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Research Article

Pregnancy Associated Glycoproteins are an Option in Early Pregnancy Diagnosis in Cattle

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Abstract

Introduction: Determining pregnancy in a short time after breeding not only increases productivity in cattle but also is important in terms of shortening the time between two calving periods. This study aimed to comparatively investigate the effectiveness of using visual PAGs ELISA test as an alternative to ultrasonography used in determining early pregnancies in Simmental breed cattle.

Methods: This study was carried out with 88 Simmental heifers and 75 uniparous cattle. On the 28th day following artificial insemination, the pregnancies of the animals were checked by both the rapid visual pregnancy test kit that provides results based on PAGs levels and an ultrasonography device with a 5-MHz linear probe, and the results of both methods were compared.

Results: When the results obtained in this study in terms of the pregnancies on the 28th day were compared, according to the visual PAGs ELISA test, 78 of the 163 cattle were determined as pregnant, while 71 of them were determined as pregnant with the method of ultrasonography. While the results were numerically different, the difference was not statistically significant ($P > 0.05$). In the control examinations made on the 35th day, the pregnancy results were determined as 77/163.

Conclusion: It was observed that pregnancies could be determined with accuracy on the 28th day after fertilization as long as contamination between wells is checked while applying the visual ELISA PAGs test that provides results based on maternal PAGs levels. It was concluded that the test may be utilised as a different option alongside ultrasonography in determining the diagnostic limits of early pregnancy, and its accuracy rate is 100% especially in determination of individuals that are negative for pregnancy.

Keywords: *Cattle; Pregnancy Associated Glycoproteins; Pregnancy; Ultrasonography*

Introduction

Early and correct identification of pregnancy is an important component of reproductive management at dairy cattle industry [18]. Determining pregnancy in a short time after breeding not only increases productivity in cattle but also is important in terms of shortening the time between two calving periods [17]. This is why pregnancy diagnosis methods used in determination of early pregnancies are desired to have a high accuracy rate and be practical, easily applicable and inexpensive [15]. While several pregnancy identification methods have been used in cattle, transrectal ultrasonography has been one of the most prominent methods since 1980s [22]. However, it is stated that mistakes may be made in identification of pregnancy by ultrasonography on the 27th - 28th days [9]. By the formation of pregnancy, in

many animal species and humans, some specific proteins and hormones either appear for the first time or show an increase in quantity in the maternal blood circulation. The reason for this change in the maternal blood circulation is caused by the foetus and placenta rather than the mother [6]. With the emergence of pregnancy and by the time a maternal connection is achieved between the mother and the calf, pregnancy associated glycoproteins (PAGs) are introduced to the maternal blood circulation by secretion in cotyledons and mononucleate and binucleate trophoblastic cells between intercotyledons [24]. PAGs have been determined for the first time as the pregnancy-specific protein B as a member of placenta-originated antigens which are a broad family [5]. It is stated that its increase in the maternal blood circulation especially on the 24th day and onwards after fertilization is an important marker for assessment of pregnancy and embryo vitality [25]. Moreover, it has been reported that test results taken on determination of PAGs may show differences among species and even among different breeds in the same species [16] and even the number of offspring during pregnancy is effective [8]. In recent years, several studies have focused on determination of pregnancies based on PAGs levels in the maternal blood, and highly effective results have been obtained [23].

Aim of the Study

This study aimed to comparatively investigate the effectiveness of using visual ELISA PAGs as an alternative to ultrasonography used in determining early pregnancies in Simmental breed cattle.

Methods

Study material and synchronisation program

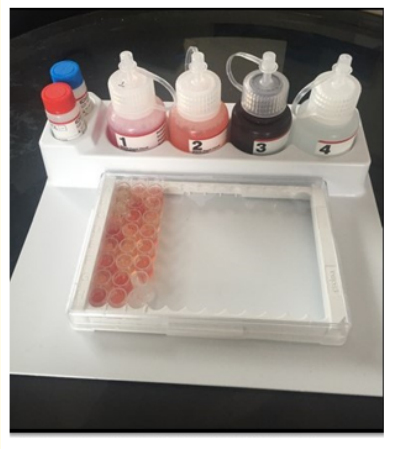
This study was carried out with 88 Simmental heifers at the ages ranging between 14 and 20 months (mean: 17.1 months) and 75 uniparous cattle at the ages ranging between 26 and 30 months (mean: 29.4 months) under THS livestock in Gölbaşı in Ankara, Turkey. The animals at the farm were those that did not have any genital or contagious diseases, and at the same time, this farm had a disease-free certificate. The animals were housed in a semi-open barn and fed with a mixed feed given by calculation based on their dry matter needs. Their water needs were met ad libitum. Before artificial insemination, a part of the heifers (n: 56) were synchronised with the protocol named Day 5 (GnRH-5d-PGF2α-1d-PGF2α-44h TAI and GnRH), while the others (n: 32) were synchronized by PGF2α (PGF2α-11d-PGF2α-81h TAI and GnRH) applied with an 11-day interval. In the cattle (n: 75), for the purpose of synchronisation, the Ovsynch protocol (GnRH-7d-PGF2α-2d-GnRH-18h TAI) was used. After synchronisation, artificial insemination was applied on the animals with the recto-vaginal method. For artificial insemination, sperm obtained from two different commercial companies (Masttering Genetic, Hohenzell, Austria; Coopex, Bezançon, France) was used. While organizing the animal experiments and during the study, the protocols were carried out based on the European Parliament and European Council directives of 2010/63/EU on the use of animals for experimental purposes.

Pregnancy determination

On the 28th day following artificial insemination, the pregnancies of the animals were checked by both the rapid visual PAGs ELISA test kit (IDEXX Europe, Hoofddorp, the Netherlands) that provides results based on PAGs levels and an ultrasonography device with a 5-MHz linear probe (ImaGo S, IMV, France), and the results of both methods were compared. Determination of the allantois fluid and foetus in ultrasonography was assessed as positive pregnancy. By conducting the ultrasonography process applied on the 28th day again on the 35th day, whether or not there were false results was checked.

For the PAGs test, blood was collected from vena coccygea with the help of vacuum tubes and vacutainer, and it was kept at room temperature for 3 hours to obtain blood serum. After waiting, by tempering with the tubes, it was ensured that the blood serum ascended to the top of the tube. The test kits were normally kept in a refrigerator at 4°C and brought to room temperature (22°C) by taking out of the refrigerator before usage. Afterwards, the plate that contained the wells where the test would be carried out was prepared. So that

one of the first two wells would include negative control and the other positive control at the amounts of 100 µL, the other wells were filled individually with the blood serums obtained from the animals at the same amounts by using an automated pipette. Afterwards, by putting 3 drops of reagent 1 (detector solution) on each of these wells, and by ensuring that there would be no contamination between wells, the solutions inside these wells were mixed with slow movements, and 7 minutes were given for the reaction, after which the wells that were used for testing were washed with twice-distilled water for three times. After this washing process, the same wells were added respectively reagent 2 (conjugate solution), reagent 3 (3, 3', 5, 5'-tetramethylbenzidine; TMB substrate), and the process was repeated for the same duration in the same way. After the washing process of the reagent 3 solution, by putting reagent 4 (stop solution), the test was ended (Picture 1). After this, by comparing the colour changes in the wells representing each animal to the positive and negative control wells, the pregnancy statuses of the animals were assessed. Colour changes from light blue to dark blue as in the positive control group in the wells were accepted as positive pregnancy. No colour change as in the negative control group was accepted as negative for pregnancy (Picture 2). The obtained results were re-checked by ultrasonography on the 35th day.



Picture 1: *Visual pags elisa test kit.*

Picture 2: *Colour changes of samples.*

Statistical analysis

Analysis of the data were performed by using a computer software SPSS for windows (version 22.0). Firstly, determined the groups normality and homogeneity test. Than effect of methods (PAGs; ultrasonografi) on pregnancy rates were computed using chi-square analysis. The correlation of methods was tested with Pearson correlation test. The level of significance was held at $P < 0.01$ to show statistically significant differences among variables.

Results and Discussion

When the results obtained in this study in terms of the pregnancies on the 28th day were compared, according to the visual PAGs ELISA test, 78 of the 163 cattle were determined as pregnant, while 71 of them were determined as pregnant with the method of ultrasonography. While the results were numerically different, the difference was not statistically significant ($P > 0.05$). In the control examinations made on the 35th day, the pregnancy results were determined as 77/163. While the specificity and reliability of both methods were high, it was determined that the presence of PAGs is a significant pregnancy identification method in assessing pregnancy on the 28th day. In agreement with our study, in their study with Zebu Nelore breed cattle, Bragança., *et al*. (2018) reported that the ELISA kit providing results based on PAGs levels was a highly effective method in the correct identification of pregnancies in the early period, and it provided more accurate results especially in comparison to ultrasonography on the 25th day. It is notified that early embryonic deaths in cattle occur usually between the 7th and 28th days of pregnancy [10], while late embryonic and early foetal deaths occur between the 28th and 66th days, and they have a range of 8 - 15% [20]. A study on beef cattle and heifers stated that pregnancies could be determined by determining PAGs after the 24th day, and it is an effective method in especially determination of early embryonic deaths in the first 30 days [17]. Breukelman., *et al*. (2005) reported that ultrasonography may be used to determine embryonic deaths in Holstein-Friesian heifers, but on the other hand, determining PAGs is a highly useful and helpful method in revealing embryo viability. A study where the course of PAGs was examined in buffalos in the pregnancy period revealed that PAGs showed an increase in the case of pregnancy occurrence from fertilization until the 105th day of pregnancy, while it stayed stable and constant until labour [2], whereas another study on Holstein Friesian cattle argued that PAGs levels continued to increase until labour [26]. On the other hand, a group of researchers asserted that it would be more appropriate to make PAGs-related pregnancy diagnosis in a later period than the 28th day so that the results are not misleading in animals getting pregnant earlier than the 60th postpartum day [13]. Besides, in compatibility with the findings of this study, Perenyi., *et al.* (2002) stated that PAGs determined in the maternal blood circulation especially in the early pregnancy period is a significant marker of pregnancy, while Green., *et al.* (2005) reported that PAGs determination may be carried out in determination of pregnancy without confusions after the 28th day. In a way to support our results, these studies have revealed that PAG, which is a placenta-specific antigen, may be used to identify pregnancy on the 28th day of pregnancy, and its sensitivity is high (98%) especially in determination of those that are not pregnant.

Findings in accordance with our results were also obtained in studies on different species. Accordingly, in a study that comparatively assessed twin pregnancy in sheep with PAGs and ultrasonography, it was reported that PAGs is effective in determining early pregnancies and twin pregnancies (40th - 50th days), but it has a low sensitivity in determining foetal sex [1]. Another study on sheep reported that ELISA fast test kits that are used based on maternal PAGs levels may be easily used in farm conditions, they have higher specificity and accuracy especially after the 30th day, and they are found to be as reliable as transrectal ultrasonography [7]. It is stated that the earliest PAGs determination may be made on the 22nd day after insemination in sheep [14] and the 25th day in goats [11]. Although these studies conducted on sheep in determining PAGs for identifying early pregnancies were in parallel with our study, the fact that the reliable results of the test (97.56%) were taken on days 30 and onwards was assessed as a difference caused by the difference of species as also stated in the study by [21].

Conclusion

It was observed that pregnancies could be determined with accuracy on the 28th day after fertilization as long as contamination between wells is checked while applying the visual ELISA PAGs test that provides results based on maternal PAGs levels. It was concluded that the test may be utilised as a different option alongside ultrasonography in determining the diagnostic limits of early pregnancy, and its accuracy rate is 100% especially in determination of individuals that are negative for pregnancy.

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Conflict of Interest

Author have not any conflict of interest to declare.

Author Contributions

G. Bulut worked at the field U Taşdemir designed the study, worked at the field and wrote the manuscript.

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