

## EFFECT OF VITAMIN C INJECTION ON SEMEN QUALITY OF AWASSI RAMS.

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### ABSTRACT

This study was conducted in the Animal Farm, College of Agriculture, University of Baghdad, from march to may 2013, to clarify the effect of subcutaneous vitamin C injection on semen characteristics of Awassi rams. Six rams (1.5-2 years old) were randomly divided into three groups of 2 rams. Rams in T1 and T2 were subcutaneously injected, every other day with dose of 50 and 100 mg/kg body weight respectively, for 45 days while control group in T0 received normal saline. The results showed a significant increase ( $P < 0.05$ ) in the sperm concentration of T1 as compared with control group, while there were no significant differences among treated groups (T1 and T2). Group 2 showed a significant ( $P < 0.05$ ) increases in mass activity, individual motility and live sperm percentage. There was a significant decrease in individual motility and percentage of live sperm in T2 as compared to T1 and control group.

In conclusion, despite of the significant improvement of some semen trail as in T2 group, the lower dose of vit. C (less than 50 mg/kg of body weight) was recommended represented the skin tearing (T2 group) as well as the redness and sensitive skin in T1 group.

**Key words:** Vitamin C, Semen, Rams.

### INTRODUCTION

Vitamin C, is a water soluble vitamin also known ascorbate or ascorbic acid (AA), synthesized from glucose, by the active enzyme L-gulonolactone oxidase (Linster & Van Schaftingen, 2007), L- ascorbic acid and L-dehydroascorbic acid are physiologically active forms of vitamin C (Thurnham, 2000). Vitamin C has been associated with fertility, and it may have It has also been shown to be important for reproduction (Akmal et al, 2006). Deficient ascorbate in man have been associated with low sperm counts, agglutination, increased number of abnormal sperm, and reduced fertility, (Wilson, 1954; Harris *et al.*, 1979; Dawson *et al.*, 1990). The (AA) level of

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seminal plasma were found to be positively correlated with sperm concentration, sperm motility, normal sperm morphology. and closely related to male fertility(Ko *et al* 2012). There is beneficial effect of subcutaneous injections of (AA)to sub-fertile bulls (Phillips *et al.*, 1940) and stallions (Ralston *et al.*, 1988)in addition to increased its plasma and seminal concentrations (Fazeli *et al.*, 2010). In rabbits, supplementation with vitamins C and E reduced the production of free radicals and improved semen quality (total sperm output, sperm concentration and ejaculate volume) (Yousef *et al.* 2003). The small beneficial effect of feeding of (AA) reported in rams (Asghari, 1999) and goats (Heidari, 2002) might be due to denaturing of this vitamin in the rumen(Hidiroglou *et al.*, 1997).Sonmez and Demirci (2003) reported that intramuscular injection of 20 mg (AA)per kg live weight for 30 days increased semen volume, sperm concentration and motility of Rams,So Vitamin C is a physiological additives because it help the normal development of physiological functions of deferent activity in the body(Peris and Calafat, 2005). So this experiment was done to evaluate the subcutaneous injection of Vit. C on some physiological activity of semen of Awassi rams.

## MATERIALS AND METHODS

Six mature Awassi rams (1.5-2 years of age) weighing 40-55kg were used in this study, The study was carried out from march to may 2013. Animals were housed at the Animal Farm of the College of Agriculture, University of Baghdad,. All rams were in good health. They were maintained in identical nutritional and managerial condition throughout the period of study.

The rams were randomly divided into three groups (2 rams each) and received subcutaneous injections of either 0, 50 or 100 mg vitamin C per kg live weight once every two day for 45 days. The experiment was started on march 16, and data were collected from march 31, to 30 may, 2013. Vitamin C (L(+)-ascorbic acid, Carlo Erba-Reagenti Vale De Reuil) was dissolved in distilled water and filtered through a 25  $\mu$ m filter before injection. The control animals were injected with normal saline.

A total number of 48 ejaculates were collected from the rams using an electro ejaculator twice weekly.

Ejaculates were evaluated and accepted, if the following criteria were met: volume varying between 0.4-1.0 ml; sperm concentration of  $1-2 \times 10^9$  sperm/ml;the motile sperms percentage higher than 70% and less than 12% dead sperm in total.

### Semen analysis

Semen collection 15 days after the first injection. Fresh semen samples were kept in a water bath at 35°C, and seminal parameters (volume, pH, sperm concentration, individual motility, percent of live sperm, percent of sperm showing morphological abnormalities) were evaluated within 15 min (Evans and Maxwell, 1989). Semen volume was determined by direct reading the graduated tubes. Semen pH was measured immediately using a digital pH meter (pH 211 Microprocessor, Hanna, Italy). Mass motility was estimated immediately after semen collection using a microscope (Olympus, Japan) (x100 magnification), fitted with a warm stage at 37°C (Zamfirescu & Sonea, 2004). Sperm concentration was determined by Neubaur hemocytometer, diluting a small drop of the semen with 2% eosin solution (1:200), with the sperm counted in five squares of one chamber (*Smith and Mayer 1955*). The percentages of individual motility were determined microscopically (×400) by using a score ranging from 0 to 100% (*Chemineau et al, 1991*). The percentages of live sperm were determined by observing 200 spermatozoa, after eosin-nigrosin staining, using a light microscope at ×400 magnification (Evans and Maxwell, 1989). the final score being the mean of three successive estimations

### Statistical Analysis

Statistical analysis was performed by using SAS program (SAS, 2010). The significant level was set at  $P \leq 0.05$ . Duncan's multiple range test (Duncan, 1955) was used for comparisons among means. The statistical model used was:

$$Y_{ij} = \mu + \tau_i + \varepsilon_{ij}$$

Where:

$Y_{ij}$ .....Observed value

$\mu$ .....Overall mean

$\tau_i$ .....The effect of the treatment (i=1,2,3)

$\varepsilon_{ij}$ .....Error

### RESULTS AND DISCUSSION

In this study, the effect of vitamin C administration as antioxidant on some semen characteristics in Awassi rams was investigated. The results of this experiment were listed in Tables 1 and 2 confirm that there was a significant decrease ( $P < 0.05$ ) in the ejaculate volume in both treated groups, while there were no significant differences showed among treated groups. There were no significant differences in the pH of seminal plasma among all groups.

**Table 1:** *Effects of vitamin C injection on ejaculate volume , pH and sperm Concentration of Awassi rams.*

Treatment	Ejaculate volume (ml)	Seminal pH	Sperm Conc. (*10 <sup>9</sup> )
T0	0.68 <sup>A</sup> ±0.034	6.99 <sup>A</sup> ±0.022	2.53 <sup>B</sup> ±0.032
T1	0.61 <sup>AB</sup> ±0.031	7.08 <sup>A</sup> ±0.035	2.60 <sup>A</sup> ±0.014
T2	0.57 <sup>B</sup> ± 0.029	7.01 <sup>A</sup> ± 0.032	2.55 <sup>AB</sup> ±0.017

T0=control group , T1= subcutaneous injections of 50 mg/kg Vit. C , T2= subcutaneous injections of 100 mg/kg Vit. C

Different capital letter in the column means significantly different (  $p \leq 0.05$  ).

**Table 2:** *Effects of vitamin C injection on mass activity, individual motility and sperm viability of Awassi rams.*

Treatment	Mass. Activity (%)	Ind.Motility (%)	Live (%)
T0	72.50 <sup>AB</sup> ±0.913	71.88 <sup>B</sup> ±0.774	89.63 <sup>B</sup> ±0.109
T1	74.06 <sup>A</sup> ±1.309	78.13 <sup>A</sup> ±1.360	91.55 <sup>A</sup> ± 0.182
T2	70.00 <sup>B</sup> ±0.913	68.75 <sup>C</sup> ±0.854	88.99 <sup>C</sup> ±0.203

T0=Control group , T1= subcutaneous injections of 50 mg/kg Vit. C , T2= subcutaneous injections of 100 mg/kg Vit. C

Different capital letter in the column means significantly different (  $p \leq 0.05$  ).

The sperm concentration were significantly increased ( $P < 0.05$ ) in rams of T1 compared to control group, Similarly, Sonmez. and Demirci (2003) reported that intramuscular injection of 20 mg Vit.C / kg body weight for 30 days increased sperm concentration in rams Yousef *et al.*(2003) reported that adding ascorbic acid to drinking water for 12 weeks increased sperm concentration of male rabbits..while there were no significant differences showed among T2 and control group and between the two treatment groups(2 and 3).

The results of sperm motility (Table 2) demonstrates that rams in T1 showed a significant ( $P < 0.05$ ) increase, which agreed with Sonmez and Demirci (2003) when they found that the intramuscular injection of ascorbic acid for 30 days increased the semen quality of rams. However short-term administration of vitamin C did not affect semen parameters of rams (Sonmez and Tanyildizi, 2008). Fazeli *et al.* (2010) found the importance of subcutaneous vitamin C injections for 90 days in increased of about 15% in sperm motility of buck.

Our observations indicating that the vitamin C was able to improve some sperm parameters. Furthermore, the motility of sperm cells would be preserved by the binding of antioxidant to endoperoxides (Luvoni, 2006).

The mechanism of Vitamin C effect may due to inhibition of cell impair by binding to the free radical and neutralizing its unpaired electron mediated by a tocopheryl-quinone' formation (Schuh, *et al.* 2004). and improve ram sperm parameter after the addition of vitamins C and E to semen diluents (Thuwanuta *et al.* 2011).

However, in this study higher doses of Vit. C 100mg/kg (T2) showed significant decrease in the individual motility and percentages of live sperm compared to rams of T1 and control groups, as well as it cause skin tearing, and this may be due to the harmful effect of high vitamin C concentrations to sperm motility (Beconi *et al.* 1993), and may have damaging pro-oxidant effects which actually reduce sperm motility (Abel *et al.*, 1983).

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## تأثير حقن فيتامين C على نوعية السائل المنوي للكباش العواسي .

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### المستخلص

أجريت هذه الدراسة في الحقل الحيواني التابع لكلية الزراعة/ جامعة بغداد في أبو غريب للفترة من آذار ولغاية مايس 2013 ، لمعرفة تأثير الحقن تحت الجلد لفيتامين C على بعض صفات السائل المنوي للكباش العواسي. استخدمت ستة كباش بعمر 1.5-2 سنة وقسمت الى ثلاث مجاميع وبواقع كباشين لكل مجموعة. كباش المجموعتين الثانية والثالثة حقنت بـ 50 و 100 ملغم فيتامين C /كغم من وزن الجسم بين يوم وآخر لمدة 45 يوما ، المجموعة الأولى مجموعة سيطرة وحقنت بالمحلول الفسيولوجي. تم جمع السائل المنوي من الكباش باستخدام التحفيز الكهربائي. اظهرت نتائج هذه التجربة زيادة معنوية ( $P<0.05$ ) في تركيز النطف لكباش المجموعة الثانية مقارنة بمجموعة السيطرة ، فيما لم تظهر فروق معنوية بين المجموعة الثالثة والسيطرة وكذلك بين المجموعة الثانية والثالثة. المجموعة الثانية اظهرت زيادة معنوية في الحركة الجماعية والفردية للنطف ونسبة النطف الحية. كما لوحظ حصول انخفاض معنوي في الحركة الفردية ونسب النطف الحية لكباش المجموعة الثالثة مقارنة بالمجموعة الثانية ومجموعة السيطرة.

ويمكن الاستنتاج بانه على الرغم من التحسن المعنوي لبعض صفات السائل المنوي للمجموعة الثانية، الا اننا نوصي باستخدام تراكيز اقل من 50 ملغم فيتامين C /كغم من وزن الجسم بسبب التأثيرات السلبية التي لوحظت عند استخدام التراكيز العالية في المجموعة الثالثة (تهتك الجلد) وتحسس واحمرار الجلد (تهرش) لدى المجموعة الثانية .

الكلمات المفتاحية: فيتامين C ، السائل المنوي ، الكباش .