesterone.

At estrus, progesterone values were 0.27-0.91 ng/ml, with high variation coefficients, demonstrating thus the great individual variability or the presence of many corpora lutea on the ovaries. Embryonic death was highlighted in 8.11% of the females and occurred in an interval of 25-35 days.

**Conclusion**

The results clearly demonstrate that, for greater accuracy of diagnosis of pregnancy is necessary to know the moment "0" (estrus) of the female. Diagnostic accuracy can be influenced by fetal losses occurred after 30 days of fertile mating, but can be easily noticed when monitoring pregnant females is correct.

The results obtained demonstrated that the precision of the correct diagnosis for pregnancy and non-pregnancy on days 14-36 after mating was 92.85%, while the RIA-PAG error was 7.15%. Certainly, large PAG concentrations appear after embryonic nidation in females. Increases of over 3-5 ng/ml on day 7 after fertilization occurred very rarely; one of the causes for this may be the presence of more than one preimplantational embryos in the uterus. In veterinary practice, PAG concentration may become useful both to determine pregnancy and for monitoring trophoblast function.

**Acknowledgements**

This paper was accomplished within the project PN2 51057 financed by Education, Research, Youth and Sport Ministry -Bucharest. We would like to thank Ms. Olivia Chirobocea for the translation of this paper from Romanian into English.

**References**


