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Short Communication

SEASONAL VARIATIONS IN PROGESTERONE SECRETIONS IN PREGNANT AND NON-PREGNANT AARDI GOATS IN THE SEMI-ARID REGION OF SAUDI ARABIA

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ABSTRACT

In this study, the effects of heat stress on the secretory activity of corpus luteum were evaluated by measuring serum progesterone (P4) concentrations on day 15, 23, and 35 after natural mating. Thirty healthy mature Aardi goats were examined during summer and another 30 during winter, using the Ovsynch protocol and natural mating with tested fertile bucks. Pregnancy was verified with ultrasonography at 23 and 35 days. Pregnancy rates did not differ between summer (54.1%) and winter (56.9%). However, serum P4 was significantly ($p<0.001$) higher in pregnant than non-pregnant goats at all time-points, irrespective of season. Serum P4 did not differ between summer and winter groups in pregnant goats but differed significantly ($p<0.001$) between two seasons in non-pregnant goats on days 15, 23, and 35 post mating. In conclusion, conception did not differ between summer and winter. Heat stress significantly alters corpus luteum activity, as reflected by decreased serum P4 concentrations in non-pregnant goats during summer, but it did not affect pregnant goats.

Key word: Corpus luteum, Summer, Winter, Ovsynch, Progesterone, Aardi goats.

INTRODUCTION

The number of goats in Saudi Arabia is estimated around 3.6 million (General Authority of Statistics, 2016). Aardi goats are one of the pure breeds of goats in Saudi Arabia. They are characterized by black body color, long drooping ears, and a white face with coarse hairs (Alamer, 2006). Although, there are other lesser breeds, Aardi goats are more tolerant to harsh environment and known to produce steadily supply of milk when compared to others (Al Saiady *et al*., 2007). There is a need for more insights in the field of reproduction using advanced techniques to help farmers decrease their cost and increase their productivity since Aardi goats are used for the production of milk and meat. Early detection of pregnancy has a major economic value and a very important tool in livestock management (Abdullah *et al*., 2014). Recognizing non-pregnant animals would lead to better management in decreasing costs related to feed and labor while giving more attention to the feeding and care of pregnant animals.

High environmental temperature has negative effects on most reproductive functions in farm animals. The environmental temperature in Saudi Arabia can be higher than 45°C during summer, and the conception rate can fall below 10% in dairy cattle (Al-Hassan, 2002). Goats are no exception: heat stress decreases plasma estradiol (E2), follicular E2, aromatase activity, and LH receptor levels leading to delayed ovulation (Ozawa *et al*., 2005). Wolfenson *et al*., 2000, reported that P4 secretion by luteal cells during summer was decreased in cows and ewes (Macias-Cruz *et al*., 2016) under heat stress, which is reflected by a lower plasma P4 concentration. Low circulating P4 has been associated with lower fertility (Al-Katanani, *et al*., 1999) and pregnancy rate (Denicol *et al*., 2012), while supplementing P4 before artificial insemination increased pregnancy rate in cattle (Chebel *et al*., 2010).

Serum P4 concentrations were used in an indirect method to determine the stage of the estrous cycle and to predict pregnancy after insemination (Wiltbank *et al*., 2012; Broes and LeBlanc, 2014). However, serum or milk P4 concentrations are not a direct indication of pregnancy but just a mere indication of the presence of a functional CL, and it has been used as a tool to diagnose a cycling cow or goat.

Serum P4 concentrations show conflicting results during heat stress. Some studies show that serum P4 concentrations are higher in summer than in winter (Marai *et al*., 2004; Sejian *et al*. 2011), while some other workers reported decreased serum P4 in summer than in winter (Macias-Cruz *et al*., 2016) or unchanged levels (Ozawa *et al*., 2005; Li *et al*., 2016). According to Younas *et al*. (1993), cows under evaporative cooling during summer have higher serum P4 concentrations than cows under shade only.

The aim of the present study was to evaluate the CL functions via serum P4 concentrations in Aardi goats to at 15, 23 and 35 days post natural mating to help identify non-pregnant goats. In addition, to evaluate the effect of heat stress on CL functions by measuring P4 concentrations in summer and winter months in pregnant and non-pregnant goats. Furthermore, evaluating the merge of Ovsynch protocol with natural mating in order to make it easier to use as a tool in reproductive management.

MATERIALS AND METHODS

The present study was conducted at an experimental station of the Department of Animal Production of King Saud University, Riyadh, Saudi Arabia (+24º 48' N +46º 31' E). The study was conducted for 70 days, including 35 days during summer (from May 30 to July 3) and 35 days during winter (from November 24 to December 29). All procedures were approved by the Faculty Research Ethics Committee at King Saud University.

Animal and management: Healthy, mature Aardi goats averaging 1 to 1.5 years old were used in this study (30 goats during summer and another 30 during winter). The daily diet for each animal consisted of a commercial total mixed diet (ME 1950 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. The experimental goats were subjected to Ovsynch protocol (Nur *et al*., 2013). They received an 8-μg intramuscular injection of GnRH (GnRH Receptal®, Intervet/Schering-Plough, MSD, Animal Health) at day 0. At day 7, goats received a10-mg intramuscular injection of prostaglandin-F2α (PGF2α, PGF Lutalyse, Pfizer Animal Health (Zoetis)). A second GnRH dose was given at 48 hrs after PGF2α injection. Six bucks that had been screened for fertility were introduced to the synchronized goats for natural mating. Estrous behavior was monitored, and day 1 of pregnancy was calculated after 48 hrs of expressing estrus.

Measurements: Blood samples (10 ml) were collected in plain tubes via jugular venipuncture on days 15, 23, and 35 after mating. Blood samples were placed on ice and transferred to the laboratory for processing immediately after collection. At 1h after blood sample collection, sera were separated by centrifugation at 3000 rpm for 30 min and 4°C, transferred into 1.5-mL Eppendorf tubes, and stored at -20ºC until assaying for P4.

Enzyme-linked immunosorbent assay (ELISA) was used to assess serum P4 (ng/mL) using commercial kits (Human, Germany) and microtiter plates. The assays were performed according to the manufacturer's instructions, and an automatic ELISA microplate reader (Stat Fax 4200, Awareness Technology Inc., USA) was used to read absorbencies. Samples were analyzed in duplicates in the same assay. Intraassay CV ranged from 4.5 to 7.2%, and interassay CV ranged from 7.4 to 10.7%.

A real-time B-mode ultrasonography machine (Prosound 2, ALOKA, Japan) with a multi-frequency linear trans-rectal probe (UST 660-7.5, ALOKA, Japan) was used at day 23 post mating, and observations were confirmed at day 35 using a multi-frequency convex trans-abdominal probe (UST-9137C, ALOKA, Japan). All pregnancies were followed until kidding.

Statistical analysis: A statistical analysis system (SAS Inst., Inc., Cary, NC) was used for data analysis. Data were subjected to ANOVA using $\alpha = 0.05$ using GLM procedure of SAS. The initial model included treatment (summer vs. winter), and then between pregnant vs. non pregnant goats. Means and their pooled SEs are presented unless otherwise indicated. Progesterone concentrations were compared between pregnant and non-pregnant, summer vs. winter within pregnant and summer vs. within non-pregnant goats by student t-test.

RESULTS AND DISCUSSION

The pregnancy rate in goats showed no difference between summer and winter groups (54.1% vs. 56.9%). These pregnancy rates are higher than 24% reported in Saanen goats (Nur *et al*., 2013). However, higher pregnancy rates (78.9%) were also reported in Alpine goats under an average temperature of 20.9ºC in Brazil (Fonseca *et al*., 2005). Other studies reported pregnancy rates ranging from 50 to 62%, using controlled internal drug release (CIDR) and a P4 sponge accompanied by artificial insemination and use of pregnant mare serum gonadotropin (Nadiope *et al*., 2012). It is important to note that kids and goats are not under the same stress as lactating animals.

Serum P4 concentrations in pregnant and non-pregnant Aardi goats at 15, 23, and 35 days post mating are shown in Table 1. The results show that the mean serum P4 concentrations were higher ($p<0.001$) at each time point after mating in pregnant goats compared to their non-pregnant counterparts. The main source of P4 in pregnant goats is the CL (Sawada *et al*., 1994), and its concentrations in plasma are high from day 10 until day 140 of pregnancy (Sawada *et al*, 1994). Therefore, it was not unexpected to find that pregnant goats had higher serum P4 concentrations. However, there might be a reason for high P4 concentrations on day 23 in non-pregnant goats due to the fact that not all goats ovulate within 48 hours after treatment.

It is estimated that about 85% of cows treated with the Ovsynch protocol ovulate (Colazo and Mapletoft, 2014), which means that many animals at 23 days might not be in a new estrous cycle. In addition, early embryonic death extends the estrous cycle and delays the start of the next cycle. Serum P4 concentrations and ultrasound have been used previously as a screening method for early detection of pregnancy in goats (Fricke, 2002; Singh *et al*., 2004). As mentioned, serum P4 concentrations reflect an active CL but not necessarily pregnancy in goats, which makes it a better tool for identification of non-pregnant animals when serum P4 concentrations are low.

The results in Table 2 show P4 serum concentrations in pregnant goats during summer and winter at days 15, 23 and 35 post mating. These results showed non-significant differences in serum P4 concentrations between summer and winter at days 15, 23, and 35 post mating. However, there was a trend of slightly higher serum P4 concentrations during winter months, which might be related to the effect of heat stress on the functional CL during summer, when less P4 is secreted. Researchers observed conflicting results regarding serum P4 concentrations during heat stress. However, some researchers report that heat stress during summer months has a negative effect on the CL, which leads to lower production of P4 during summer (Macias-Cruz *et al*., 2016). These heat stresses on P4 secretions from CL during summer months were significantly apparent in non-pregnant Aardi goats, which had lower serum P4 concentration ($p<0.001$) in summer than in winter at 15, 23 and 35 days post mating (Table 3).

Table 1. Serum progesterone (P4, ng/mL) in pregnant and non-pregnant Aardi goats at days 15, 23, and 35 after natural mating. Values are expressed as means ± SE.

Days post mating	Pregnant	Non-Pregnant
15	11.48 ± 0.97^a	6.04 ± 1.13^b
23	12.10 ± 1.25^a	4.23 ± 0.97^b
35	13.14 ± 1.32^a	6.63 ± 0.86^b

$^{a-b}$ Values within a row with different superscripts are significantly different ($p<0.001$).

Table 2. Serum progesterone (P4, ng/mL) in pregnant Aardi goats during summer and winter at days 15, 23, and 35 after natural mating. Values are expressed as means ± SE.

Days post mating	Summer	Winter
15	11.33 ± 1.33	11.62 ± 0.61
23	11.20± 0.91	12.99± 1.59
35	12.48± 0.84	13.80± 1.80

Table 3. Serum progesterone (P4, ng/mL) in non-pregnant Aardi goats during summer and winter at days 15, 23, and 35 after natural mating. Values are expressed as means ± SE.

Days post mating	Summer	Winter
15	4.69 ± 0.60^a	7.39 ± 1.66^b
23	1.54 ± 1.32^a	6.93 ± 1.61^b
35	3.97 ± 1.51^a	9.29 ± 1.22^b

$^{a-b}$ Values within a row with different superscripts are significantly different ($p<0.001$).

Conclusion: Serum P4 concentrations were higher in pregnant than non-pregnant goats at 15, 23, and 35 days post natural mating, which suggests a possibility of using such tests to detect non-pregnant goats, even though P4 concentrations reflect only a functional CL. The days post mating can be used as a tool to help identify non-pregnant female goats in combination with ultrasound at day 35. This study confirms that the use of ultrasound is greatly beneficial at day 35 post mating. Even with the negative effect of heat stress on P4 in non-pregnant goats, there was no effect on the pregnancy rate during summer in comparison to winter months. In addition, combining the Ovsynch program with natural mating is a new approach that can reduce reproductive management without negatively affecting the pregnancy rate of Aardi goats in the local environment of Saudi Arabia.

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