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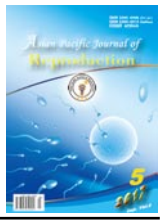

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Seasonal variations in serum pregnancy associated glycoproteins during early pregnancy in Aardi goats in central Saudi Arabia

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ABSTRACT

Objective: To accurately detect pregnancy in local Aardi goats of Saudi Arabia before day 30 of pregnancy by using serum caprine pregnancy associated glycoproteins (caPAG) concentrations and to evaluate the effects of heat stress on early pregnancy and on caPAG concentrations in pregnant and non-pregnant goats. **Methods:** This study was to confirm the reliability of our methods in different seasons of the year. A new protocol to evaluate and improve Aardi goats' reproductive performance was implemented during summer and winter months. A total of 60 healthy mature Aardi goats were used (30 and 30 in the summer and winter, respectively). All were synchronized using an ovsynch protocol and then naturally mated (NM) to mature tested bucks. Conception rates and caPAG were measured and pregnancy was verified by ultrasonography. **Results:** Regardless of season, however, on day 23 and 35 post-NM, caPAGs were higher in sera of pregnant (P<0.05) than in non-pregnant goats, while there was no difference in caPAG concentrations on day 15. On day 23 and 35 post-NM, serum caPAG concentrations were higher (P<0.001) in the pregnant winter group than in the pregnant summer group, while no differences were found in non-pregnant goats. Measuring serum caPAG concentrations proved to be easy and accurate in assessing early stages of pregnancy (as early as 23 days post-NM) during both summer and winter seasons. **Conclusions:** It can be concluded that pregnancy rates were not affected by seasonal differences. The modified ovsynch program can be advantageous and worthwhile for its ease of use. When we add the accuracy of caPAG tests, such a program will help in detecting pregnancies in Aardi goats as early as 23 days post-NM, regardless of the season.

1. Introduction

Aardi goats are one of the major pure breeds in Saudi Arabia. The others are Bishi, Jabaly, and Tohami, and all are raised to provide milk and meat[1-3]. Pure Aardi goats have distinctive features, including black body color, white faces with coarse hair, and long white drooping ears[1]. Increasing knowledge of reproduction in Aardi goats using new technology will help farmers reap the benefits of raising such a breed in an optimum amount of time with the least effort.

One of the major livestock reproduction management techniques is the early detection of pregnancy. The economic value of early pregnancy detection is extremely important since it is advantageous to be able to detect the pregnancy sooner in order to monitor detection of both unsuccessful mating and artificial insemination, identify infertility cases such as culling non-pregnant animals, and help resolve pregnancy-related problems[4-6]. It is best to

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be able diagnose pregnancy at or near the time of the end of the goats' 21 day-long estrous cycle. However, even with the use of ultrasonography, early pregnancy diagnosis is best performed between days 30 and 40 of gestation using the transabdominal or transrectal approach to achieve the greatest confidence in diagnoses[7].

The need for reliable, accurate, simple, inexpensive and easy-to-administer methods of early pregnancy diagnosis became more important with the use of new breeding techniques such as artificial insemination[8]. The central region of Saudi Arabia is a desert and is hot during the day, cold at night, and has low levels of rainfall. Even in the shade, the outdoor temperature is high enough to exert stress on the animals. In the Riyadh region, summer temperatures can reach above 45 ℃ while the relative humidity can be less than 10%[9]. Heat stress has been associated with decreased fertility especially in dairy cattle[10]. High temperatures affect the developing embryo and can lead to lower conception rates. It is reported that high temperatures lowered conception rates in cows more than in heifers, since lactating cows were usually unable to maintain normal body temperature under heat stress conditions because of the high rates of lactation-associated internal heat production[11,12].

Observing signs of estrus about 21 d after breeding followed by non-return to estrus is the simplest pregnancy diagnostic method. However, it is an inaccurate diagnostic method due to many factors; one of which is goats failing to return to estrus at the expected time, even though they are not pregnant. Additionally, some goats have luteal cysts and/or a prolonged luteal phase, which makes this method unreliable[13-15].

Several diagnostic methods for pregnancy with high sensitivity and specificity ease of implementation, and lower costs have been applied in domestic animals in an attempt to resolve early pregnancy issues. In goats, many methods have been applied, including radiography, abdominal palpation, ultrasonography, and hormonal and pregnancy-associated glycoproteins (PAG) assays.

One of the most accurate methods for early pregnancy diagnosis in goats and other farm animals is the use of trans-abdominal or rectal ultrasonography (US), which identifies and visualizes placentomes and embryonic heartbeats[8,16-20]. In addition, technological advances in US have made it possible to detect pregnancy in goats as early as day 17 to day 19, with an accuracy of 66%, which reaches 100% by day 34[8]. However, US machines are expensive, limited to mostly veterinary clinics, and need skilled technicians to operate them properly[19,21]. PAG are produced and secreted from the binucleate cells of trophoectoderms that migrate from the embryo to maternal tissue during placentation[21-27]. Three placental caprine PAG (caPAG) were purified with different amino acid sequences and molecular masses (62, 59, and 55 kDa)[28]. These antigen-specific pregnancy-associated proteins were observed around 16-18 d after post-breeding in goats and significantly increased after 19-23 d[29,30]. Using serum caPAG detection, in contrast to other unreliable methods such as serum P4 assays or non-return to estrus, allowed us to accurately determine which goats are pregnant. In addition, the caPAG test has shown its potential to be highly suitable for field practice[31]. Pregnancy status assessed through detection of placental PAG in blood is now used for pregnancy detection in sheep and cattle[32-36].

In sheep serum, caPAG enzyme-linked immunosorbent assay (ELISA) was considered an effective method for the early diagnosis of pregnancy when performed from day 30 of gestation[33]. The latest studies using caPAG ELISA tests on African dwarf goats' blood samples collected from slaughter houses showed high accuracy (87%) in detecting pregnancy[37].

Nevertheless, to our knowledge, studies on the diagnostic reliability of caPAG in detecting early pregnancy in Aardi goats in the Saudi Arabian climate are lacking. Using the ovsynch protocol in combination with natural mating, the present study was designed to evaluate and compare conception rates during summer and winter periods in Aardi goats. In addition, we aimed to evaluate the use of serum caPAG as a reliable tool for pregnancy diagnosis as early as 23 days post-NM in Aardi goats during summer and winter periods, which was verified by other methods.

2. Materials and methods

All procedures described in this experiment were approved by the Faculty Research Ethics Committee at King Saud University.

2.1. Location

The present study was conducted for 70 d at the Experimental Station (+24° 48′ N +46° 31′ E) affiliated with the Department of Animal Production, College of Food and Agriculture Sciences, King Saud University, Riyadh, Saudi Arabia. The study was conducted for 35 d during the summer (from May 30th to July 3rd) and 35 d during the winter (from November 24th to December 29th).

2.2. Animals and management

A total of 60 healthy mature Aardi goats (averaging 1-1.5 years old) were evaluated. Thirty goats during the summer and another 30 during the winter were screened for pregnancy after natural mating (NM) with six bucks. At first, semen was collected from the bucks and examined for quality before they were introduced to synchronized goats. The ovsynch protocol in combination with NM was used for synchronization, in which they received the first 8 μg intramuscular injection of GnRH (GnRH Receptal®, Intervet/ Schering-Plough, MSD, Animal Health) on day 0[38,39]. On day 7, they received 10 mg intramuscular injection of Prostaglandin-$F_{2\alpha}$ [$PGF_{2\alpha}$, PGF Lutalyse, Pfizer Animal Health (Zoetis)]. Thereafter, they received a second 8 μg intramuscular injection of GnRH 48 h after the $PGF_{2\alpha}$ injection. On the same day of the 2nd GnRH treatment, bucks were introduced to female goats. Female goats were monitored to detect estrus behavior. Day 1 of pregnancy was calculated 48 h after the start of estrus. The daily food ration for each animal consisted of a commercial total mixed ration (Metabolizable

energy 1 950 kcal/kg, crude protein 13%, crude fat 2%, crude fiber 10%, ash 8% on DM basis; Al-wafi pellets, ARASCO, KSA). Feed was offered twice daily, while water was offered ad libitum.

2.3. Measurements

Blood samples (10 mL) were collected into plain tubes via jugular venipuncture at day 15, 23, and 35 post-NM. Collected samples were placed inside an ice box and immediately transferred to the laboratory to be processed. Within approximately 1 h after collection, sera were separated by centrifugation at 3 000 r/min for 30 min at 4 ℃, transferred into 1.5 mL Eppendorf tubes, and then stored at -20 ℃ until assayed for caPAG.

ELISA kits were used for serum caPAG (ng/mL) using a commercial kit (IDEXX Laboratories, Inc., United States) and micro-titrimetric plates. The assay was performed according to the manufacturer's instructions, and an automatic photometer plate reader was used for absorbance measurements.

For US, a real-time B-mode machine (Prosound 2, ALOKA, Japan) with multi-frequency linear trans-rectal probe (UST 660-7.5, ALOKA, Japan) was used on day 23 post NM, and the reading was confirmed on day 35 using a multi-frequency convex trans-abdominal probe (UST-9137C, ALOKA, Japan). All pregnancies were followed until kidding, and the collected data (number and gender) were then confirmed after kidding.

2.4. Statistical analysis

Data were analyzed using the statistical analysis system (SAS Inst., Inc., Cary, NC). Data were subjected to ANOVA using $\alpha = 0.05$. Means showed significant differences in ANOVA. $P<0.05$ indicated statistical significance. Means and their pooled SEs are presented unless otherwise indicated. Conception rates were compared between summer and winter and between pregnant and non-pregnant goats by Student t-test and presented using SigmaPlot software (SigmaPlot v12.5, Systat Software Inc., San Jose, CA, USA).

3. Results

Results show that there were no significant differences between winter and summer pregnancy groups (56.94±1.39 *vs.* 54.09±1.46). Measuring serum caPAG concentrations showed significant differences between pregnant and non-pregnant goats. There were no differences between pregnant and non-pregnant goats on day 15 post-NM, while it was clear that serum caPAG concentrations increased ($P<0.001$) on day 23 (1.08 ng/mL *vs.* 0.11 ng/mL) and 35 (3.11 ng/mL *vs.* 0.31 ng/mL) post-NM in pregnant goats compared to non-pregnant ones. Pregnancy was verified by ultrasonography on day 35 post NM. Serum caPAG concentrations at early pregnancy in winter and summer goat groups are shown in Table 1. Results show that on day 15 post-NM, there were no differences in caPAG serum concentrations in pregnant Aardi between the summer and winter groups. However, caPAG concentrations were higher ($P<0.001$) in pregnant goats' serum during the winter than those during the summer on day 23 (1.59 ng/mL *vs.* 0.59 ng/mL) and day 35 (3.40 ng/mL *vs.* 2.87 ng/mL) post-NM. Furthermore, caPAG concentrations in non-pregnant goats were very low and there were no significant differences found between the summer and winter groups (Table 1) on days 15, 23, and 35 post-NM.

Table 1
Serum pregnancy associated glycoproteins in pregnant and non-pregnant Aardi goats (means±SE) (caPAG ng/mL).

Days post NM	Pregnant		Non-pregnant	
	Summer	Winter	Summer	Winter
15	0.08±0.04	0.05±0.02	0.03±0.01	0.03±0.01
23	0.59±0.13[a]	1.59±0.36[b]	0.08±0.02	0.04±0.03
35	2.87±0.08[a]	3.40±0.04[b]	0.04±0.01	0.02±0.01

[a,b]values within a row with different superscripts are significantly different ($P<0.001$).

4. Discussion

Pregnancy rates in our treatment groups of Aardi goats (54%-56%) were high in comparison to other studies which showed a 24% pregnancy rate in Saanen goats[39]. Other studies using controlled internal drug release and sponge of P4 accompanied with artificial insemination and using pregnant mare serum gonadotropin, had pregnancy rates ranging from 50% to 62%[40]. It is of importance to note that kids and heifers are not under the same stress as lactating animals, since lactating animals cannot maintain normal body temperatures under heat stress conditions because of the high lactation-associated internal heat production[11]. While the conception rate in our study was not as high as we wanted, its ease of implementation was encouraging. The protocol (ovsynch in combination with NM) is easy to setup and follow with minimal interference to daily routines of animal management. While we do not have data of pregnancy rates in Aardi goats under arid and semi-arid grazing conditions in central Saudi Arabia, we presume that our animals had better pregnancy rates since we used tested bucks, and goats were kept under shade, dewormed, vaccinated and were under a better feeding management.

The reliability of caPAG concentrations in serum early pregnancy detection in goats before day 23 post NM had been evaluated in earlier studies[41]. Starting on 23 days post NM, it was found that serum caPAG concentrations were very accurate (specificity of 100%) in detecting pregnancy. At day 23 and 35 post NM, our results indicated that pregnant Aardi goats had higher serum caPAG concentrations than non-pregnant goats, and all pregnancies were followed up until kidding. In fact, researchers were able to detect caPAG in pregnant goats as early at day 18 post insemination. Concentrations reached 3-5 ng/mL between day 21 and 22 and increased to 30-50 ng/mL during the 5-8 wk of pregnancy[42–44]. Thereafter, caPAG serum concentrations started to decrease by the 9th week of pregnancy, reached 16-32 ng/mL between 12-17 wk, and continued to decrease until parturition[28,41,45]. We did not analyze

later periods of pregnancy since we were only interested in early pregnancy diagnosis. However, our results indicated that using serum caPAG concentrations at day 23 post NM was a reliable tool for early pregnancy detection in Aardi goats.

As stated earlier, using an ELISA for caPAG detection is very reliable on day 23 post NM and can be used to detect pregnancy as early as 16 days post-NM[30]. Because caPAGs are produced by the placenta during early pregnancy and then enter the maternal circulation, any detrimental effects on the placenta can affect caPAG secretions. It was demonstrated that chronic heat exposure lowered circulating placental hormone concentrations, and heat exposure resulted in reduced fetal development and placental size in ewes[46]. Our results show that heat stress has impacted the serum concentrations of caPAG negatively, but not to the extent of affecting the reliability of using it as a tool for early pregnancy detection. In agreement with our results, it was reported that cows had higher PAG concentrations throughout cooler gestation periods when compared to hot seasons of the year[47].

It is of importance to note that these caPAG concentrations in non-pregnant Aardi goats are very low when compared to pregnant ones regardless of the season, which makes the use of caPAG ELISA tests for pregnancy diagnosis very important. This indicates the viability of using caPAG for the diagnosis of early pregnant and non-pregnant goats.

Early pregnancy diagnosis of Aardi goats under local environmental conditions is of great importance for improving reproductive management. Even with the negative effects of heat stress on caPAG concentrations in pregnant goats, there were no effects on the conception rates during the summer when compared to winter months. In addition, combining caPAG tests with the ovsynch program is a new approach which shows it can simplify reproductive management without negatively affecting conception rates of Aardi goats under local environmental conditions in Saudi Arabia. To our knowledge, this is the first study verified by US that compared summer to winter early pregnancy rates using the ovsynch program with natural mating and serum caPAG tests to detect early pregnancy during both seasons. These results show that the modified ovsynch program can be advantageous and worthwhile for its ease of use. When we add the accuracy of caPAG tests, such a program will help in detecting pregnancies in Aardi goats as early as 23 days post-NM, regardless of the season.

Conflict of interest statement

The authors declare that they have no conflict of interest.

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