

REPRODUCTIVE PERFORMANCE, OVARIAN VASCULARIZATION, FOLLICULAR DYNAMICS, AND BLOOD HORMONES AFTER ESTROUS SYNCHRONIZATION OF SA'IDI GOATS

M.A. Abdel-Ghani^{1*}, M. Hayder², N.S. Abou-Khalil³, D.R. Derar¹, F.F. AbouAmmou², M.H. El-Shafie², T.M.M. Abdel-khalek², H. Hamdon⁴

1- Department of Theriogenology, Faculty of Veterinary Medicine, Assuit University, Assuit, 71526, Egypt, 2-Animal Production Research Institute, Agriculture Research Center, Dokki, Giza, 12311, Egypt, 3- Department of Medical Physiology, Faculty of Medicine, Assiut University, Assuit, 71526, Egypt, 4- Animal Production Dept., Faculty of Agriculture, Assiut University, New Valley Branch, El kharga, New Valley, 3201, Egypt

*Correspondence: M.A. Abdel-Ghani (e-mail: mohammed_ali76@hotmail.com)

SUMMARY

The objectives were to determine the effects of estrus synchronization on reproductive performance of Sa'idi goats, and to examine the relationship between follicular blood flow (FBF) of ovulatory follicle and serum concentrations of progesterone P4, Estradiol-17 β (E2) and nitric oxide (NO). Group 1 (sa'idi goats) was received an intravaginal sponge for 14 d (LT group), and was injected with 500 IU PMSG at the time of sponge removal. Group 2 (sa'idi goats) was received an intravaginal sponge for 5 d (ST group), and was injected with 500 IU PMS and 2.5 mg of Dinoprost at the time of sponge removal. Arbitrary blood flow scale was used to assess the FBF in color Doppler images. There was no difference observed between the ST and LT groups in terms of reproductive parameters. The multiple kidding rates were different ($P < 0.05$), being 61.5% LT group and 46.2% ST group respectively. The arbitrary blood flow was increased with the increase in the diameter of ovarian follicles. The ST group had greater ($P < 0.05$) blood flow scores than LT group on Day 0. The P4 concentrations were similar ($P > 0.05$) between the two groups. On Day 2, 3 and 4, concentrations of E2 were greater in ST group ($P < 0.05$) than in LT group. The NO concentration tended to be different ($P < 0.05$) in LT group before sponge withdrawal compared with ST group. In conclusion, there is a positive relationship between E2 and NO, but E2 and NO concentrations were closely related to the FBF.

Keywords: color doppler ultrasonography, estrous synchronization, follicular blood flow, reproductive performance

INTRODUCTION

Estrous synchronization, particularly in ruminants, is a valuable management tool that has been successfully employed to improve the production of milk and meat (Hashemi *et al.*, 2006). In addition, as the popularity of goat production continues to increase, pressure to develop efficient and cost-effective methods for estrus synchronization in goats becomes more important. Therefore, with estrous synchronization, producers are able to more efficiently use reproductive biotechnologies for reproductive management, including AI and embryo transfer, so that genetic material is more easily obtained or transferred domestically and internationally (Whitley and Jackson, 2004).

Transrectal color Doppler ultrasonography is a useful, and noninvasive technique that has been successfully used for evaluating ovarian vascular function, allowing a visual observation of the blood flow in the wall of follicles (Brannstrom *et al.*, 1998) and corpus luteum (Acosta *et al.*, 2003). This image has facilitated haemodynamic

studies and reproducible measurements (Acosta *et al.*, 2002). The use of color Doppler ultrasonography to investigate the ovarian responses after hormonal stimulation has recently been studied in sheep (El-sherry *et al.*, 2013), horses (Witt *et al.*, 2012) and cows (Matsui and Miyamoto, 2009). In addition, follicle blood flow assessment by Doppler ultrasonography has been used in mares to study the role of follicle vascularity in maturity of the preovulatory follicle after hCG treatment (Gastal *et al.*, 2006 and Ginther *et al.*, 2007) and oocyte maturity (Ginther *et al.*, 2007).

Moreover, nitric oxide (NO) is a potent vasodilator agent that is involved in folliculogenesis and ovulation (Tamanini *et al.*, 2003) and vasculature, capillary area density, and capillary number density were positively correlated with NO production (Moonmanee *et al.*, 2013). In addition, NO stimulates the synthesis of both PGE and PGF2 α that cause inflammation in the preovulatory follicle and induce rupture (Faletti *et al.*, 1999) with other

locally produced substances, growth factors and cytokines (Tamanini *et al.*, 2003)

The objectives of the current study was to compare the changes in reproductive performance, sex hormones, follicular dynamics and blood flow (BF) within the ovulatory follicle after short-term and long-term progesterone based synchronization protocols of Saïdi goats.

MATERIALS AND METHODS

Animals and experimental design:

Adult female goats ($n = 30$) with almost similar age (1.5-3 years) and weight (20-23 Kg) were selected from the lot maintained at Mallawi Animal Production Research Station, El-Minia, Egypt, (latitude 28°07'N and 30°33'E) during the first three weeks of October (Autumn). The Saïdi goats are a local Egyptian breed found in the Upper Egypt. The animals are kept mainly for meat production. The goats were maintained graze on in pens during all the day. Water was available *ad libitum*. The management of the goats did not change throughout the experimental period. The bucks were separated from goats. The goats were randomly divided into two equal groups, and were registered by numbering on their ears. Group 1 (Long-term progestagen treatment-PMSG): the animals were received an intravaginal Progestagen impregnated sponge (40 mg fluorogestone acetate, GFA, Chronogest®, Intervet, International, boxmeer, Netherland) for 14 days, and were injected with 500 IU PMSG (Folligon, Intervet) at the time of sponge removal. Group 2 (Short-term progestagen treatment-PMSG-PGF2 α): the animals were received an intravaginal Progestagen impregnated sponge for 5 days. At the time of sponge removal, 500 IU PMSG and 2.5 mg of Dinoprost (Lutalyse, Pfizer manufacturing, Purts, Belgium) were injected intramuscularly.

Applications of hormones used for the estrous synchronization were made between 18:00 and 19:30 h in all groups. Starting from the first hour of removal of the intravaginal sponges in all groups, the occurrence of behavioral estrus signs was monitored every four h interval and for five d using teaser bucks ($n = 6$). The goats were considered to be in estrus only if they stood while being mounted by the bucks (standing behavior). A buck was introduced in the herd during the experiment for breeding purpose. The mating was scheduled every 12 h until does refuse to be mounted by bucks. The mated goats were recorded and kept under close observation to detect the number of abortions until parturition. Duration of estrus was defined as the interval between the onset and the end of estrus signs. The end of estrus was the time when the doe refuse to be further mounted. The day of Progestagen withdrawal considered as Day 0.

The reproductive parameters were calculated following the treatment according to Karaca *et al.* (2010) and Simões *et al.* (2006) were as follows:

* Onset of estrus (interval from sponge's removal to time of first estrus identification).

* Estrus response (number of goats showing oestrus/total number of goats treated in each group X 100).

* Onset of follicular wave: First observation of at least one follicle ≥ 3 mm diameter, followed by a growing follicular wave resulting in a follicle with a minimal diameter of 5 mm.

* Day of maximum follicular diameter: The first day when the dominant follicle reached its maximum diameter (>5 mm).

* End of follicular wave: The day when the number of follicles <3 mm increased and the number of follicles ≥ 3 mm decreased by the same proportion.

* Duration of wave: Interval between the onset and the end of a follicular wave.

* Pregnancy rate (number of pregnant goats/number of mated goats in each group X 100).

* Abortion rate (number of aborted goats/number of pregnant goats in each group X 100).

* Kidding rate (number of kidding goats/number of pregnant goats in each group X 100).

* Multiple kidding rates (number of goats kidding twin or triplet/number of kidding goats in each group X 100).

* Litter size (number of total kids/number of kidding goats kidded in each group).

Doppler ultrasound monitoring of follicular development:

Ultrasound scanning was performed by the same operator every 24 h until ovulation using a Doppler ultrasound scanner (Mylab30, Piemedical, Netherlands), equipped with a 6–8 MHz endorectal linear probe (Lv513). Examinations were conducted on the standing goat. The urinary bladder was used as a guide to find the uterine horn. The probe was rotated laterally 90° clockwise and 180° anticlockwise to scan the ovaries and genitalia. The size and number of follicles larger was detected and profiled by retrospective evaluation of ovarian sketches that provided topographical and the diameter changes for each follicle. The follicular dynamics and ovulation rate were recorded. Following morphological evaluation of the ovarian findings, the power flow mode of the ultrasound scanner was activated for blood flow mapping. Color signals were used to evaluate the blood flow around the entire perimeter of the follicle.

An arbitrary-scale was used to assess the follicular blood flow in Doppler image. The scale was scored accordingly to Oliveira *et al.* (2014): (0) non-detectable, (1) small, (2) moderate, (3) intense blood flow (Figure1).

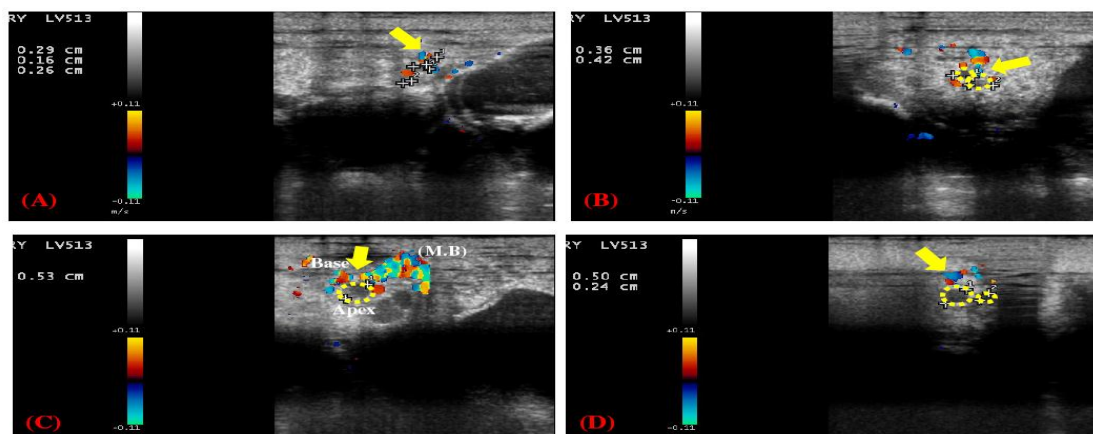


Fig. 1. Color Doppler images of arbitrary blood flow (yellow arrows) and follicular diameter. (A) Showing the growing follicle and (B) showing selected follicle. (C) Showing a prominent changes in the regional blood flow of the follicle with a marked increase of the flow to the base of the follicle and a concomitant decrease of blood flow to the apex (the base is ischemic) before ovulation; M. B represented the main blood branch that nourish the follicle. (D) Showing an insufficient vascular supply that could act as the trigger that leads to follicular atresia

Blood collection and hormonal determination

The blood samples were collected via jugular venipuncture at the time of sponge insertion, then every three days till the time of sponge removal (Day 0). At Day 0, the blood samples were collected daily for four d just after each Doppler scan. The blood samples were allowed to clot at room temperature then centrifuged within half an hour after collection and were stored at -20°C . The hormones were assayed in blood serum. Circulating concentrations of P4 and E2 were determined by ELISA kits (BioChek, Inc., Foster City, CA 94404, USA). The lowest detectable level of P4 in this test is 0.05 ng/ml and 10pg/ml for E2. The NO was analyzed by Colorimetric Determination of Nitrite (Biodiagnostic, catalog No. TA2532, Egypt). The final products of NO *in vivo* are nitrite (NO_2) and nitrate (NO_3). There is an exogenous source of NO_3 from the diet, so the index of NO production is the NO_2 . The biodiagnostic nitrite assay kit provides an accurate and convenient method for measurement of endogenous nitrite concentration as indicator of endogenous NO production in biological fluids.

Statistical analysis:

All data were expressed as mean \pm SD. The comparisons of mean values were performed by Kruskal-Wallis ANOVA on Ranks followed by Dunn's multiple comparisons. All statistics were calculated with the help of either JMP v5.0.1 (SAS campus drive, Cary, NC, USA) or Graphpad Prism v5 software (Graphpad Software, Inc., San Diego, CA). Differences of $P < 0.05$ were regarded as significant.

RESULTS

Reproductive performance and follicular growth:

The reproductive performances of the goats were presented in Table (1). Estrus was detected in 86.7% and 93.3% of the does for the LT and ST groups, respectively. The onset and end of estrous symptoms were 25.3 ± 10.1 to 65.5 ± 14.4 , and 25.0 ± 8.9 to 76.5 ± 17.3 h, for the LT and ST groups, respectively ($P > 0.05$). The duration of heat in LT group (Table 1) was shorter ($P > 0.05$) than in that ST group. The percentage of goats that showed estrus 48 h following the sponge withdrawal in LT group was 100%, however, 100% of the goats showed estrus 32 h following the sponge withdrawal in ST group (Figure 2).

The diameter of recruited follicles was 2.5 ± 0.3 mm for LT group and 2.8 ± 0.2 mm for ST group (Figure 3). In LT group, the maximum follicular diameter was 6.0 ± 0.4 mm and 6.0 ± 0.5 mm in ST group. There was no difference ($P > 0.05$) between two the groups in the size of recruited and ovulatory follicles.

The multiple kidding rates were different ($P < 0.05$); being higher in LT group than ST group. However, the pregnancy rate, kidding rate and litter size were similar ($P > 0.05$) (Table 2).

Blood flow:

Arbitrary blood flow scores were set out in figure (4). The Arbitrary blood flow was increased with the increase in the diameter of the follicles in both groups of the studied goats. The ST group had greater ($P < 0.05$) blood flow scores than LT group on Day 0, however, There was no difference ($P > 0.05$) between two the groups after day 0.

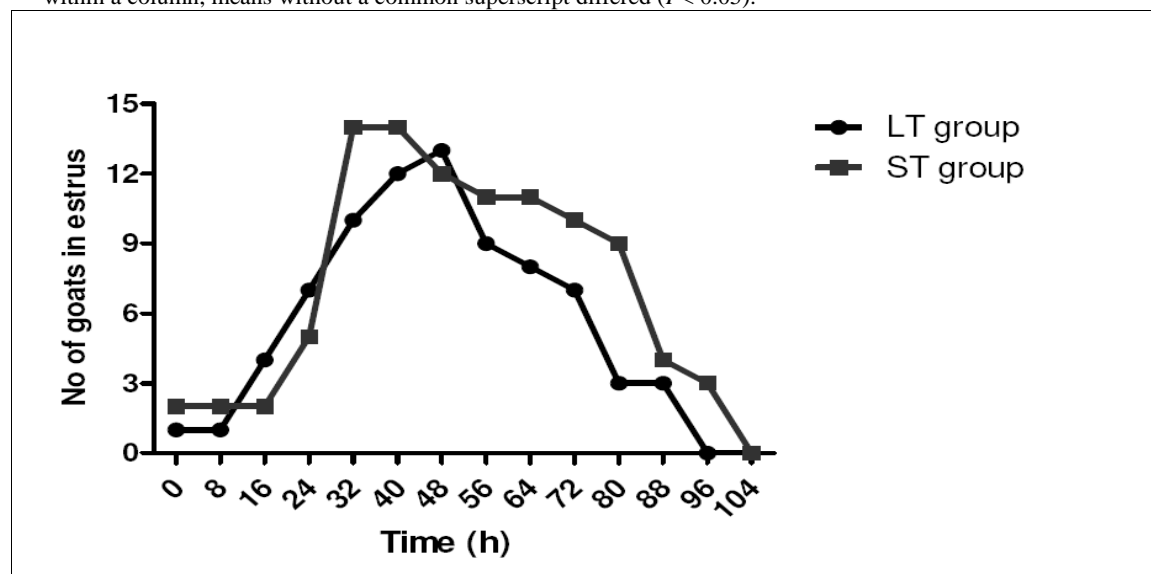
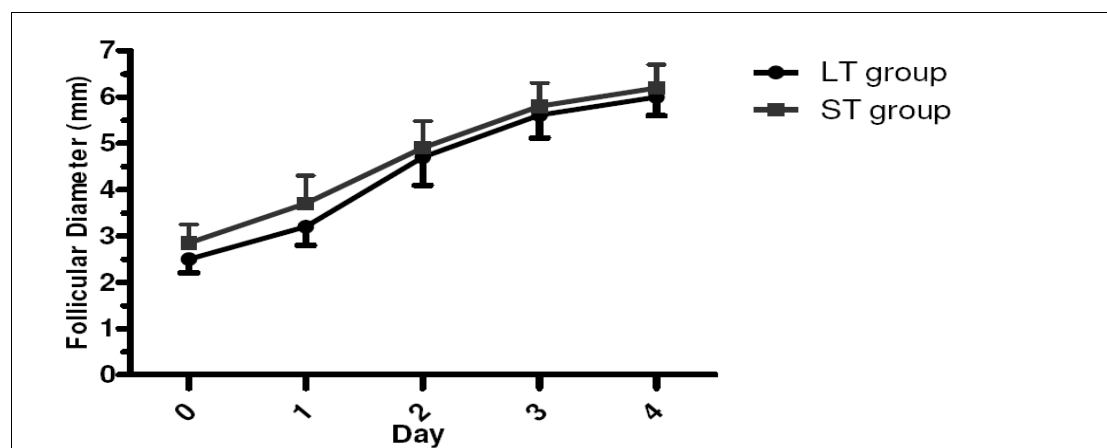
Table 1. Reproductive performance (mean \pm SD) of Sa'idi goats after long-term (LT group) and short-term (ST group) progesterone treatment

Reproductive parameters	LT group	ST group
Estrus response (%)	86.7%	93.3%
Onset of heat (h)	25.3 \pm 10.1	25.0 \pm 8.9
End of heat (h)	65.5 \pm 14.4	76.5 \pm 17.3
Duration of heat (h)	40.7 \pm 15.0	52.3 \pm 18.7
Onset of the wave (d)	0.4 \pm 0.5	0.3 \pm 0.5
First day of maximum follicular diameter	4.1 \pm 0.3	4.5 \pm 0.5
Growing phase (d)	3.8 \pm 0.5	4.2 \pm 0.9
Maximum follicular diameter (mm)	6.2 \pm 0.5	6.0 \pm 0.4
End of wave (d)	4.9 \pm 0.4	5.1 \pm 0.4
Length of wave (d)	4. 5 \pm 0.8	4. 8 \pm 0.4

Table 2. The pregnancy rate, abortion rate, kidding rate, multiple kidding rate and litter sizes of Sa'idi goats after long-term (LT group) and short-term (ST group) progesterone treatment

Treatment	Pregnancy rate	Abortion rate	Kidding rate	Multiple kidding rate	Litter size
LT group no.	8/13	1/8	7/8	7/7	22/7
%	(61.5%)	(12.5%) ^a	(87.5%)	(100%) ^a	(3.1)
ST group no.	6/13	0/6	6/6	3/6	11/6
%	(46.2%)	(0.0%) ^b	(100%)	(50%) ^b	(1.8)

^{a-b} within a column, means without a common superscript differed ($P < 0.05$).

**Fig. 2. Distribution of the onset of estrus after long-term progesterone treatment (LT group) and short-term progesterone treatment (ST group) of sa'idi goats.****Fig. 3. Mean \pm SD. Changes in diameter of the ovulatory follicular (mean \pm SD) induced long-term progesterone treatment (LT group) or short-term progesterone treatment (ST group) of Egyptian goats.**

Blood serum hormones concentration:

The P4 concentrations in blood serum were almost similar ($P>0.05$) in the two groups (Figure 5). On Day 2, 3, and 4 the concentrations of estradiol-17 β (E2) were greater in ST group ($P<0.05$) than that in LT group (Figure 6). The E2 concentrations were almost similar ($P>0.05$)

in the two groups before sponge withdrawal. In contrast, the concentration of the NO tended to differ ($P<0.05$) in LT group before sponge withdrawal compared with ST group (Figure 7). However, there was no difference ($P>0.05$) between the two groups in the concentrations of the NO after sponge withdrawal.

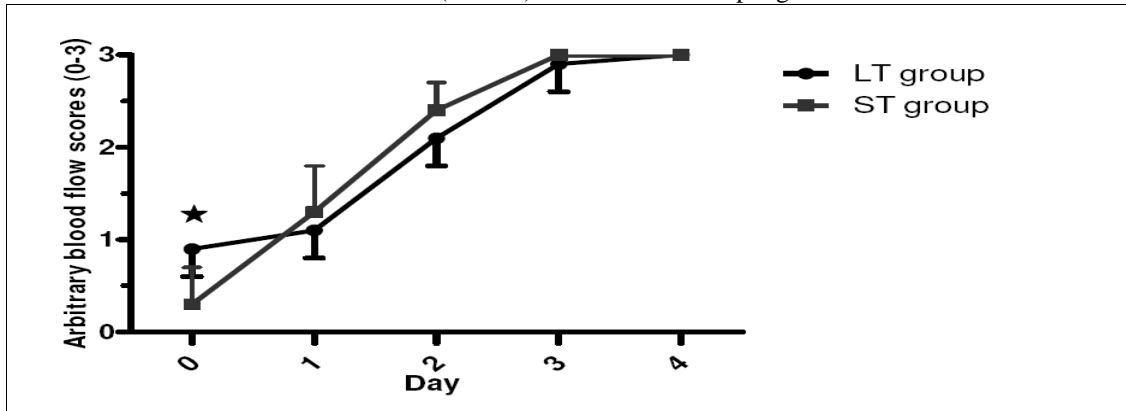


Fig. 4. scored scale (mean \pm SD) of ovarian blood flow of sa'idi goats during heat and the four subsequent days. Differences ($P<0.05$) between the two groups were denoted by \star .

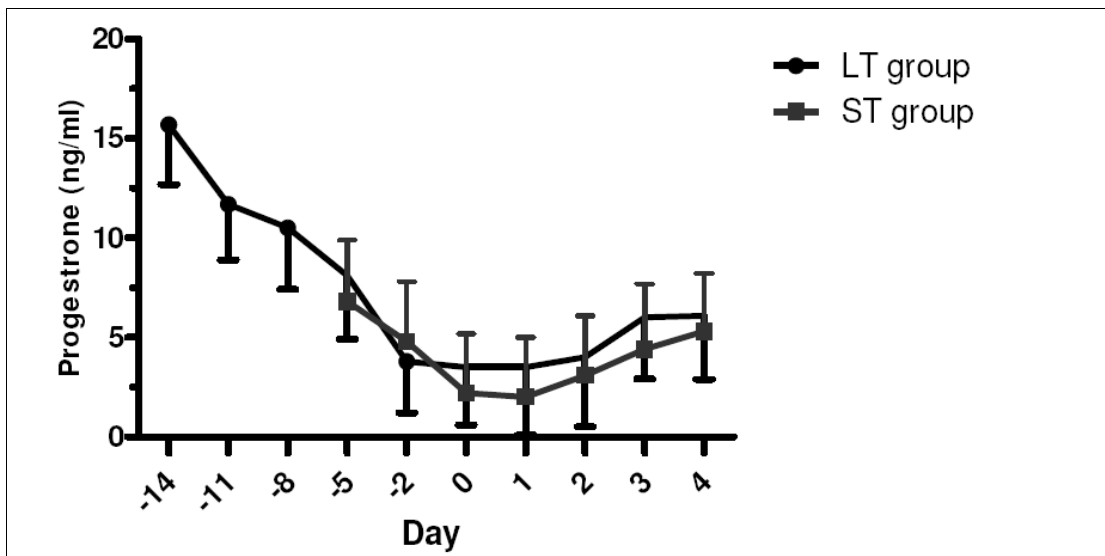


Fig. 5. Changes in plasma progesterone (P4) concentration. Differences ($P<0.05$) between the two groups were denoted by \star .

DISCUSSION

In the present study, no difference was observed between the ST and LT treatments combined with PMSG in the studied reproductive parameters (Table 1). The onset of heat in both groups was similar to those obtained in goats by Freitas *et al.*, (1997) (27.8 ± 5.0 h), Motlomelo *et al.* (2002) (30.9 ± 0.4 h) and Romano (2004) (32.9 ± 9.7 h). However, the onset of heat was longer than those of Dogan *et al.* (2005) (18.0 ± 1.9 h), who used 500 IU PMSG 2 d before the sponges removal, but were shorter than those (49.7 ± 15.5 h) of Fonseca *et al.* (2005), who used 200 IU PMSG 24 h before sponge removal. These differences may be associated with the dose of PMSG used in the

present study. The PMSGs known to reduce the interval between sponge removal and onset of estrus (Greyling *et al.*, 1985). Regueiro *et al.* (1999) showed that the use of 500 IU PMSG decreased the interval to estrus onset in Nubian, Saanen and cross-breed goats. However, (Ustuner *et al.*, 2007) reported that there was no significant effect of the type of progestagen sponges or time of PMSG administration on the time to estrus onset in ewes. On Day 2, 3, and 4 the concentrations of E2 were significantly higher in ST group than in LT group that may give explanation for the shorter duration of heat in LT group (40.7 ± 15.0 h) than in ST group.

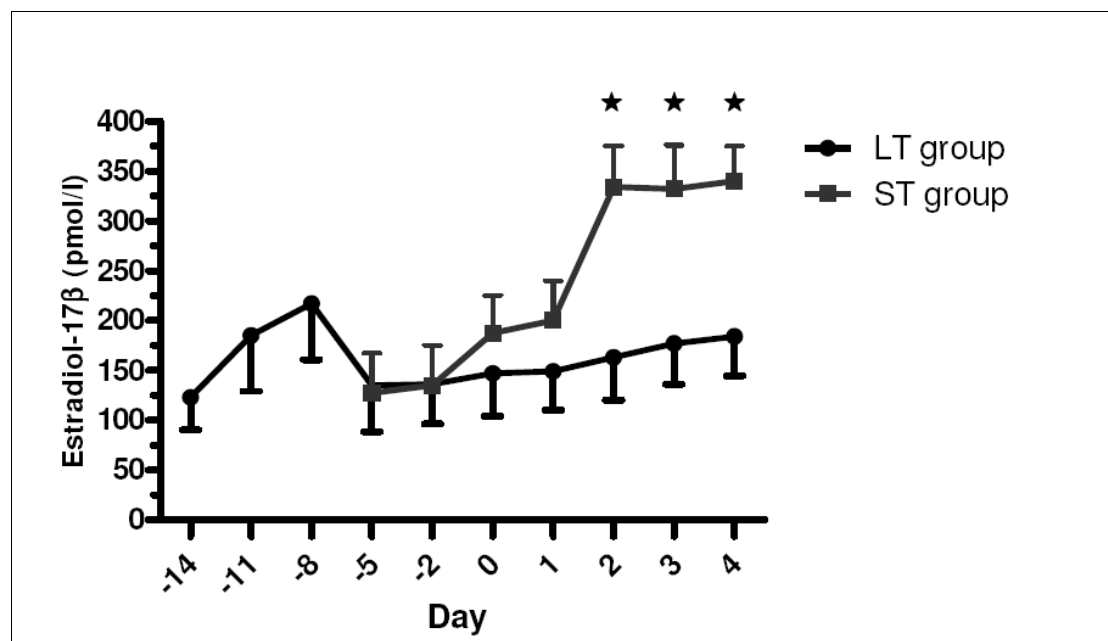


Fig. 6. Changes in plasma estradiol-17 β (mean \pm SD) in long (LT) and short (ST) progesterone treated sa'idi goats. Differences ($P < 0.05$) between the two groups were denoted by \star .

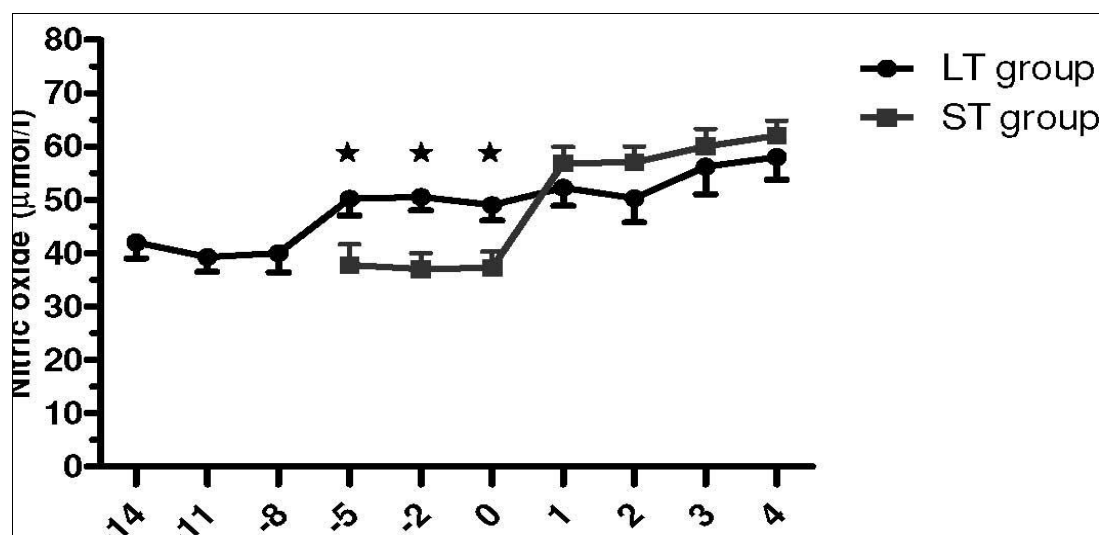


Fig. 7. Changes in plasma nitric oxide (mean \pm SD) in long (LT) and short (ST) progesterone treated sa'idi goats. Differences ($P < 0.05$) between the two groups were denoted by \star .

The results of LT progestagen treatment did not improve reproductive performance of goats, as it has been reported in ewes (Karaca *et al.*, 2009). The reason for this variation is not clear; it may be associated with the PMSG used at the time of sponge removal in goats in the onset of breeding season. It has been reported that the administration of PMSG when progestagen treatment was terminated, could compensate the deleterious effect of long-term progestagen treatment on follicular dynamics in cyclic ewes (Noël *et al.*, 1994). In addition, Barrett *et al.* (2004) revealed that, in anestrus ewes, 500 IU of PMSG given at the end of a 12 d treatment with progestogen-impregnated intravaginal sponge limited effects on the dynamics of ovarian

follicular waves. The abortion rate observed in the present study was 27.9% for LT group. In the aborted goats, no symptoms of infectious diseases were observed; this rate was comparable to the incidence of fetal loss (3-38%) that was reported in the different goat herds by England (1998).

Changes of vascularization and expression of some regulators, including angiogenic factors, are associated with follicular growth and/or atresia. Vascular network around developing follicles may be rate limiting for the selection of the dominant follicle that is destined to ovulate. In the present study, the blood flow increased from Days 0 to Day 5 in both ST and LT groups of goats as well as the follicular diameter, and

the concentrations of NO and E2 were increased with the increase of blood flow. Therefore, the marked increase in ovarian blood flow was associated with the increase of NO, E2 and follicular growth. The NO is a potent vasodilator agent that is involved in folliculogenesis and ovulation (Tamanini *et al.*, 2003). The healthy follicles have a greater ability to produce E2 during a period of relatively low serum gonadotropin concentration (Grazul-Bilska *et al.*, 2007). The E2 is recognized as the follicular growth, differentiation, and survival factor, which enhances aromatase activity, promotes expression of LH receptors, and increases sensitivity of granulosa cells to FSH and LH (Quirk *et al.*, 2004). In addition, the follicular estradiol enhanced NO production, and may cause a rapid dilation of blood vessels by activating angiogenic factor such as endothelial nitric oxide synthase (eNOS) (El-Sherry *et al.*, 2013).

The eNOS mRNA was detected in both theca and granulosa cell layers of ruminant species (Grazul-Bilska *et al.*, 2007). However, the protein was immunolocalized only in the theca and exclusively in the blood vessels of developing preovulatory and postovulatory follicles (Tessaro *et al.*, 2011). The eNOS is involved in follicular and luteal angiogenesis through the production of nitric oxide (NO) (Grazul-Bilska *et al.*, 2006). On a cellular level, successful development of ovulatory follicles requires both proliferation and differentiation of follicular cells (Feranil *et al.*, 2004). Such high proliferation of granulosa and theca cells of follicles may be closely associated with increased NO production. Thus, the expressions of eNOS in the cal layers were greater in the healthy follicle than in the atretic follicle (Moonmanee *et al.*, 2013). Therefore, the higher NO concentration was coincidental with the increase in follicular diameter and reached the maximum level at the time of ovulation as observed in our study. Furthermore, the NO is a potent vasodilator agent (Tamanini *et al.*, 2003) and vasculature, capillary area density, and capillary number density were positively correlated with NO production (Moonmanee *et al.*, 2013). Therefore, the increase in Blood flow could have been due to high concentration of NO that associated with the increase in follicular diameter. Moreover, because the granulosa layer has no blood vessels (Redmer *et al.*, 1996), the eNOS were not detected in the granulosa layer of all follicles (Moonmanee *et al.*, 2013) that may give explanation for why the blood flow was absent or reduced in recruited follicle and before theca layer formation.

Increased the follicular vascularization and permeability of blood vessels are known to allow a greater supply of growth factors, gonadotropins, and dissolved oxygen important

to the growth of follicles resulted in follicular progression, follicular fluid production and antrum formation (Van den Hurk and Zhao, 2005). In turn, insufficient vascular supply could act as the trigger that leads to follicular atresia (Augustin *et al.*, 2001). Thus, vascular supply or blood flow was significantly increased after selection of the goat dominant follicle and the vascularization of the follicular wall were greater in follicles that subsequently developed into antral follicle. Moreover, there are prominent changes in the regional blood flow of the follicle with a marked increase in the blood flow to the base of the follicle and a concomitant decrease of blood flow to the apex before ovulation. Similarly, El-Sherry *et al.* (2013) investigated the regional changes of blood flow in the ewe follicle, they demonstrated that blood flow is localized at the bottom of the follicle while the base is *ischemic*. Taking together, the NO has a role in maintaining vascularization during follicular growth and selection of the follicles for complete ovulation.

In conclusion, the estrus synchronization using LT progestagen did not have a positive impact on thereproductive performance of the Sa'idi goats, and could be replaced with ST progestagen at the beginning of breeding season. In addition, there is a positive relationship between E2 and NO, and E2. The NO concentrations were closely related to the FBF.

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التغيرات في تدفق الدم، وهرمون البروجسترون، استراديول، وأكسيد النيتريك بعد التزامن الشبقي خلال موسم التكاثر في الماعز الصعيدى

محمد عبد الغنى^{1*}، محمد حيدر²، ناصر أبو خليل³، ضرار رفعت⁴، فاتن أبوعمو⁵، محمد الشافعي⁶، طارق عبد الخالق⁷، حاتم حمدون⁸

١- قسم التوليد، كلية الطب البيطري، جامعة أسيوط، أسيوط، مصر، ٢- معهد بحوث الإنتاج الحيواني، مركز البحوث الزراعية، الدقي، الجيزة، ١٢٣١١، مصر، ٣- قسم وظائف الأعضاء الطبية، كلية الطب، جامعة أسيوط، أسيوط، ٧١٥٢٦، مصر، ٤- الحيوان قسم الإنتاج، كلية الزراعة، جامعة أسيوط، فرع الوادي الجديد، شركة الخارجية، الوادي الجديد، ٣٢٠١، مصر

وكانت الأهداف لتحديد الآثار التزامن الشبقي على الأداء التناسلي للماعز الصعيدى، ودراسة العلاقة بين تدفق الدم الجريبي (FBF) وتركيزات الدم من البروجسترون (P4)، استراديول (E₁₇) وأكسيد النيتريك (NO) تلقت مجموعة ١ إسفنجة مهبلية لمدة ١٤ يوم (مجموعة LT)، وحققوا ٥٠٠ وحدة دولية PMS في وقت إزالة الإسفنجة تلقت مجموعة ٢ إسفنجة مهبلية لمدة ٥ أيام (مجموعة ST)، وحققوا ٥٠٠ وحدة دولية PMS و ٢.٥ ملغ من دينوبروست في وقت إزالة الإسفنجة. استخدم الدوبلر الملون على تدفق الدم لتقييم FBF في الصور لوحظا يوجد فرق بين المجموعات ST و LT من حيث المعلمات الإنجابية وكانت معدلات المواليد المتعددة مختلفة. وكانت ٦١.٥% في مجموعة LT و ٤٦.٢% في مجموعة ST. زيادة تدفق الدم الجريبي مع الزيادة في القطر. وكان تدفق الدم في الفريق ST أكبر منتدفع الدم في مجموعة LT في اليوم ٠. تركيزات P4 مماثلة بين المجموعتين. في يوم ٢ و ٣ و ٤، وكانت تركيزات E2 أكبر في مجموعة ST من مجموعة LT. تميل تركيز NO لأن تختلف في مجموعة LT قبل انسحاب الإسفنجة مقارنة مع مجموعة ST. وفي الختام، هناك علاقة إيجابية بين E2 و NO، وكان تركيز NO ترتبط ارتباطا وثيقا بFBF.