

Effect of adding different levels of green tea infusion to semen extenders on some characteristics of the chilled-stored Awassi ram semen

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Abstract

The aim of this study was to assess the effect of adding different levels of green tea (GT) infusion to semen extenders on the percentage of motility, viability and abnormality of ram semen during storage at 5°C for 0, 24, 48, 72, 96, 120 and 144 hrs after collection and dilution. A total of 16 ejaculates from 3 Awassi rams were gathered by artificial vagina and extended by TRIS extender. The GT infusion was added by 0, 2, 4 and 6 ml/ 100 ml of extender which represents control group (C) and treatment groups (T1, T2 and T3) respectively.

The results demonstrated a significant increase ($P<0.05$) in the percentage of sperm motility for each of the treatments T2 and T3 as compared with control group (C) for periods 0, 24, 48, 72, 96 and 120 hrs, while the treatment T1 showed a calculation increased for the same trait as compared with the control group (C) for all periods of experiment. The percentage of sperm viability showed a calculation increased for all treated groups T1, T2 and T3 as compared with control group (C) for all periods of experiment. Otherwise, the results appeared a significant decrease ($P<0.05$) in the sperm abnormalities percentage for both treatments T2 and T3 as compared with the control group (C) for all periods of experiment, whereas the treatment T1 revealed a significant decrease ($P<0.05$) for both periods 120 and 144 hrs as compared with control group (C) for the same trait. Therefore, it was concluded that adding GT infusion to the ram semen extenders can be used to enhance semen characteristics which include motility and viability as well as reduce the abnormality of ram semen stored at 5°C.

تأثير اضافة مستويات مختلفة من نقيع الشاي الاخضر الى مخففات السائل المنوي على بعض خصائص السائل المنوي المحفوظ بالتبريد للكباش العواسية

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الخلاصة

الهدف من الدراسة الحالية هو لتقييم تأثير اضافة مستويات مختلفة من نقيع الشاي الاخضر الى مخففات السائل المنوي على النسبة المئوية لحركة وحيوية ونشوهات حيامن الاغنام العواسية عند حفظها على درجة حرارة 5 م° لمدة صفر، 24، 48، 72، 96، 120 و 144 ساعة بعد الجمع والتخفيف. تم جمع 16 قذفة من 3 كباش عواسية باستخدام المهبل الاصطناعي وتم

تخفيفها باستخدام مخفف TRIS. تم اضافة نقيع الشاي الاخضر بمقدار صفر و 2 و 4 و 6 مل/ 100 مل من المخفف والتي تمثل مجموعة السيطرة (C) وكل من المعاملات T1 و T2 و T3 على التوالي.

اشارت النتائج الى وجود زيادة معنوية ($P < 0.05$) في النسبة المئوية لحركة الحيامن لكل من المعاملات T2 و T3 مقارنة مع مجموعة السيطرة (C) للفترات 0، 24، 48، 72، 96 و 120 ساعة، في حين اشارت المعاملة الاولى T1 الى وجود زيادة حسابية لنفس الصفة مقارنة مع مجموعة السيطرة (C) لكل فترات التجربة. وقد اظهرت النسبة المئوية لحيوية الحيامن زيادة حسابية لجميع المعاملات T1، T2 و T3 مقارنة مع مجموعة السيطرة (C) لكل فترات التجربة. علاوة على ذلك، فقد اظهرت النتائج وجود انخفاض معنوي ($P < 0.05$) في النسبة المئوية لتشوهات الحيامن لكل من المعاملتين T2 و T3 مقارنة مع مجموعة السيطرة (C) لجميع فترات التجربة، في حين اشارت المعاملة الاولى الى وجود انخفاض معنوي لكل من الفترتين 120 و 144 ساعة مقارنة مع مجموعة السيطرة (C) لنفس الصفة. لذا نستنتج بان اضافة نقيع الشاي الاخضر الى مخففات السائل المنوي للكباش العواسية يمكن ان يستخدم لتحسين خصائص السائل المنوي والتي تشمل الحركة والحيوية بالاضافة الى تقليل تشوهات حيامن الكباش المحفوظة على درجة حرارة 5 م°.

Introduction

Using of deliquescent semen stored between 0-5°C allowing the use of semen for a longer period as compared with fresh semen, also using of cooled semen led to higher pregnancy rates when placed at the cervix compared with frozen semen (Maxwell and Salamon, 1993; Salamon and Maxwell, 2000).

It is recommended that the semen conserved at 5°C must be used within a maximum of 24 hrs from gathering for intracervical artificial insemination and within a maximum of 6 days for intrauterine insemination (Salamon *et al.*, 1979).

Spermatozoa have the ability to produce reactive oxygen species (ROS) (Aitken and Baker, 2004) such as superoxide anions (O_2^-), hydroxyl radicals (OH) and hydrogen peroxide (H_2O_2) (Gholinezhad and Hosseinzadeh, 2011; Ho *et al.*, 1996), which lead to oxidative stress that appears as a consequence of the extreme ROS production and results in a decrease of intracellular ATP levels which initiates lipid peroxidation in the plasma membrane of sperm (Almeida and Ball, 2005), these ROS affect negatively on sperm motility and impair its fertilizing ability (Zarghami and Khosrowbeygi, 2005; Fraczek *et al.*, 2007). Mammalian sperm

plasma membranes contain extremely high concentrations of long-chain (C_{22}) polyunsaturated fatty acids, and through the process of sperm conservation the unsaturated fatty acids of the sperm membrane binds with oxygen and form numerous peroxide bonds (Budai *et al.* 2014). Therefore and due to Insufficient defensive mechanisms for the spermatozoa they are highly susceptible to lipid peroxidation (Jones and Mann, 1973; Aitken *et al.*, 1993; Hesham *et al.*, 2008), which contributes to the damage of the plasma membrane of sperm (Deichsel *et al.*, 2008). Living organisms have natural protective analogous known as ROS scavengers (antioxidants) that extinguish the unfavorable effects of ROS (Knapen *et al.*, 1999), these antioxidants include enzymes like superoxide dismutase, catalase, ascorbate peroxidase, guaiacol peroxidase, glutathione reductase, monodehydroascorbate reductase, dehydroascorbate reductase, and non-enzymatic antioxidants like ascorbic acid, reduced glutathione, α -tocopherol, carotenoids and flavonoids (Kaushik and Aryadeep, 2014). Therefore, an antioxidant that decrease oxidative stress and ameliorate sperm motility can improve the management of male infertility (Verma and Kanwar, 1999), and preserve spermatozoa from the prejudice caused by free radicals (Gallardo, 2007).

GT consists of about 30% polyphenols (dry basis), like flavanols, flavandiol, phenol acids and flavonoids (An *et al.*, 2004). Polyphenols have been well-known to have different splendid biological activities such as inhibition of oxidation, especially the retardant effect of GT polyphenols on lipid oxidation was more than that of the synthetic antioxidant (Chen *et al.*, 1996; Wanasundara and Shahidi, 1998). Polyphenols in GT can counteract free radicals and may decrease or even help to block some of the damage they cause (Thasleema, 2013). Epigallocatechin gallate (EGCG) is the predominant catechin present in GT leaves (48–55% of total polyphenols) (Ho *et al.*, 1997; Kodama *et al.*, 2010). These polyphenols have a vigorous antioxidant activity (Dufresne and Farnworth, 2001) and are effective scavengers of ROS superoxide, hydrogen peroxide, hydroxyl radicals, and nitric oxide produced by various chemicals (Schroeder *et al.*, 2003).

There are no reports on the effect of GT infusion on the ram semen. Thus, the goal of the present study was to examine the effects of adding different levels of GT infusion to the extenders on the ram semen stored at 5°C.

Materials and Methods

Animals and management

This study was conducted at the Animal Farm, Department of Animal Resources, College of Agriculture, University of Baghdad during the breeding season (February to March- 2010). Semen samples from 3 mature Awassi rams (the native breed in Iraq) aged between 2-2.5 years with proven fertility were used in this study. The rams were maintained under uniform feed system and housing.

Preparing of GT infusion

GT was prepared by infusing 10 gm of dried GT (Lipton GT, packed in the United Arab Emirates by Unilever Gulf FZE and arrangement with the Trade Marks proprietor, London – United Kingdom) in 100 ml of distilled water, then the infusion putted in an incubator at 38°C for 45 min to dissolve most active materials of GT.

Semen collection, dilution and cooling

A total number of 16 ejaculates were gathered 2 times weekly by artificial vagina. All ejaculates were assessed using light microscope within a maximum period of 10 minutes after collection. The ejaculates were used only if the volume was ≥ 0.75 ml and had a sperm concentration $\geq 2.5 \times 10^9$ spermatozoa/ml and $\geq 70\%$ motile sperms. Semen was diluted using a TRIS extender (Salamon and Maxwell, 2000) in a solution of double-distilled water contains TRIS (3.63 gm), glucose (0.5 gm), citric acid (1.99 gm), and complemented with egg yolk 10% (v/v). Antibiotics were added to the extender which include penicillin and streptomycin (100.000 IU and 100 mg) respectively. All chemicals were bought from Sigma Chemical Co. (St. Louis, MO, USA). Semen was preserved in a water bath at 35 °C for 15-20 minutes until dilution. GT infusion was added by 0, 2, 4 and 6 ml/ 100 ml of extender which represents each of the treatment C, T1, T2 and T3 respectively. The pH of extenders after dilution was 6.8 and the osmotic pressure was about 300-320 mOsm. Cooling of semen achieved by putting the straws in a beaker of water (500 ml) at room temperature and place in a refrigerator for 3 hrs to allow slow cooling to 5°C (Curry, 1995). At this time, the motility, viability and abnormality was evaluated which represents 0 time of experiment, consequently after 24, 48, 72,

96, 120 and 144 hrs.

Semen evaluation

The percentage of sperm motility were confirmed using a light microscope (x400 magnification), fitted with a warm stage at 37°C. The estimation of motility was performed in 5 separate microscopic fields for each sample. The viability was evaluated by eosin-nigrosin staining, and at least 200 spermatozoa from different fields on each slide were examined with a microscope (x400 magnification). The unstained sperm was categorized as alive and that demonstrated any pink coloration was graded as dead. The abnormality percentages of sperms were also assessed using oil lens (x1000 magnification), these abnormalities including giant head, detached head, acrosome anomalies and abnormal of midpiece and tail.

Statistical analysis

The differences of motility, viability and abnormality of ram spermatozoa during its storage periods were evaluated by analysis of variance. Data analysis was performed using SAS software program (SAS, 2001). Variation between treatments were analyzed by Duncan's Multiple Range Test) Duncan, 1955).

Results

The results of the present study demonstrate that there was a significant increase in the sperm motility percentage for the treatments T2 and T3 as compared with the control group C for each of the storage periods 0, 24, 48, 72, 96 and 120 hours (Table 1). As for the treatment T1 there was a calculation increase in the sperm motility percentage for all experiment periods compared with the control group C.

The results of sperm viability were summarized in table (2) which revealed that there were no significant differences in the percentage of viable spermatozoa between all treatments, although there was a calculated increase for the treatments T1, T2 and T3 compared with control group (C) for all periods of experiment.

Throughout the experiment periods, the percentage of abnormality for ram semen were decreased significantly ($P < 0.05$) for the treatment T3 compared with control group (C), while the treatment T2 showing a significant decrease ($P < 0.05$) in the periods of 24, 72, 96, 120 and 144 hrs. Also, the periods 120 and 144 hours appeared a significant decrease in the percentage of sperm abnormality which were 13.00 and 14.00% for T1 compared with 15.00 and 17.00% for control group respectively (table 3).

Table (1): effect of adding different levels of GT infusion to semen extenders on the percentage of sperm motility (Means±SE)

Treatment	Time						
	0 Time	24 Hour	48 Hour	72 Hour	96 Hour	120 Hour	144 Hour
C	80.50±1.65 C	62.50±5.95 C	51.25±5.15 B	46.25±4.73 C	42.50±4.33 C	37.50±6.40 B	32.75±6.20 B
T1	83.25±1.18 BC	67.75±4.69 CB	58.75±2.39 B	56.00±2.44 CB	55.00±2.88 B	47.75±6.00 B	37.75±5.42 B
T2	85.50±0.95 B	79.25±1.49 AB	73.00±1.22 A	67.25±1.65 AB	64.25±1.49 AB	62.50±1.44 A	43.25±8.35 AB
T3	90.25±1.18 A	83.25±1.18 A	76.25±2.39 A	73.00±4.72 A	69.00±3.80 A	64.25±3.25 A	59.75±4.30 A

Means with different letters in the same column are significantly different ($p < 0.05$)

Table (2): effect of adding different levels of GT infusion to semen extenders on the percentage of sperm viability (Means±SE)

Treatment	Time						
	0 Time	24 Hour	48 Hour	72 Hour	96 Hour	120 Hour	144 Hour
C	84.50±3.66 A	81.00±4.26 A	76.50±6.76 A	74.00±8.01 A	71.25±8.26 A	68.25±9.20 A	65.50±10.24 A
T1	85.25±3.35 A	81.75±4.71 A	79.00±5.11 A	75.50±6.65 A	73.25±7.65 A	70.75±8.19 A	68.75±9.25 A
T2	85.00±3.34 A	83.00±4.26 A	81.00±4.26 A	79.00±4.33 A	75.50±6.13 A	73.00±6.63 A	70.75±7.38 A
T3	88.50±2.17 A	82.75±3.44 A	81.25±3.72 A	79.75±3.90 A	77.00±5.11 A	74.50±6.55 A	73.00±6.79 A

Table (3): effect of adding different levels of GT infusion to semen extenders on the percentage of sperm abnormality (Means±SE)

Treatment	Time						
	0 Time	24 Hour	48 Hour	72 Hour	96 Hour	120 Hour	144 Hour
C	8.50±0.28 A	9.75±0.25 A	10.75±0.62 A	12.25±0.75 A	14.00±0.70 A	15.00±0.70 A	17.00±0.70 A
T1	8.50±0.28 A	9.00±0.40 AB	10.00±0.00 A	11.00±0.40 AB	12.25±0.75 AB	13.00±0.57 B	14.75±1.03 B
T2	7.75±0.25 AB	8.75±0.25 B	10.00±0.40 A	10.75±0.25 B	11.25±0.47 CB	11.75±0.25 B	12.50±0.50 C
T3	7.00±0.40 B	7.75±0.25 C	8.50±0.28 B	9.25±0.25 C	10.25±0.25 C	11.50±0.28 B	11.75±0.25 C

Means with different letters in the same column are significantly different ($p < 0.05$)

Discussion

The enhancement of sperm motility that's founded in the present study may be due to beneficial effect of GT antioxidants on the levels of reactive oxygen species (ROS) that's affect negatively on sperm functions and cause lipid peroxidation. This is confirmed with the results of Duru *et.al.* (2000) and Ozmen *et.al.* (2007) which reported that's lipid peroxidation of sperm plasma membrane by ROS induces the destruction or modification of cellular components that influence sperm function, fertilizing ability and even survival. Also, increasing of sperm motility that's found in the present study agreed with the results of Aitken and Fisher (1994); de Lamirande and Gagnon (1995) who noticed that increasing of ROS levels is correlated with reduction of sperm motility. This is consistent with the findings of Pahune, *et al.* (2013) who

observed that seminal antioxidant ability is restrained in infertile men with high ROS levels compared to men with normal levels of ROS. Accordingly, the enhancement of sperm motility after adding different levels of GT infusion to semen extenders might be induced by effective ROS reduction.

Also, the enhancement of sperm viability and reduction in the percentage of sperm abnormalities for the groups treated with different levels of GT infusion may be due to the beneficial effect of GT antioxidants on sperm functions, in addition to protect the plasma membrane of sperm from attacking free radicals, this possibility agreed with the results of Zahedifar and Baharara (2015) who mentioned that free radicals enter to the cell membrane and induce lipid peroxidation, but tea polyphenols degenerate the outer part of hydroxyl radicals and minimize its adverse effects in the cell. This hypothesis match with the findings of Mariane *et al.* (2015)

which indicated that GT infusion attenuated the reproductive system toxicity in male mice, as well as ensuring the safety of sperm and fertility. Also, the enhancement of sperm functions after adding different levels of GT infusion may ascribe to positive effects of GT antioxidants on reduction of lipid peroxidation, and this conform with the results of Budai *et al.* (2014) who indicated that's lipid peroxidation leads to lopsided oxidative stress that causes various disorders of sperm cells and acrosome loss.

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