Improving Reproductive Performance by Glucose Injection in Damascus Does Goat during Early Summer

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Abstract: Goats are seasonally polyestrous having estrous activity during late summer, fall, and winter and showing no activity during summer and spring. The objective of the present study was to improve reproductive performance of Damascus doe goats in early summer including: estrous activity (EA), ovarian follicular (OF) growth, corpus luteum (CL) development, and progesterone (P_4) profile by injection of glucose (Glu). A total of twelve apparently healthy Damascus doe goats were used in this experiment and were classified randomly into two equal groups. Animals in group A were injected by Glu via j.v.; each animal of the treated group received 94.584 g Glu daily for nine days before the expected day of ovulation. The second group (B) was injected with saline solution and used as control. All animals in both groups were synchronized by $PGF_2\alpha$ (cloprostenol) three times (10 days between each interval and other) with notice that Glu was injected in the second interval. Blood samples were collected from each animal; the blood was then centrifuged and the serum was analyzed for P_4 determination. All does were subjected to ultrasonographic examination on days 5, 9, and 19 after the third injection of $PGF_2\alpha$ and post-treatment by Glu. The results revealed that Glu injection achieved estrous activity higher than in the control (100% vs. 50 %, $p>0.05$). All animals showed the estrous activity through 24-72 hours after each dose of $PGF_2\alpha$ and post-treatment by glucose. The number of follicles (≤5mm) in the treated group was higher than in the control group (111 vs. 94 follicle, $p>0.05$), while the follicular diameter did not differ between the two groups. Left ovary was more active than in right ovary (107 vs. 98 follicle, $p>0.05$) and the ovulation rate detected from the number of corpora lutea and progesterone level was higher ($p>0.05$) in the treated group than in the control. Moreover, the ovulation was significantly higher in the right ovary than in the left ovary (19 vs. 9 follicles). Corpus luteum diameter in the treated group was significantly larger than in the control group (1.2±0.11 cm vs. 0.97±0.13 cm, $p>0.05$). The average progesterone concentration increased significantly (2.36±0.84 ng/ml) in the treated animals than in the control (0.96±0.23 ng/ml). It could be concluded that Glu treatment led to improvement of number of estruses, ovarian follicles, corpora lutea and progesterone concentration in Damascus doe goats during early summer. Therefore, treatment by energy-yielding nutrient (glucose injection) on the estrous and ovarian activity may be recommended in periods of reproductive activity impairment in goats.
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1. Introduction:

The estrous activity of Damascus does goat occurs in autumn and winter and not in spring and early summer (Mahmoud, 2010). This means that ovarian activity during spring and early summer in these animals is lacked. Away from the use of known hormonal treatments (gonadotropins) which evoke on the ovarian activity, high energy-yielding nutrients are used for increasing ovulation rate in sheep (Teleni *et al.*,1989b, Downing & Scaramuzzi, 1991) and rising FSH levels during the estrous cycle (Rhind *et al.*, 1985, Rhind & McNeilly 1986). In contrast, findings obtained by Findlay & Cumming (1976), Rhind *et al.* (1989), Xu *et al.* (1989), Smith and Stewart (1990) were not compromised with the previous reports. Whereas, Vin˜oles *et al.* (2002) reported that effect of nutrition on daily follicular development led to an increase in ovulation rate and FSH secretion and a decrease in estradiol-17β concentration during the follicular phase of the estrous cycle in ewes with a high body condition. Further, ewes fed high energy-yielding nutrients from days 8 to 14 of the estrous cycle owned increased ovulation rate by 14% (Vin˜oles, 2003). For this reason, we prefer in this experiment to use the high energy-yielding nutrients (glucose injection) to test if it is able to resume the estrous and ovarian activity of Damascus does in early summer or not, since administration of exogenous hormones is a costly treatment and may cause severe health problems (Grøndahl, 2008).

The physiological mechanism of immediate energy-yielding nutrients (glucose) on the follicular development has been investigated. Glucose may work in direct way on the ovary and metabolic hormones such as insulin-like growth factor I (IGF-I),

since the glucose transporter proteins and specific receptors for this hormone are found in the ovarian follicles (Williams *et al.*, 2001, Mun˜oz-Gutie´rrez *et al.*, 2004). Scaramuzzi and Radford 1983, Souza *et al.*, 1997, Monget and Martin 1997, Scaramuzzi *et al.*, 1999, Williams *et al.*, 2001, reported that feeding on glucose increased Insulin and IGF-I levels which in turn supported follicular responsiveness to gonadotrophins. Additionally, metabolic hormones may also regulate the steroid synthesis (Scaramuzzi *et al.*, 1999). So, our hypothesis in this study is to test glucose injection in the synchronized doe goat on estrous activity resumption, ovulation rate, CL development and progesterone concentration during early summer under Upper Egypt (Assiut) climatic conditions.

2. Material and Methods

Animal and managements:

This experiment was carried out in goat barn, Animal Production Farm, Faculty of Agriculture, Assuit University.

A total of twelve apparently healthy does with mean body weight of 32 ± 0.8 kg were used in this experiment and classified randomly into two equal groups (n = 6 for each). Animals in group A were considered as treated group while B was considered as control group for A. Both groups were housed in semi-pen pens and fed maintenance ration according to NRC (1985).

Glucose injection

Each animal in the treated group was injected i.v. by 94.584 g Glu (Algomohorya Medical Company, Egypt). Glu was calculated from the dose used by Teleni *et al.*, 1989a (525 mMole/day), which was dissolved in 264 ml distal water and given to each animal daily for nine days before the expected day of ovulation. The conversion from mMole to grams is as follows:

$525 \times 10^{-3} \times 180.16$ (molecular weight of glucose, $C_6H_{12}O_6$) = 94.584 g.

Hence, 94.854 g glucose should be dissolved in 264 ml distal water.

$$\frac{264 \text{ ml}}{24 \text{ hours}} = 11 \text{ ml / hour}$$

To avoid stress on animal, 44 ml glucose/4 hours/6 times/day for 9 days was injected in each animal. While animals in group B (control) were infused by normal saline solution (Algomohorya Medical Company, Egypt).

Estrous synchronization

All does were synchronized by prostaglandin analogue (Cloprostenol, estroPLAN® injection, Parnell Laboratories, PTY.LTD, AUST) and each doe was injected three times by 125 µg i.m. $PGF_2\alpha$ according to Nuti *et al.*, 1992, Kusina *et al.*, 2001 Cueto *et al.*, 2006, Khanum *et al.*, 2007 and Fernandez-Moro *et al.*, 2008). The first injection was given on day 10/7/2007, the second injection was given 10 days after the first injection, while the third injection was followed after 10 days of the last one as shown in Table (1).

Estrus detection

After each injection of $PGF_2\alpha$, estrus was detected by using two fertile bucks to run with the does for 30 minutes for detection the estrous does. Each buck was allowed to run with a half number of does for 15 minutes then after that, each one was replaced by the other. Estrus detection was checked daily at 8:00 a.m. for three days throughout the experiment. Estrous signs such as vaginal discharge, vulva swelling and tail twitching and mounting have been taken into consideration during determination of estrous (Mauleon and Dauzier, 1965 and Cerbito *et al.*, 1995).

Blood collection

Time table of blood samples collection, injection of glucose and synchronization by $PGF_{2\alpha}$ are presented in Table (1). Blood samples were withdrawn from j.v. at 8.0 a.m. during and after each injection by $PGF_{2\alpha}$. and post-treatment by Glu, then left overnight in the refrigerator (at 4 °C) to clot, and then centrifuged at 2000 g for 20 minutes to harvest the sera, after that the sera were preserved in eppendorf tube (4 ml) and stored at -20 °C until hormonal assay (Mori and Kona, 1984 and Burfening and Berardinelli, 1986).

Table 1. Shows days of blood samples collection and injections of $PGF_{2\alpha}$ and Glu.

Date of treatment	Days of blood samples collection
10 / 7 / 2007 (1st injection of PGF2α)	1, 2, 3 (during estrus) 4, 6 and 11 (post-estrus)
20 / 7 / 2007 (2nd injection of PGF2α with Glu injection)	1, 2, 3 (during estrus), 4, 6 and 11 (post-estrus)
30 / 7 / 2007 (3rd injection of $PGF_2\alpha$ and post injection of Glu)	1, 2 (during estrus), 4, 8, 12, and 16 (post-estrus)
Ultrasonography (sonar)	5, 9 and 19 after Glu injection and $PGF_2\alpha$.

Hormonal assay

Progesterone was assayed by ELISA through the kits purchased from DRG, Instruments GmbH, Germany (2005). The DRG progesterone enzyme immunoassay kit provides materials for the

quantitative determination of progesterone in serum and plasma (DRG, 2005).

Ultrasonographic (sonar) examination

All does were examined by sonar (ultrasonography) on days 5, 9 and 19 after treatment by Glu and the 3rd injection of $PGF_{2\alpha}$. Trans-rectal ultrasonography was done on all animals utilizing a real-time B-mode echo camera (Pie Medical, 100 LC, Maastricht, The Netherlands) connected to a 6/8 MHz changeable transducer. The transducer was fitted in a self-manufactured connector to favor its manipulation in the rectum. The transducer was manipulated externally, with the doe in standing position. At each examination, the number, diameter and relative position of all follicles ≥ 2 mm in diameter and corpora lutea were recorded and sketched on ovarian charts to analyze the pattern of growth of follicles.

Statistical analysis

Quantitative data were analyzed by analysis of variance using the General linear model procedures (GLM) (SAS, 1996). Differences between means were tested using Duncan's multiple range test (Duncan, 1955). Number of estruses, ovarian follicles, and corpora lutea was statistically analyzed by Chi-square analysis.

3. Results

Estrous activity:

Fig. 1. Shows that the percentage of estrus detected after the 1st injection of $PGF_{2\alpha}$ and before treating by Glu was significantly higher (50 %) in non-treated does than in treated does (33 %). In the 2nd dose of $PGF_{2\alpha}$ accompanied with Glu injection, the estrus percentage was significantly higher (100%) in treated does than in non-treated does (50%). After the 3rd dose of $PGF_{2\alpha}$, and post-treatment by Glu, estrus percentage was significantly higher in treated does (100%) than in non-treated does (83 %). In addition, all does exhibited the estrus through 24-72 hrs after each dose of $PGF_{2\alpha}$ and even post-treatment by glucose. These data suggests that synchronized does treated by Glu achieved high estrous activity, and this should be used for rising reproductive efficiency in the animal farms.

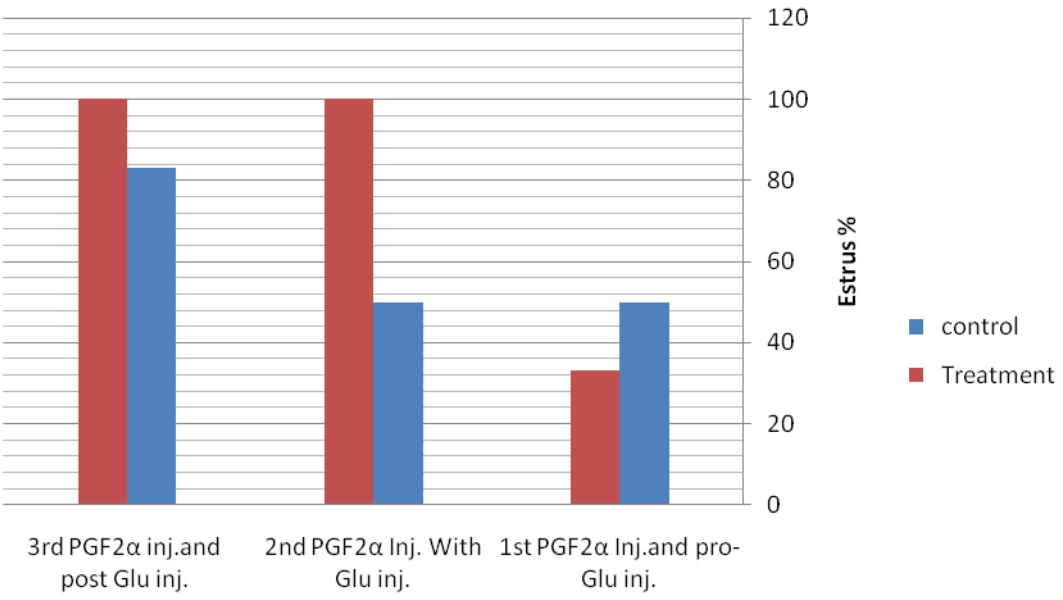

Figure 1. Estrus percent in the does synchronized by 3 times of $PGF_2\alpha$ and post treatment by Glu.

P_4 concentration (ng/ml blood serum)

After the 1st dose of $PGF_{2\alpha}$ and pre-treatment by Glu, P_4 concentration was almost semi-equal in the two groups (0.23±0.04 in treated and 0.27±0.02 ng/ml, in control) as in the 2nd dose of $PGF_{2\alpha}$ accompanied with Glu (0.36±0.07 and 0.33±0.04 ng/ml, in treated and control does, respectively). While in the 3rd dose of $PGF_{2\alpha}$, and post-treatment by Glu, P4 level increased significantly ($P<0.05$) in the treated does (2.36±0.84 ng/ml) than in the control group (0.96±0.23 ng/ml) (Fig. 2).

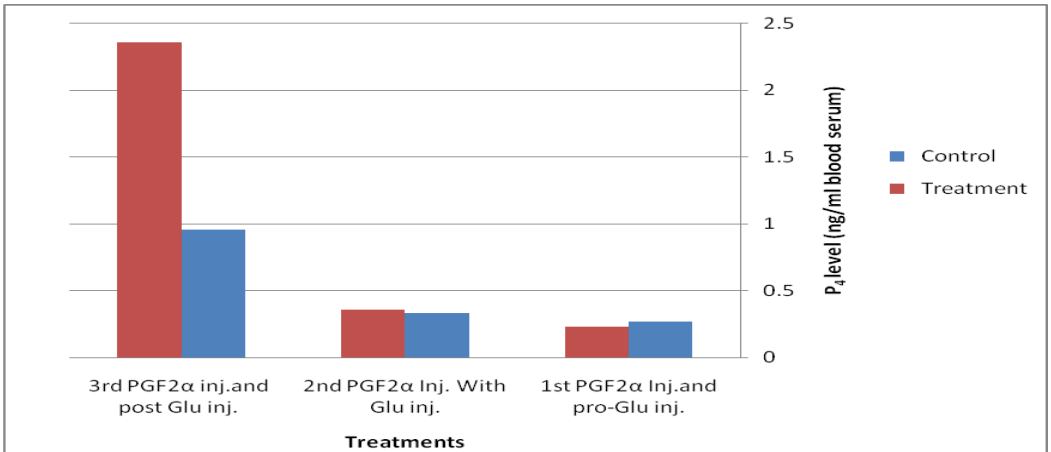

Figure 2.P_4 concentration pre and post treatment of Glu in the synchronized does by $PGF_2\alpha$ analogue.

These data also indicate that P_4 profile in the synchronized does treated by Glu remained approximately similar to levels in the normal estrous cycle, it was < 1.0 ng/ml on day of estrus, then rose gradually until reached > 1.0 ng/ml by 8 days post estrus, and the peak level occurred on day 16 of the cycle, since this level was significantly higher in the treated does than in the control group (5.86±1.73 vs. 3.62±1.21 ng/ml, respectively). These results suggest that the increased P_4 concentration in the does treated by Glue may be utilized for enhancing pregnancy.

Number and diameter (Cm) of OF

In Fig. 3, does injected by Glu showed sensible increase in numbers of ovarian follicles (n=111 F) compared with that non-treated does (n=94 F). Generally, numbers of OF decreased gradually during the three examinations of sonar and reached the minimal number on day 19 in the two groups (23 OF, control and 31 OF, treatment). Follicles observed in ovaries of both groups were ≤ 0.5 cm in diameter (Fig. 3). Despite the significance was absent in the two groups, left ovaries were significantly higher in follicular production than in right ovaries (107 vs. 98 follicles) (Table 2 and Fig. 4), moreover, the left ovaries of treated does were also more active than the right ovaries in OF production (61 OF vs. 50 OF) (Table 3 and Fig. 4).

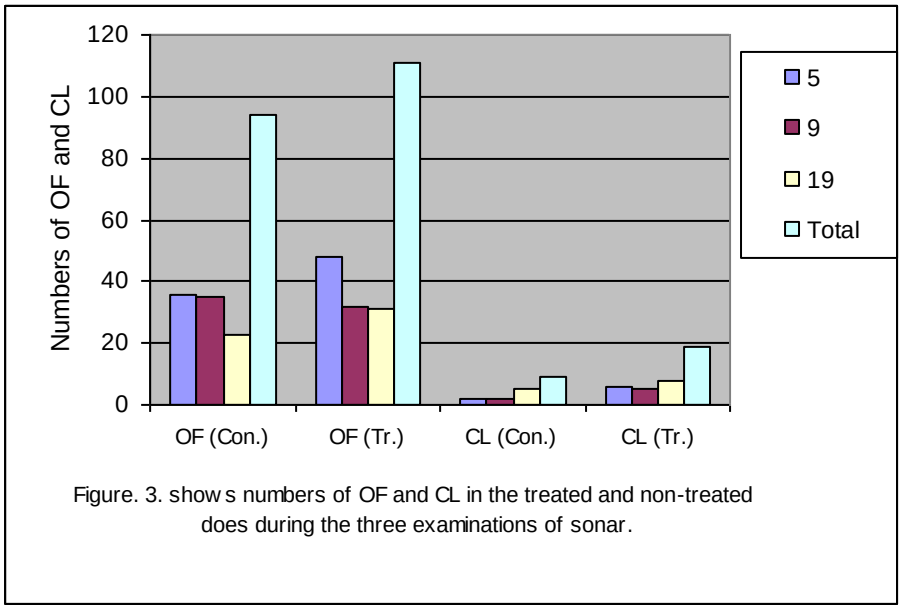

Figure. 3. shows numbers of OF and CL in the treated and non-treated does during the three examinations of sonar.

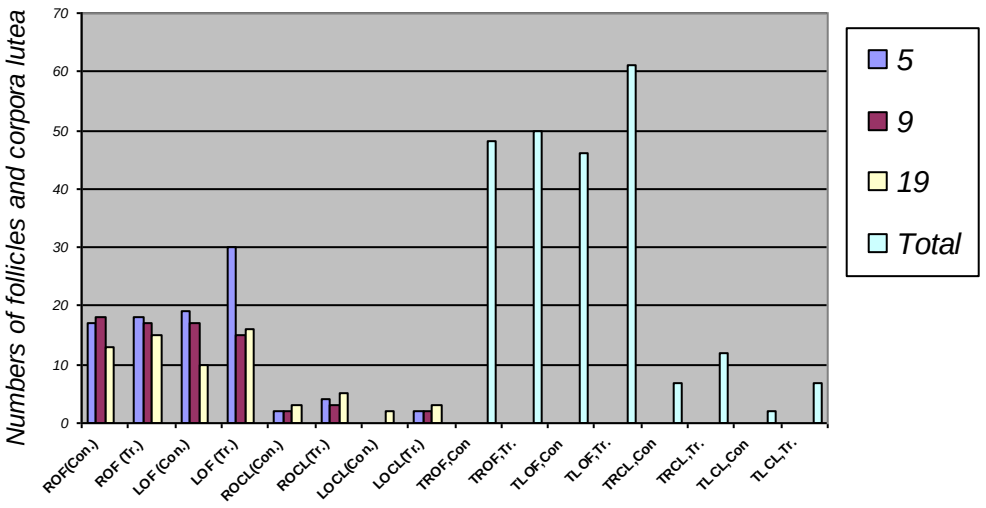

Figure 4. Shows numbers of OF and CL observed in the right and left ovaries (RO & LO) of treated and non-treated does during the three examinations of sonar.

Table 2. Shows mean ± SE diameters of OF and CL in the treated and non-treated does during the three examinations of sonar.

Sonar day	No. does/group	OF diameter (cm)		CL diameter (cm)	
		Control	Treatment	Control	Treatment
5	6	0.39±0.02	0.35±0.01	1.04±0.15	1.20±0.15
9	6	0.34±0.02	0.37±0.02	0.85±0.12	1.29±0.09
19	6	0.32±0.03	0.32±0.03	1.03±0.13	1.10±0.08
Overall mean		0.35±0.02	0.35±0.02	0.97±0.13[b]	1.2±0.11[a]

Values are least-squares means (L.S.M) ±standard error of L.S.M

[A and b] Means in the same row with different superscripts are significantly different (P<0.05)

Table 3. Mean ± SE diameters of OF and CL (cm) observed in right and left ovaries of treated and non-treated does.

Sonar Day	RO				LO			
	Control		Treatment		Control		Treatment	
	OF	CL	OF	CL	OF	CL	OF	CL
5	**0.42±0.03 n=17**	1.04±0.15 n=2	**0.36±0.03 n=18**	1.27±0.23 n=4	**0.36±0.03 n=19**	-	**0.35±0.02 n=30**	1.05±0.08 n=2
9	**0.38±0.04 n=18**	0.85±0.12 n=2	**0.38±0.03 n=17**	1.32±0.16 n=3	**0.31±0.03 n=17**	-	**0.36±0.04 n=15**	1.24±0.02 n=2
19	**0.35±0.03 n=13**	1.13±0.20 n=3	**0.35±0.04 n=15**	0.97±0.07 n=5	**0.27±0.05 n=10**	0.88±0.06 n=2	**0.30±0.04 n=16**	1.32±0.05 n=3
Overall mean	**0.39±0.02 n=48**	1.02±0.10[b] n=7	**0.36±0.02 n=50**	1.16±0.09[a] n=12	**0.32±0.02 n=46**	0.88±0.06[b] n=2	**0.34±0.02 n=61**	1.22±0.05[a] n=7

Values are least-squares means (L.S.M) ±standard error of L.S.M

[A and b] Means in the same row with different superscripts are significantly different (P<0.05)

Number and diameter (Cm) of CL

Sonar examination showed that numbers of CL during the three examinations increased

significantly (P <0.05) to 6, 5 and 8 in the treated does compared with the control (2, 2 and 5 CL) (Fig. 3). This increase was associated with increasing P_4 concentration and number of OF produced from the treated does compared with the control as shown in Table 2. The average CL diameter in the treated does was significantly (P<0.05) larger than in the control group (1.2±0.11 vs. 0.97±0.13 cm). Furthermore, the average number of CL in the right ovary was significantly higher than that found in the left ovary (19 vs. 9, P<0.05), also the right ovaries of treated does contained large numbers of CL than in the left ovaries (12 CL vs. 7 CL) (Table 3 and Fig. 4). These data also denote that when the number of OF increases in one ovary the diameter of these follicles decreases in the same ovary or increases in the other ovary. Similarly, when numbers of CL increase in one ovary, their diameters would decrease in the same ovary or increase in the other ovary (Table 3 and Fig. 4). Otherwise, the average CL diameter increased slightly in the treated does compared with the control. However, Images (5-8) taken by sonar show the variability in CL size in the treated and non-treated does.

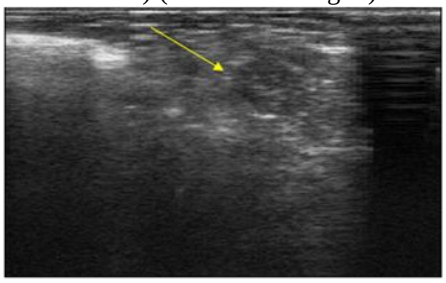

Fig 6. The arrow denotes CL size on day 5 (treatment).

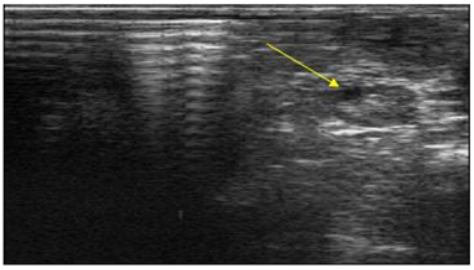

Fig 5. The arrow denotes CL size on day 5 (control).

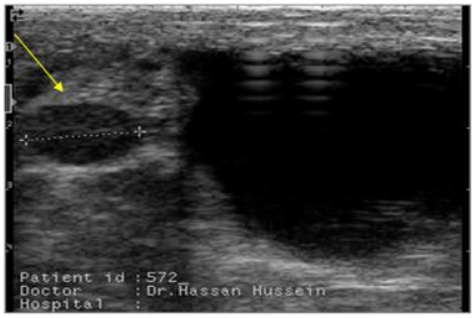

Fig 7. The arrow denotes CL size on day 19 (control).

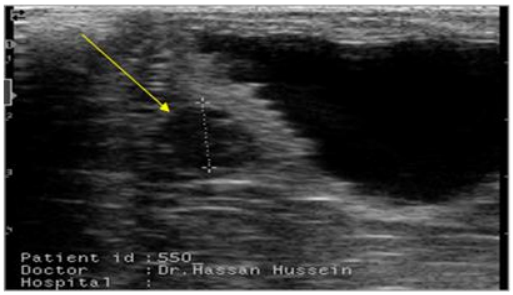

Fig 8. The arrow denotes CL size on day 19 (treatment)

4. Discussion:

It is obvious from the results that all does treated by Glu came into estrus compared with non-treated does. This means that Glu improved expression of estrus in the synchronized does. These results are similar to that obtained by Funston *et al.* (1995) who indicated that expression of estrus was prevented after depletion of glucose (2-deoxyglucose) from ewes. Ott *et al.* (1980) showed that the synchronized does goat with two injections of $PGF_{2\alpha}$ (11 days apart) 70 % of them came into estrus by 54 hrs after the 1st injection and 94 % after the 2nd injection by 53 hrs. El-Amrawi *et al.* (1993) treated cycling Saanen does with the same protocol as in the study of Ott *et al.* (1980), and reported that all does came into estrus within 48 h after the 1st injection, and 80 % became pregnant following breeding. In one study on West African dwarf goats, Akusu *et al.* (1986) reported that most goats came into estrus by 42 and 59 hrs after the 2nd injection of $PGF_{2\alpha}$, analogue, respectively. Regarding Fig. 2. P_4 concentration remained under 1.0 ng/ml after the 1st dose of PGF_2 and the 2nd dose of PGF_2 accompanied by Glu, while after the 3rd dose of PGF_2 accompanied by Glu, P_4 level rose to > 1.0 ng/ml in the treated does compared to the control group. Furthermore, P_4 level reached the peak on day 16 of the cycle, but it was significantly higher in the treated does than in no-treated group. This increase may refer to the large numbers and sizes of Cl observed in the treated group. So, the immediate effect of Glu may be accompanied by an increase in P_4 concentration.

These results are similar to that obtained by Gaafar *et al.* (2005) who indicated that plasma P_4 concentrations were 0.5±0.1 ng/ml, 0.4±0.3, 2.3±0.6 and 3.5±0.5 ng/ml at days 13-14 before the estrus, day 0 (estrus), day 3 and days 5-14 of the estrous cycle of Damascus does, respectively. They indicated also that the decline in plasma P_4 concentration was noted 24-72 hrs before the estrus comes. Thorburn and Schneider (1972) indicated that during the breeding season, P_4 concentration in the blood plasma decreased during the follicular phase (< 1.0 ng/ml) of the estrus cycle and remained at this level until reached > 1.0 ng/ml at beginning of luteal phase (Chemineau, 2004 and Yu *et al.,* 2005).

The present results showed also that treatment by Gl had a vital role in recruitment follicles especially in LO than in RO, these results are similar to that reported in previous studies in sheep (Teleni *et al.* 1984, Teleni *et al.* 1985, Teleni *et al.*1989_a, Teleni *et al.*, 1989_b and Downing *et al.*, 1995). They indicated that intravenous infusion of glucose improved ovulation rate in ewes. Teleni *et al.* (1989_b) pointed out that the increase in the ovulation rate was strongly associated with feeding on Glu and Lupin, in other study, ovulation rate was 1.64 in Merino ewes injected i.v. by Glu (Teleni *et al.,* 1984 and Teleni *et al.,* 1985). Teleni (1989_a) indicated that all ewes treated with Glu or Glu + acetate + lupin for 9 days before the expected time of ovulation led to a significant increase in the ovulation rate (approximately 25 %, P<0.001) than the control group. Downing *et al.* (1995) showed that there was a strong relationship between Glu infusion and ovulation rate in sheep. Ovulation rate increased to 2.4±0.3 vs 2.0±0.0 when ewes were administrated Glu with amount of 60-65 mM/h for five days in the end 3- 4 days of luteal phase of the estrous cycle. Mun˜oz-Gutie´rrez *et al.* (2002) and Mun˜oz-Gutie´rrez *et al.* (2004) reported that ewes infused by glucose and fed on lupin tended to have more follicles and high ovulation rate. San-Martin *et al.* (1968) demonstrated that left ovaries of *Lama pacos* ovulated more frequently than the right post mating or administration HCG. Letelier *et al.* (2008) reported that ovulation rate increased in ewes supplied by glucogenic mixture at first administration. They indicated also that the higher ovulation rate found in the treated ewes may be related to an increased developmental competence of their follicles. However, the cause of inequality of ovarian function is unknown, but some previous studies clarified that inequality may refer to invasion of autonomic nerves in the ovarian stroma which regulate the steroid hormones secretion and follicular growth. Thomson et al (2001) indicated that the difference between right and left ovaries may refer to anatomical and physiological differences that regulate their functions and induce ovulation. In contrast, Potashnik *et al.* (1987), Check *et al.* (1991), Jarvela *et al.* (2000) reported that right ovaries had higher ovulations than left ovaries, the reason refers to the anatomical difference in the ovarian veins which affect blood flow characteristics into ovaries (which was observed in the left ovaries more than the right ovary). The hormonal effect has also effect on ovulation rate; previous studies indicated that the increase in ovulation rate may be related to an increase in FSH and LH levels which in turn develop the ovarian follicles. Funston *et al.* (1995) reported that depletion of Glu (2-deoxyglucose) available in ewes blocked the formation of yellow body and suppressed releasing LH from the pituitary gland. Therefore, glucose could be considered as a stimulator of hypothalamic gonadotrophic releasing factors. Downing and Scaramuzzi (1991) indicated that short-term energy inputs (4–6 days) or, more specifically, infusion of glucose via i.v. increased ovulation rate. Williams *et al.* (2001) indicated that the presence of glucose transporters (GLUT1 and GLUT4) in the granulosa and theca cells may modify the follicular function within the ovary of ewe. There is also other factor may affect ovulation rate as reported by Vin˜oles *et al.* (2002), they found that ovulation rate and FSH concentration during the follicular phase were higher in ewes recognized by high body condition than in low body condition. On the other hand, Srewart (1990) indicated that role of immediate nutrition in increasing ovulation rate was not constant. In summary, the present results indicate that glucose supports the estrous activity of Damascus does during early summer in Upper Egypt (Assiut governorate) and modifies the ovarian activity by increasing number and size of OF and CL. Further efforts are needed to figure out role of Glu on recruitments follicles in the slimmed and fatten animals, and how Gl compensate lack of animal's appetite which occurs during hot summer in sub-tropical countries?

Conclusion

In order to resume the estrous activity during early summer, Damascus does goat are needed to be treated by glucose, since the treatment by Glu in the present experiment improved expression of estrus, aided in improvement of numbers and sizes of OF and Cl and increased progesterone production which will be useful in enhancement of early stage of pregnancy in future. Thus, treatment by energy-yielding nutrient (glucose injection) on ovulation rate in Damascus does goat may be recommended in periods of reproductive activity impairment.

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