Effects of Licorice Extract on Sperm Motility of Chilled Stored Ram Semen

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Abstract

This study was designed to investigate the effects of licorice extract (*Glycyrrhiza glabra L.*) addition to semen diluters on ram sperm progressive motility during storage at 5 ˚C for 72 h. Semen was collected from 3 proven Awassi rams. Licorice extract powder was added at levels of 1, 5, 10, 50 and 100 µg per ml. of diluter. Diluter containing no licorice extracts served as control (0).Progressive motility was estimated subjectively after dilution (0h), and at 24, 48, and 72 h of storage. The experiment was replicated 2 times with egg yolk-tris (EYT) diluter and 2 times with yolk- glucose-citrate diluter. Progressive motility increased significantly ($p < 0.01$) in levels of licorice extract 1, 5, 10, 50 and 100 µg / ml in both diluters, during all storage periods. The means of progressive motility were 72.5 ± 1.02 %, 72.08 ± 1.05, 70.90 ± 2.05 % and 66.25 ± 3.15 % respectively, compared to the control (0) 61.45 ± 16.2 %. Levels 1, 5 and 10 µg /ml were superior ($p < 0.01$) to levels 50 and 100 µg /ml. In conclusion, the addition of licorice extract to the diluter improved ram sperm progressive motility during cooled storage at 5 ˚C.

Key words: Licorice extract; diluter; ram semen; progressive motility.

Introduction

Licorice is the name applied to the roots and rhizomes of *Glycyrrhiza* species and has been used for medicinal purposes for at least 4000 years [1]. *Glycyrrhiza glabra L.* is one of the very important nutraceuticals, contains some 400 bioactive phytochemicals [2], and has many documented bioactivities such as: steroid like activity [3], powerful antioxidants activity [4], antibacterial activity [5] and antiviral activity [6].

In traditional herbal medicine, licorice was considered as a natural source of sex hormones and used to: strengthen female reproductive system [7], treat some women's sterility cases in Japan and China [8,9], strengthen male reproductive system, improve sperm count as well as semen viscosity in Ayurvedic medicine [10], and improve erection [11,12].

Our previous study results indicated that treatment with licorice improved reproductive performance of ram lambs and rams [13].

Many additives were used to improve preserved semen quality such as: dyes [14], caffeine [15], sugars [16], aromatic compounds [17], chelating agents [18], antioxidants [19], selenium [20], seminal plasma [21] and soybean [22].

Licorice contains many phytochemicals which may have ameliorating effects on semen quality, so this study was designed to investigate the possible effects of licorice extract addition to the diluter on sperm motility of chilled stored ram semen.

Material and Methods

Two types of diluters were used, egg yolk- tris (EYT) [23] and yolk- glucose-citrate [24]. Licorice extract powder *(Glycyrrhiza glabra L.)* purchased from local company *(Al-Ahliah Company for Aromatic Odors & Flavorings Production Ltd., Baghdad, Iraq)* was added at

levels of 1, 5, 10, 50 and 100 µg per ml. of diluter. Diluter containing no licorice extracts served as control (0). Diluters were prepared the day prior to use, allowing large participle to settle overnight at 5˚C, so that the supernatant could be used. Before use, each diluter was warmed to 37 ˚C.

Fresh semen was collected by an artificial vagina from Awasi rams. Semen of 3 proven rams was pooled, mixed and kept in a water bath at 37 ˚C.

Semen was diluted 1:10 (vol. /vol.) to different diluters, then gradually cooled by putting the tube of diluted semen in about 500 ml of 37 ˚C water containers and placed in a 5 ˚C refrigerator for 2 hours to reduce cold shock [24], then stored at 5 ˚C for 72 h.

Progressive motility was estimated subjectively after dilution(0h), and at 24, 48, 72 h of storage, by diluting a drop of semen with 0.9 % sodium chloride solution in a warm slide, mounting it with cover slip and examining it under a microscope at X 400 magnification, using a 100-point scale for linear movement [25]. The data were expressed in percentage of total cells. Motility estimations were performed from 5 different fields in each sample by the same person throughout the study; the mean value averaged from 5 successive estimations was used as the final motility score.

The experiment was replicated 2 times with egg yolk-tris (EYT) diluter and 2 times with yolk- glucose-citrate diluter.

The Statistical Analysis System [26] general linear model was used to analyze the data. Differences among treatment means were compared for statistical significance, using Duncan's multiple range test [27].

Results

Progressive motility increased significantly ($p < 0.01$) in levels of licorice extract 1, 5, 10, 50 and 100 µg / ml in both diluters, during all storage periods. The means of progressive motility were 72.5 ± 1.02 %, 72.08 ± 1.05, 70.90 ± 2.05 % and 66.25 ± 3.15 % respectively, compared to the control (0) 61.45 ± 16.2 %. (fig1). Levels 1, 5 and 10 µg /ml were superior ($p < 0.01$) to levels 50 and 100 µg /ml (fig1).

Diluter type had a significant effect ($p < 0.01$) on sperm motility. Overall the percentage of motile sperm in EYT diluter (66.48 ± 1.21 %) was higher than that in yolk-glucose citrate diluter (64.37± 1.44 %).

Sperm motility tended to decline significantly ($p < 0.01$) as the length of storage period increased. The means of progressive motility were 80.00 ± 2.04 % after dilution (0h), 68.75 ±3.15 % 61.25 ± 4.27% and 50.62 ±4.61 %, at 24, 48 and 72 h after cooling, respectively.

Discussion

Although the history of licorice is as old as the history of medicine and of confection [28] and references to licorice date back to approximately 2500 B.C on Assyrian clay tablets and Egyptian papyri [29], this study, to the best of our knowledge, is the first indication for a positive effect of licorice (*Glycyrrhiza glabra L.*) on sperm motility of chilled stored ram semen.

Sperm motility is regarded as a manifestation of sperm functional competence [30] and licorice extract improved ($p < 0.01$) progressive motility especially in low levels (1, 5, 10 µg /ml diluter). Progressive motility is the most important individual quality test, because fertility is highly correlated with number of motile sperm inseminated [24].

The precise role of the components of diluters (sugars, proteins, a range of additives, etc.) in preserving the integrity and the fertilizing potential of spermatozoa is still far from being understood, and the composition of the diluents has been improved by more or less empirical studies [31,32,33].

Licorice may be considered as a versatile additive. First, an important reason for the decrease in motility during the storage of semen is the formation of lipid peroxides from oxygen radicals [34]. The sperm plasma membrane contains a high amount of unsaturated fatty acids and is therefore particularly susceptible to peroxidative damage [35]. The lipid peroxidation destroys the structure of the lipid matrix in the membranes of spermatozoa, and it is associated with a loss of motility and membrane integrity [36]. Semen contains appreciable amounts of antioxidants that balance lipid peroxidation and prevent excessive peroxide formation [37] but the endogenous antioxidative capacity of semen may be insufficient during storage or dilution [38]. The addition of antioxidants is well known to improve the viability and motility of liquid storage or cryopreserved ram sperm cells [19, 39]. Licorice has powerful antioxidant activity, it is contains seven antioxidant compounds, four isoflavans, two chalcones and an isoflavone [4], and these compounds were shown to be effective to protect biological systems against various oxidative stresses [40] via a mechanism involving scavenging of free radicals [41]. Second, licorice contains many sugars such as fructose and sucrose [11]. Fructose is one of the principle energy substrate for spermatozoa [24] and an activator factor of mammal spermatozoa [42], sucrose are reported to be effective in stabilizing sperm membrane bilayers during storage [43]. Third, Licorice has antimicrobial activity, and the antimicrobial agents are routinely added to semen diluters to control many semen organisms which are pathogenic and compete with sperm for nutrients and produce metabolic by-products that have an adverse effect on livability of the sperm [24].

The decrease in sperm motility at high levels of licorice extract in this study (50 and 100 µg /ml) is possibly caused by high osmolality; hyper osmotic environment can inhibit sperm motility [44]. Further researches are needed to design an isotonic diluter with high level of licorice extract.

Overall progressive motility was better in EYT. Different diluents for semen have been used to improve fertility rate. EYT extender was recommended by many researchers and commonly used for artificial insemination [23, 45].

Over storage period, sperm motility declined significantly ($p < 0.01$) probably due to the accumulation of the toxic products of sperms metabolism [38].

The study findings may contribute to the recent attempts to design defined semen diluter and move away from animal-based cryoprotectants, which may pose hygienic risks and are difficult to standardize [46].

Finally there are several factors affecting the phytochemistry of the licorice root such as geographical location, soil condition, time of harvesting and the environmental factors [47] and this should be considered when applying such treatment widely.

In conclusion, the addition of licorice extract to the diluter improved ram sperm progressive motility during cooled storage at 5 ˚C.

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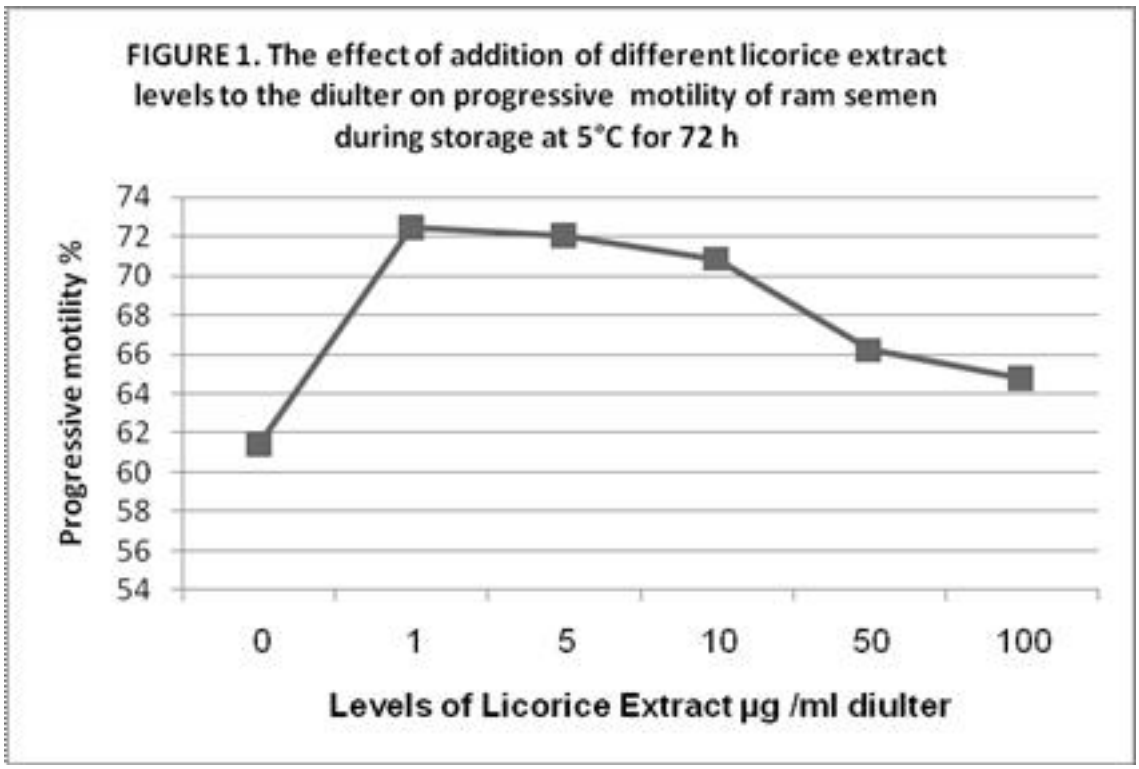

تأثير مستخلص عرق السوس في قيم الحركة الفردية للحيامن في السائل المنوي المبرد للكباش

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الخلاصة

صممت هذه التجربة لدراسة تأثير إضافة مستخلص عرق السوس (*Glycyrrhiza glabra L.*) إلى مخففات السائل المنوي على قيم الحركة الفردية لحيامن الكباش خلال حفظ السائل المنوي بدرجة 5 م° مدة 72 ساعة . جمع السائل المنوي من ثلاثة من كباش العواسي المختبرة .أضيف مستخلص عرق السوس بمستويات 1 و 5 و 10 و 50 و 100 مايكروغرام / مل من المخفف .استعمل المخفف غير المحتوي على مستخلص عرق السوس مجموعة مقارنة (0). تم تقدير قيم الحركة الفردية بعد التخفيف (0h) ، و بعد ،24 و ،48 و 72 ساعة من الحفظ بدرجة 5 م° . كررت التجربة مرتين باستعمال مخفف الترس – صفار البيض (EYT) ومرتين باستعمال مخفف السترات –الكلوكوز– صفار البيض .ازدادت قيم الحركة الفردية معنويا ($p < 0.01$) في عينات السائل المنوي المخففة والمضاف إليها مستخلص عرق السوس بمستويات 1 و 5 و 10 و 50 و 100 مايكروغرام / مل في كلا المخففين خلال كل مدد الحفظ . كانت متوسطات قيم الحركة الفردية 72.5± 1.02 % و 72.08± 1.05 % و 70.90± 2.05 % و 66.25 ± 3.15 % على التوالي مقارنة بمجموعة المقارنة (0) 61.45 ± 16.2%. وتفوقت المستويات 1 و 5 و 10 مايكوغرام / مل في المخفف معنويا ($p < 0.01$) على المستويين 50 و 100 مايكوغرام / مل في قيم الحركة الفردية . نستنتج من نتائج هذه الدراسة ان إضافة مستخلص عرق السوس إلى المخفف قد حسن من قيم الحركة الفردية لحيامن الكباش خلال مدة الحفظ المبرد بدرجة 5 م ° .