Serum concentration of pregnancy – associated glycoproteins (PAGs) as a predictor for embryonic / fetal losses and fetal numbers in cross-bred ewes.

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Abstract: Two blood samples were simultaneously collected, with 2 transrectal ultrasonographic (US) examinations on Days 28 and 45 post-breeding, from 61 pregnant cross-bred ewes (Romanov x Rahmany) to measure the serum PAGs concentration as a predictor for embryonic/fetal losses. Also, the variation in the serum PAGs concentration among ewes on Day 45 post-breeding was tested as a predictor for fetal number at the time of lambing. Eighteen ewes (29.5%) out of the 61 were detected to have embryonic / fetal losses on the basis of US detection of embryonic / fetal heart beats on Days 28 which disappeared later on Day 45 post-breeding. The lamb (s) number / ewe (Singleton; twins & triplet) at the day of lambing was analyzed in relation to retrospective serum PAGs concentrations on Day 45 post-breeding. The serum concentrations of PAGs on Day 28 compared with those on Day 45 showed a decrease ($P < 0.05$) in ewes that had embryonic/ fetal losses but did not show differences ($P > 0.05$) in ewes that had no embryonic / fetal losses. The serum concentrations of PAGs in ewes carrying triplet were higher ($P < 0.001$) than in those carrying twins and in same respect were higher ($P < 0.05$) in ewes carrying twins compared with those carrying singleton. It is concluded that measuring the serum PAGs concentration might be used for predicting embryonic / fetal losses and detecting fetal numbers as well.

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Key words: Ewes, PAGs, ultrasonography, embryonic / fetal losses, fetal number.

1. Introduction:

Early pregnancy diagnosis and determination of fetal numbers are essential tools for improving productive and reproductive performance of sheep. They enable sheep breeders to rebreed non-pregnant ewes and cull ewes not respond to various fertility treatments ***(Amer, 2010; Redden and Passavan, 2013; Reese et al., 2016 and Silva Chaves et al., 2017)***. Determination of fetal numbers helps sheep breeders to adjust nutritional requirements to accommodate fetal numbers and reduce risk of pregnancy toxaemia ***(Karen et al., 2006)***. Also, determination of fetal numbers helps herdman to improve lamb survival at the time of lambing through avoiding / manipulating dystocia and improving neonatal management ***(Rowland et al, 1992).***

The accuracy of the most currently used pregnancy diagnosis methods, except ultrasonography, depends on the pregnancy stage. Also, their efficiency for determination of fetal number is poor ***(Gonzalez et al., 2004 and Karen et al, 2001)***.

Although ultrasonography remains the gold standard method for pregnancy diagnosis and determination of fetal numbers ***(Karen et al., 2001 and Reese et al., 2016)***, its efficiency is always dependent on the expertise of the operator and that the learning time is slow as mastering the skill and the art of ultrasonography requires patience, diligence and persistence ***(Des Coteau et al, 2010).***

Pregnancy associated glycoproteins (PAGs), as a pregnancy markers has been used for pregnancy diagnosis in ruminants ***(Sousa et al, 2006 and Youngquist, 2006)*** including cattle ***(Friedrich and Holtz, 2010)***; sheep ***(Karen et al, 2003)***, goat ***(Gonzalez et al, 1999)*** and buffalo ***(Karen et al, 2011 and Barbato et al, 2017)***.

The cross reactivity among various anti-PAGs anti-sera and PAGs from various ruminant species facilitates the wide application of various RIA and ELISA kit systems for diagnosis of early pregnancy ***(Barbato et al., 2017)***. A RIA. System, which used antisera raised against caprine PAGs and purified bovine PAGs as a tracer, was used for detecting PAG molecule and pregnancy diagnosis in buffalo ***(Karen et al, 2007 and Barbato et al, 2009)***. Also, visual ELISA test, used for detection of bovine PAGs, was efficiently used for early pregnancy diagnosis in sheep from day 30 of gestation ***(Silva Chaves et al, 2017)***.

The concentration of ovine PAG might be influenced by fetal numbers. Measuring serum

concentration of PAGs beyond day 40 can be used to differentiate between single and twin pregnancies in sheep. Ewes carrying twins had a higher mean ovine PAG than those carrying singleton ***(Ranilla et al, 1997)***. Sequential measuring serum concentration of PAGs can be used for diagnosis of embryonic mortality in ruminants ***(Sousa et al, 2006)***.

Increased serum concentrations of PAGs are generally predictive of increased embryo survival, making PAGs a likely marker for evaluating embryo viability and placental competence ***(Pohler et al., 2016 and Reese et al., 2016***). Serum PAGs concentrations continues to increase in pregnant cows throughout normal gestation thus a marked drop during early embryonic/ fetal stages permits detection of embryonic /early fetal deaths (***Mouiche et al., 2016***).

The research hypothesis of the current study was to test the efficiency of detecting the variation in the serum PAGs concentration between days 28 and 45 as a predictor for diagnosing embryonic / fetal losses as well as to test validity of serum PAGs concentration on Day 45 as a predictor for lamb (s) numbers at the time of lambing.

The aim of the present study was to measure the serum concentration of PAGs as predictors for embryonic / fetal losses less than day 45 of gestation and fetal number in cross-bred ewes.

2. Materials and methods:

1. Animal and experimental design:

The study was conducted at Mehallet-Mousa experimental farm of the Animal Production Research Institute resident at the North of Nile Delta, Egypt. Seventy cross-bred ewes (50% Romanov x 50% local Rahmany fat-tailed breed), with an average age of 21-28 months and weight of 35-40kg, were used in the present study. The animals were subjected to a synchronization program and bred by hand-mate methods using four highly fertile rams of the same breed. The breeding date was recorded (day 0).

Blood samples were collected on Day 17-18 post-mating for progesterone assay to exclude non-pregnant ewes and on Day 28 and 45 post-mating for assay of pregnancy associated glycoproteins (PAGs). Blood samples (5ml) were collected by jugular vein puncture into plain vacutainers tubes. The blood samples were centrifuged at 1500 XG for 15 minutes and the collected sera were stored at -20^{o}C until measurement of progesterone (P4) and pregnancy associated glycoproteins (PAGs).

2. Pregnancy diagnosis and determination of embryonic / fetal viability:

2.1. Progesterone assay:

Ewes, that had a serum progesterone concentration $\leq$ 1ng/mL on Day 17-18 after mating, were considered non-pregnant ***(Shemesh et al., 1979)***. Nine 0ut of 70 ewes were found non-pregnant and excluded from the experiment.

2.2. Ultrasonography:

Two transrectal ultrasonographic examinations were conducted on Days 28 and 45 post-mating using an ultrasound device (ULTRASCAN 900, Alliance Int, Qubec, Canada) equipped with a 5MHz linear transducer (length 118mmx diameter 23 mm) by the same operator. However the first ultrasonographic examination done on Day 28 aimed to confirm pregnancy (presence of embryonic vesicle containing embryo proper) and judge embryonic viability (through counting embryos having heart beats) ***(Santiago-Moreno et al, 1995a)***. The second ultrasonographic examination aimed to count viable fetus (Es) / ewe by detecting fetal heart beats ***(Dawson et al, 1994)***. A comparison was held between number of viable embryo (s) on Day 28 and number of viable foetus (es) on Day 45 per ewe to differentiate between ewes that had no embryonic / fetal losses and ewes that had embryonic / fetal losses. Embryonic / fetal losses were considered in a ewe when the embryonic heart beats were detected on Day 28 but no fetal heart beats were detected on Day 45 of gestation in the same ewe.

2.3. Determination of fetal numbers:

At lambing, the number of lambs per ewe was counted and ewes were categorized into ewes giving birth to singleton, twins and triplets.

2.4. Serum concentration of PAGS:

The serum concentrations of PAGs was quantified on Day 28 (**Pohler et al., 2016**) and day 45 post-breeding in ewes (**Gonzalez et al., 2000**) that was detected to have late embryonic/early fetal and those have not losses on the basis of the ultrasonographic examination done on the same days. The number of lamb (s) per ewe at the time of lambing was analyzed in relation to retrospective serum concentrations of PAGs on Day 45 post-breeding to test the efficiency of PAGs on that day as a predictor for fetal number on the day of lambing.

3. Assays:

3.1. Progesterone assay:

Serum progesterone concentrations (ng/ml) were detected by using goat progesterone ELISA kit-supplied by Lifespan Bioscience, Inc. 2401 fourth venu suite 900. Seatle, Was 98121, North America. The assay was conducted according to the assay procedures described in the enclosed catalog No. 1-S-F 10141. The intra–assay CV was 15% and inter-assay CV was 15%. The sensitivity of the test than 0.2 ng/ml.

3.2. PAGs assay:

The serum concentrations of pregnancy associated glycoproteins (PAGs) were quantified by

enzyme-linked immunosorbent assay (ELISA) using goat pregnancy specific protein B (PSBP) ELISA kit MBs 706839 supplied by lifespan bioscience, Inc 2401 fourth Avenue suite 900 Seattle, Was 98121 North America. The assay was conducted according to the procedures described in the enclosed catalog. The intra-assay precision (CV%) was < 15%. The inter-assay of precision (CV%) was < 15%.

Statistical analysis:

One-way ANOVE (SPSS software version 16.0, Inc., Chicago, USA) was used to test the differences in the serum concentrations of pregnancy associated glycoproteins (PAGs) between Days 28 and 45 in ewes diagnosed to have embryonic/fetal mortalities and ewes had not on the basis of ulstrasonographic examinations conducted on the same days. Also, the differences in the serum PAGs concentration on the Day 45 between ewes that had or had not diagnosed to have embryonic/fetal mortalities were analyzed. The variations in the serum PAGs concentrations among ewes that gave birth to singles, twins and triples at lambing were also tested using one way ANOVA using SPSS software version 16 (SPSS Inc. Chicago IL, USA).

3. Results:

Serum concentration of PAGs in ewes with and without embryonic/fetal losses:

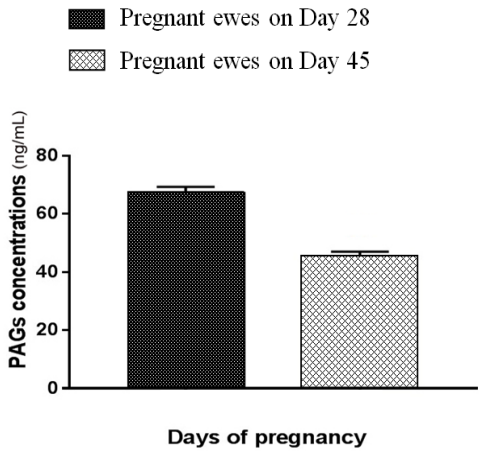

Figure 1A: Serum concentrations of PAGs (ng/ml) in ewes that had embryonic/fetal losses on Days 28 and 45 post-breeding. The serum PAGs concentrations decreased (P < 0.01) from 67.77±1.51 on Day 28 to 45.48±1.57 on Day 45.

In ewes that had embryonic / fetal losses, the serum concentrations of PAGs on Day 28 post-breeding (67.77±1.51ng/ml) showed a decrease (P < 0.01) on Day 45 (45.48±1.57ng/ml) figure 1A. On the other hand, there was no difference (P > 0.05) between serum concentrations of PAGs on Day 28 (67.77±1.51ng/ml) and on Days 45 (63.54±3.06ng/ml) post-breeding in pregnant ewes that had no embryonic/fetal losses (figure 1B).

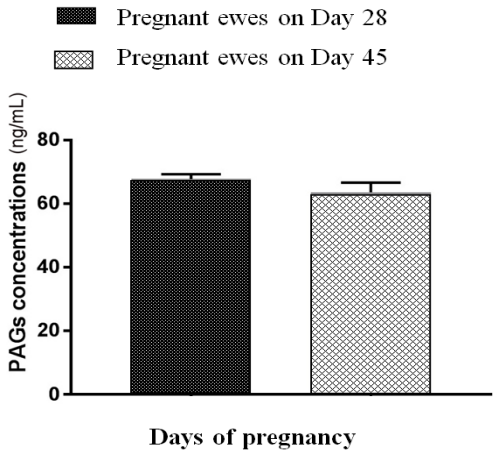

Figure 1B: Serum concentrations in ewes that had no embryonic/fetal losses on Days 28 and 45 post-breeding. The serum PAGs concentrations showed no difference (P > 0.05) between days 28 and 45 post-breeding.

3.1.2. Serum concentration of PAGs in pregnant ewes that had and had not embryonic/fetal death:

The serum concentrations of PAGs on Day 45 in ewes that had embryonic/fetal losses (45.48±1.57ng/ml) showed a decrease (P < 0.01) compared with pregnant ewes that had no embryonic/fetal losses (72.82±1.76 ng/ml, figure 1C).

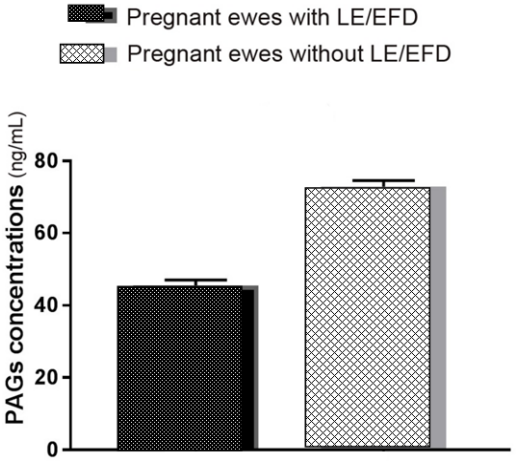

Figure 1C: The serum concentrations of PAGs in pregnant ewes that had early embryonic / late fetal losses and those had not. The serum PAGs concentrations on Day 45 showed a decrease (P<0.01) in ewes that had late embryonic / early fetal losses (LE/EFD) or had not.

3.2. Serum concentrations of PAGs in ewes carrying singles, twins and triplets:

The serum concentrations of PAGs in ewes carrying triplets (58.23±2.64 ng/ml) or twins (48.55±2.86 ng/ml) were greater (P < 0.001) than in ewes carrying singles (34.77±1.53 ng/ml). Also, the serum concentrations of PAGs in ewes carrying triplets (58.23+2.64 ng/ml) were higher (P <0.05) than in ewes carrying twins (48.55±2.86 ng/ml, Figure 2).

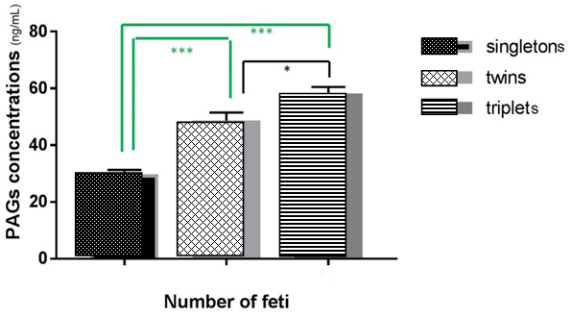

Figure 2:The serum concentrations of PAGs in ewes carrying singles, twins and triplets on Day 45 post-breeding. There was an increase (P < 0.001) in the serum PAGs concentrations in ewes carrying triplets or twins compared with those carrying singles. Also, there was an increase (P<0.05) in ewes carrying triplets compared with those carrying twins.

4. Discussion:

Early pregnancy diagnosis is an integral part of the reproductive program in sheep. With the exception of ultrasonography, the efficiency of most pregnancy diagnosis methods in detecting late embryonic / early fetal mortalities and fetal numbers is very poor. The emergence of measuring serum PAGs concentration, as a promising method for diagnosing embryonic / fetal mortalities and detecting fetal numbers, might constitute a reasonable substitute for ultrasonography.

The recorded embryonic / fetal loss in the present study was 29.50%, which came in agreement with ***Bolet (1986)*** who recorded a fetal death mortality rate of 30% in sheep. Measuring serum concentration of PAGs can be used for diagnosis of embryonic mortality in ruminants ***(Sousa et al., 2006).*** In pregnant cows that experiencing a normal pregnancy, serum PAGs concentration continues to increase steadily throughout normal pregnancy, thus a drop in the serum concentration during early pregnancy permits the detection of embryonic death ***(Whitlock and Maxwell, 2008 and Mouiche et al., 2016)***. The reduced serum concentration of PAGs on Day 45 compared with that on Day 28 post-breeding in ewes that had embryonic /fetal losses suggested the presence of correlation between embryonic/fetal mortality and serum concentration of PAGs. On the other hand, the absence of such reduction in the serum concentration of PAGs in ewes that had no embryonic/fetal mortalities ensure the existence of such relation. Our results might be supported by ***Zarrouk et al (1999)*** who reported that sequential measurements of PAGs in goats helped in determining the onset of the disturbance of trophoblastic activity associated with the death of the foetus. The same author demonstrated that PAGs fell under the positive pregnancy threshold in goats carrying singleton when trophoblastic cells died.

A similar pattern of serum PAGs concentration was observed in pregnant cows vs cows experiencing embryonic mortality as while the serum concentration of PAGs continues to increase from day 35 to 60 of gestation in pregnant cows, it dropped sharply in cows experiencing embryonic mortality ***(Mouiche et al, 2016). Pohler et al, (2013)*** recorded higher serum PAGs concentrations in cows that maintained the pregnancy than cows that underwent pregnancy loss after fetal heart beats were detected in viable fetuses at day 28.

The increased serum PAGs concentration on Day 45 in ewes that gave birth to triplets compared with those gave birth to twins or singles and in ewes that give birth to twins compared with those gave birth to singles at lambing indicated that fetal numbers influence serum concentration of PAGs. In accordance with the results of the present study, the serum concentrations of PAGs were higher in ewes ***(Karen et al, 2006 and Redden and Passavan 2013)*** and goat ***(Gonzalz et al, 2000)*** carrying twins than ewes and goat carrying single pregnancies respectively. Also ***Vandaele et al (2005)*** recorded an increase in RIA detected serum PAGs concentrations in texel ewes carrying multiple compared with those carrying singles on Day 45. However the increased serum concentration of PAGs in ewes carrying multiples compared with those carrying singles may be explained in the light of results of ***Alexander et al (1974)*** who reported that ewes carrying multiples has greater placental masses than ewes carrying singles. On the other hand ***Karen et al, (2006)*** reported that despite high accuracy in PAGs RIA for diagnosing early pregnancy in sheep, the accuracy for determination of fetal numbers are relatively low.

It could be concluded the decrease in serum concentrations of PAGs between day 28 and 45 may be predictive for embryonic/fetal mortalities in sheep. Also the increase in serum concentration in PAGs in ewes carrying multiples compared with those carrying singles on Day 45 suggest the predictive value of serum concentration of PAGs on Day 45 as a biomarkers for determation of fetal numbers in sheep.

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