

**FUNCIÓN DE LAS GLICOPROTEÍNAS ASOCIADAS A LA GESTACIÓN (PAGs)
Y PROGESTERONA EN DIAGNÓSTICO PRECOZ DE LA GESTACIÓN Y
MORTALIDAD EMBRIONARIA PRECOZ EN VACAS**

**ROLE OF PREGNANCY ASSOCIATED GLYCOPROTEINS (PAG) AND
PROGESTERONE IN EARLY PREGNANCY DIAGNOSIS AND EARLY EMBRYONIC
MORTALITY IN COWS**

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ABSTRACT

The aim of this study was to investigate the role of pregnancy associated glycoproteins (PAG) and progesterone in early pregnancy physiology in cows as well as possibility of using PAG as diagnostic tool in diagnosis of embryonic mortality.

In our research we used 74 cows of simmental breed aged 3-7 years, already used in reproduction and calved every year. We divided cows in 3 groups: pregnant cows (n=30), nonpregnant cows (n=20) and cows experienced embryonic mortality (n=24). According to ultrasound (17 and 35 days after AI) and laboratory findings for progesterone and PAG in sera samples collected between 12. and 45. days after AI every 72 h, we concluded:

It is impossible to determine embryonic mortality on basis of progesterone profil, but it is easy to distinguish pregnant from nonpregnant cows, if cows are supposed to be pregnant more than 21 day.

In group of cows with embryonic mortality, it occurred 17-24 days after conception in 98% cows. It was visible in drastic decrease of PAG 7,5-9 days later (7,5-9 days consists T₂ for PAG in first month of pregnancy). Using PAG for pregnancy diagnosis enables us to prove existence of alive vital embryo in uterus 24 days after conception.

RESUMEN

Este artículo examina la función de las glicoproteínas asociadas a la gestación (PAGs) y progesterona en fisiología precoz de la gestación en vacas. También trata la posibilidad de utilizar PAGs como herramienta de diagnóstico en la mortalidad embrionaria precoz.

Nuestra investigación incluyó 74 vacas Simenthal, de 3-7 años de edad, ya utilizadas en reproducción y paridas cada año. Las vacas fueron divididas en 3 grupos: vacas gravidas (n=30), vacas no gravidas (n=20) y vacas que sufrieron mortalidad embrionaria precoz (n=24). Según los resultados de las pruebas de ultrasonido (17 y 35 días después del IA) y de las comprobaciones en laboratorio de cada 3 días de suero para progesterona y PAGs (entre 12 y 45 días después del IA) concluimos:

Es imposible determinar la mortalidad embrionaria precoz solamente con un perfil de progesterona. En cambio, es fácil distinguir vacas gravidas de las que no lo están, si la gestación dura más de 21 días.

En el tercer grupo de vacas, la mortalidad embrionaria precoz se dio en el 98% de los casos entre 17-24 días después de la concepción. Se dio una visible disminución drástica de PAGs 7,5-9 días después (T₂ de PAGs en el primer mes de gestación son 7,5-9 días).

El uso de PAGs como diagnóstico de gestación nos permite mostrar la existencia de un embrión vivo y vital 24 días después de la concepción.

INTRODUCTION

Pregnancy associated glycoprotein has been found in the serum of pregnant cattle and used as a pregnancy marker (Perenyi, 2002). As pregnancy failure occurs, PAG concentrations dropped and disappeared from maternal blood. Sequential assays of these proteins between day 24 and term are useful for studying the course of pregnancy, although it does not allow discrimination between early embryonic mortality and non-fertilization (Mialon et al., 1993). As PAG molecules are the products of the trophoblastic cells, it was suggested that their determination in maternal blood can be useful for prediction of fetal well-being and help to detect early placental abnormalities, embryonic mortality or abortion (Ectors et al., 1996.). Heifers expressing pregnancy failure between days 16 and 35 after AI have shown wide variation in pregnancy specific protein 60 (PSP60) concentrations at days 28 and 35. This phenomenon was explained by the variation in the time of embryo death (Mialon et al., 1993). This author reported some cases where at day 50 ultrasonic scanning and signs of oestrus indicated that the embryo was already dead, however till this day the PSP60 concentrations were increasing. Decreasing PSP60 concentrations were observed in some animals before day 50, were according to ultrasonic examination, the conceptus was still alive.

Pregnancy specific protein B (BPSPB) test in conjunction with milk progesterone test was used to determine the time of the embryonic death (Humblot et al., 1988). In most of the early failures an elevation in the PSPB concentrations was not detectable, or only after day 24. No coefficient of correlation between PSPB and progesterone concentration was significant at any days of gestation studied. Significant difference was found in the rate of mean PSPB levels between pregnant cows and cows showing late embryonic mortality between days 24 and 30-35 after AI. In their study it was not possible to predict the occurrence of embryonic mortality.

After invitro production of embryos or cloning, the PAG follow up in plasma samples collected weekly was able to monitor embryonic or fetal mortality (Ectors et al., 1996, 1997). In that studies it was concluded that significantly increased PSP60 levels could indicate abnormal placental development.

In veterinary practice, PAG and PSPB RIAs in biological fluids are helpful to confirm clinical diagnoses established by ultrasonography (Szenci et al., 1998.) Cows presenting an initial positive and then negative ultrasonographic diagnosis expressed decreasing PAG concentrations (Szenci et al., 1998) The high progesterone and PAG concentrations confirmed the presence of a live conceptus and a functional corpus luteum at the time of the first ultrasonographic examination. After the time of embryonic mortality occurred, the PAG levels had already decreased, while progesterone concentrations have shown that the

corpus luteum was maintained in three of four cases. Following embryonic mortality using ultrasonography, PAG and PSPB concentrations decreased steadily, in one animal these protein concentrations did not reach the threshold level (Szenci et al., 1998). Mentioned authors were able to diagnose late embryonic and fetal mortality cases retrospectively in seven cows using PSPB RIA test and in four cows using PAG RIA test (Szenci et al., 2000).

Because of the large individual variations in PAG concentrations in maternal blood, only the marked decrease or disappearance in serum/plasma concentrations of these proteins can be a no controversial, predictive sign of embryonic or fetal death.

MATERIAL AND METHODS

Our research included 74 simmental cows, 3-7 years old, which were regularly used in reproduction and calved every year. According to ultrasound findings (17. and 35. day after artificial insemination; AI) the cows were divided in 3 groups: pregnant cows (n=30), nonpregnant cows (n=20) and cows suffered embryonic mortality (n=24). Cows which were suspect to be pregnant by both examinations were divided into pregnant group. Cows who were considered to be pregnant according to first ultrasound finding and nonpregnant according to the last were divided into embryonic mortality group. Cows considered to be nonpregnant by both ultrasound findings were divided into nonpregnant group. Blood samples were collected every 72 hours between 12. and 45. day after AI and level of progesterone and pregnancy associated glycoproteins (PAG) were determined. We used double RIA assay to determine level of PAG according to method described by Zoli (1992) and modified by Beckers (1995). Sensitivity and specificity of method consisted 80%. Level of progesterone was determined using ELISA method and Ovuchek commercial kits (Biomedica). Sensitivity and specificity of method consisted 86%.

RESULTS

Table 1: Middle values for PAG level in blood from day 12 till day 45 after AI every 72 h for all groups

Number of sampling	Pregnant cows	Embryonic mortality	Nonpregnant cows
1	0,894656	1,115524	0,011167
2	1,453688	0,905095	0,019889
3	2,103688	0,577143	0,006222
4	2,700969	0,41981	0
5	3,520063	0,405	0

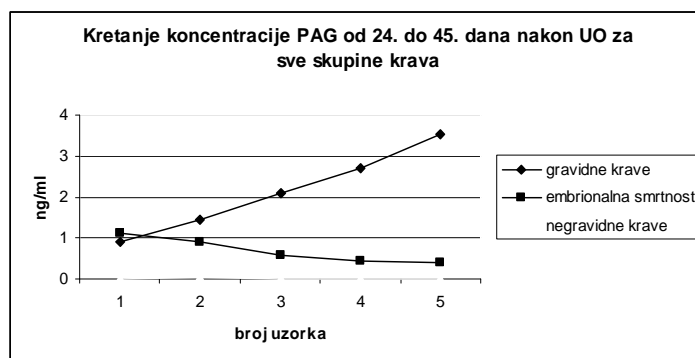
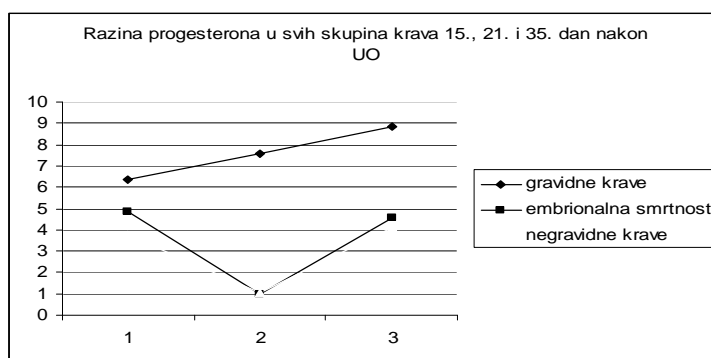


Table 2: Middle values for progesterone level in blood at day 12., 21. and 35 after AI

Number of sampling	Pregnant cows	Embryonic mortality	Nonpregnant cows
1	6,379625	1,115524	3,761444
2	7,587156	0,905095	1,019222
3	8,869406	0,577143	4,108048



CONCLUSIONS

According to ultrasound (17 and 35 days after AI) and laboratory findings for progesterone and PAG in sera samples collected between 12. and 45. days after AI every 72 h, we concluded:

It is impossible to determine embryonic mortality on basis of progesterone profil, but it is easy to distinguish pregnant from nonpregnant cows, if cows are supposed to be pregnant more than 21 day.

In group of cows with embryonic mortality, it occurred 17-24 days after conception in 98% cows. It was visible in drastic decrease of PAG 7,5-9 days later (7,5-9 days consists T_2 for PAG in first month of pregnancy). Using PAG for pregnancy diagnosis enables us to prove existence of alive vital embryo in uterus 24 days after conception.

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