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General and progressive semen motility affected by using black currant (Prunus Cerasus) extracts in rams

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***Abstract*---**This study was designed in order to investigate and determine the effect of black current (*Prunus cerasus*) extract on semen general and progressive motility of the rams. Twelve mature healthy rams was used in this study aged between 2-3 years, kept in the animal farm of the College of Veterinary Medicine, Al-Kufa University. Semen ejaculates was collected using electroejaculator methods once weekly a total of 36 ejaculates were collected among the period of the study. The black current (*Prunus cerasus*) extract was prepared by using maceration and added to the semen in five doses groups (0.25 gm/ml as G1, 0.5 gm/ml as G2, 1 gm/ml as G3, 2 gm/ml as G4, 4 gm/ml as G5) and the positive control group left without any addition. The treatment samples were evaluated under cooling for five consecutive days, the general and progressive motility were analysis using Computer Assisted Sperm Analysis (CASA). The results of the study clearly indicated that there were a significant differences ($P<0.05$) in G1 and G2 than other groups in five days of examination, with the preference to the G1 group. We conclude that the 0.25 and 0.5 gm/ml of black current extract (*Prunus cerasus*) should be used in cooling process as a part of artificial insemination in sheep.

***Keywords*---**semen, general motility, progressive motility, black current, rams.

Introduction

Antioxidants are the compound that prevent the creation of ROS or oppose their actions (Bansal and Bilaspuri, 2008). Previous studies have shown that inclusion of antioxidants in cryopreservation extenders increases factors such as motility and membrane integrity after thawing in human, ram, goat, bull, canine and boar sperm (Bucak *et al.,* 2010). Supplementation with antioxidants prior to the cryopreservation process may improve cryopreservation methods used in the sheep industry (Bucak *et al.,* 2010). The limited ability of sperm to biosynthesize, coupled with the reduction in antioxidant concentrations present in the semen after dilution, reduces the constructive effect of these endogenous anti-oxidative defenses (Allai *et al.,* 2018). Therefore, even a small addition of antioxidants to cryopreservation media may have a positive effect on sperm function (Allai *et al.,* 2018). Natural antioxidants are compounds that are obtained from natural sources such as herbs, seeds, vegetables, spices and fruits (Velasco and Williams, 2011). These natural antioxidants are often phenolic compounds or vitamins that have an appropriate molecular structure allowing them to chemically scavenge free radicals (Liang *et al.,* 2012). In plants, phenolic compounds have numerous biological roles such as antioxidants, anti-bacterial and anti-inflammatory (Manthey and Grohmann, 2001). Antioxidants obtained from natural sources such as green tea and rosemary have the ability to enhance sperm integrity, which minimizes the damage that occurs during storage**.** There are limited studies on the effect of medical plants extract as antioxidant on spermatozoa quality during and after cooling. One of well-known medicinal plants is black currant (*Prunus cerasus*), it has an excellent antioxidant activity (Piccolella *et al.*, 2008; Kirakosyan *et al.*, 2010; Damar *et al.*, 2012 and Xu *et al.*, 2016) respectively, anti-inflammatory activity (Desjardins *et al.*, 2012) as well as anti-carcinogenic activity (Al Alawi *et al.*, 2020). This study was design in order to investigate the effect of black currant *(Prunus cerasus*) adding to ram semen on general and progressive motility.

Materials and methods:

The extraction of plant was done in the artificial insemination and theriogenology laboratory, inside Veterinary Medicine College, University of Kufa, Iraq. The method of choice for the aqueous extraction was done by using maceration of the crude extracts, due to its beneficial in extracting of the thermolabile and thermostable components (Al Alawi *et al.*, 2020). The edible part of the black current Fruits were separated manually from the seeds and chopped into small pieces. The black current plant 200 gram was treated with 300 ml of water and mixed very well at room temperature. The content of bottle then was filtered through a Buchner filtration system and the water was removed by exposure to 45 °C to evaporate the water (Bustani, 2020). The remaining plant was treated again with water and the process was repeated for twice times. The extract was collected and stored in room temperature for later use.

The extender was divided into six aliquots dose of aqueous plant extract as 0% (C), 0.25 mg/ml (G1), 0.5 mg/ml (G2), 1 mg/ml (G3), 2 mg/ml (G4) and 4 mg/ml (G5), the solution had normal pH ranged between 6.8-7.2 and 425mOms osmolarity. The general and progressive motility of the semen was evaluated at

day 1, 2, 3, 4, and 5. The extender was stored after preparation at -20 °C. The extender was used in this study is a Tris- based extender includes: (for 100 ml) LDL 8%, tris: 2.40 g, fructose: 1 g, citric acid: 1..50 g, gentamycin 25 mg, penicillin 50000 IU, and D. W. until 100 ml (Baiee *et al.*, 2017). The extract was added to the extender with different doses as mentioned before and one group kept without adding the extract as a control.

Samples of semen were collected from 12 mature ram aged between 2–3 years using electro-ejaculator method (mini tube; Germany) every two weeks. Thirty six ejaculates of fresh semen were collected and evaluated at 36 ºC, general and progressive motility of sperm was done using **C**omputer **A**ssisted **S**perm **A**nalysis (**CASA**). Selected samples should be ≥70% sperm motility and ≥ 80% normal morphologyin order to be used in this study.

The extended semen groups were stored in a refrigerator at 5 °C for five days. Sperm general and progressive motility were evaluated at 1, 2, 3, 4, and 5 days of chilling. The final concentration of semen in the chilled experiment was approximately 80×10^6 sperm/ml.

The statistical analysis was done using ANOVA and LSD was used to determine the significant differences among means at level of $P<0.05$.

Results

According to Figure (1), the outcomes of the result that illustrated the value of general sperm motility in the different concentration of aqueous plant extract of *Prunus cerasus* (C, G1, G2, G3, G4 and G5). Moreover, the result showed superiority of G1 (0.25 mg/ml) among all other groups (Figure 1 and 2). In additionally the outcome showed significant increase ($P<0.05$) in the general motility of G1 at 3rd, 4th and 5th day of preservation that compared with control and other groups (Figure 1 and 2). In other hand, the general and progressive motility of G2 (0.5 mg/ml) group was higher than control in 3rd, 4th and 5th of day of preservation (Figure 1 and 2). Generally, the general and progressive motility of both G1 and G2 group higher significantly ($P<0.05$) than G4 and G5 gropes in all day of preservation (Figure 1 and 2). The motility of G1 was increased significantly ($P<0.05$) than G3 while that non-significant different between G2 and G3 groups (Figure 1 and 2).

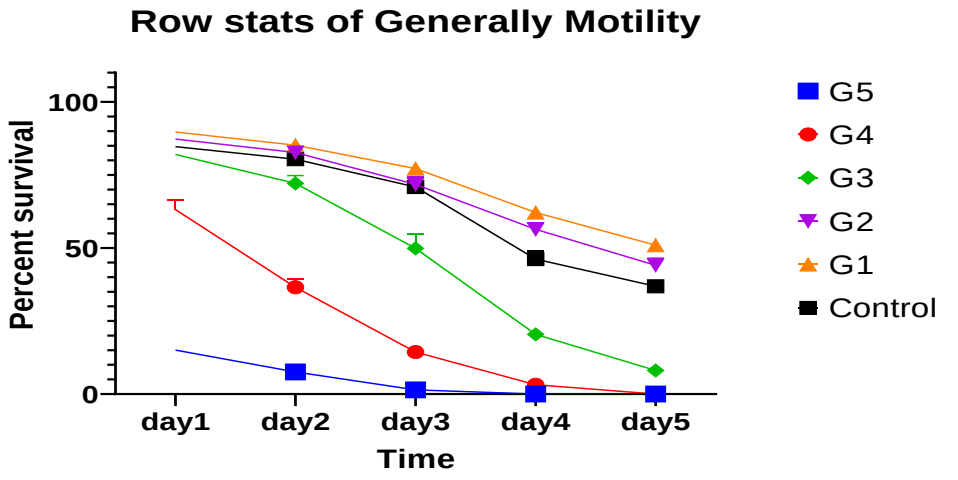

Figure 1: Comparison among groups of sperm general motility % during 5 day survival.

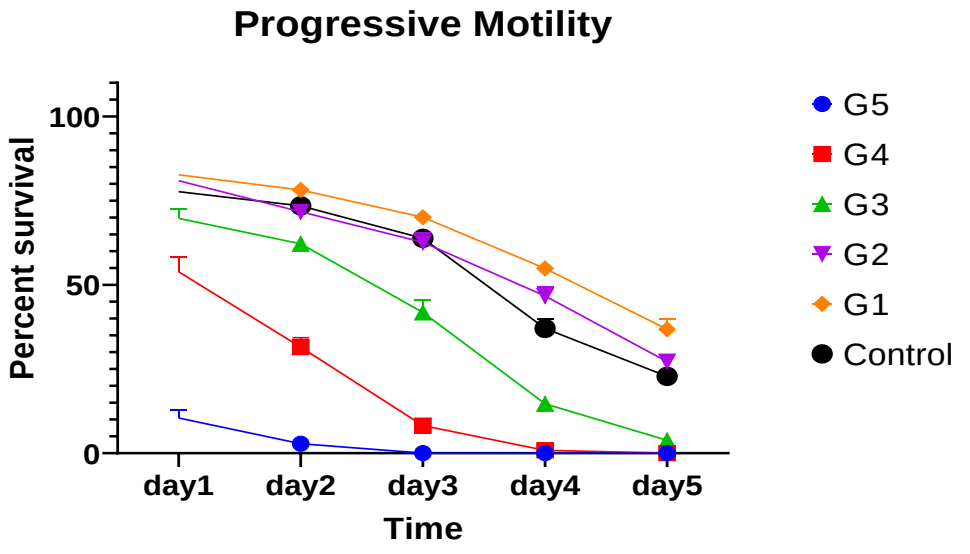

Figure 2: Comparison among groups of sperm progressive motility during 5 day survival.

Discussion

The current study found that semen diluents with 0.25 mg/ml aqueous extract of *Prunus cerasus* significantly improved the cooled sperm parameters and it was in line with the gold slandered diluents which are tris-egg yolk diluents. Thus, addition of 0.25 mg/ml of aqueous extract enhanced the functional parameters and in vivo fertility. On the other hand, oxidative stress, lipid peroxidation, and thawing effect are main causes of spermatozoal damage through preservation (Barman *et al.,* 2011 and Rizkallah*et al.,* 2022) as approbate our result in control group. Furthermore, the reduced of temperature during chilling processing which

preserved at 5 °C or less inflicts sub-lethal damage to spermatozoa, compromising sperm quality and the success of artificial breeding as described by previous studies (Mohamed *et al.,* 2020 and Bustani and Baiee 2021). The earlier studies have concluded effect of chilling temperature on the sperm motility in both general and progressive motile of rat (Varisli *et al.*, 2009), bull (Januskauskas *et al.*, 1999), rabbit (Rosato *et al.*, 2011), human (Verheyen *et al.*, 1993) and, ram (Kulaksiz *et al.*, 2012) which illustrated the temperature related in the organization of structural components of the plasma membrane have profound effects upon its properties, associated with changes in permeability, changes in membrane fluidity (Ortega *et al.*, 2019), dis-regulation of intracellular Ca+' influx (Yeste *et al.*, 2018) and changes in enzyme activity (Palhares *et al.*, 2021) finally on the ability of sperm capacitation. From the Figure (1 and 2) the result showed the improved effect of *Prunus cerasus* (G1) on the sperm motility (general and progressive). Which the past studies proved the antioxidant effect of *Prunus cerasus*on different tissue and cells (Sokół-Łętowska *et al.*, 2020), furthermore, the contain of high amount of sugars (glucose and fructose) since thesugar (glucose or fructose) offers a good protection during preservation of semen and this might be due to be a source of energy (Williams and Ford, 2001). Sperm motility doesn't depend on mitochondrial to provide the energy that used to motility action. With this in mind, studies suggested that glycolysis is an alternative pathway to produce ATP in the cytoplasm of sperm cell (Nascimento *et al.,* 2008); since, it was reported that glycolytic enzymes and an ADP/ATP carrier protein are found in the fibrous sheath of sperm tail (Krisfalusi *et al.,* 2006 and Kim *et al.,* 2007). Therefore, spermatozoa could use glycolysis to produce ATP that can be used for motility. A handful of studies reported that sperm motility was significantly increased when incubated in diluents containing sugar like glucose (du Plessis *et al.,* 2015). Generally, glycolysis and oxidative phosphorylation provide energy for sperm motility (Qiu *et al.,* 2016). As well as, sugars prevent cooling damage of sperm by affecting the pattern of ice-crystal formation and the width of the channels of unfrozen extender (Nicolajsen and Hvidt, 1994) and reduce the salt concentration of the extender that can reduce the injury due to the extender solution effects. Recent studies were indicated that spermatozoa motility depends greatly on the energy source in an extender in the form of adenosine triphosphate (ATP), which is produced from metabolism, and therefore requires a constant supply for cell function and survival (Dorado *et al.,* 2019). This study result finding broadly supports the work of other studies in this area linking motility with source of energy and production of ATP which our result showed increased significantly in the general and progressive motility in addition of aqueoussemen extender as showed in Figure (1 and 2). Moreover, the figures illustrated the superiorityof G1 significantly among all groups, This study supports evidence from previous observations (Alenezy *et al.*, 2019; Asadzadeh *et al.*, 2021 and El-Harairy *et al.*, 2018) uses of natural source of material in the extender of semen for chilling and cryopreservation of the bull sperm, in the seam area the current study found that used of vitamins are promote and increased sperm parameter (general and progressive motility) is support our result (Espina *et al.,* 2021). Also the season in Iraq had a significant effect upon animal testis specially the summer season which show the lowest reproductive activity (Ibrahim and Zaid, 2017).

It can be notable that works precisely at semen diluents with 0.25 mg/ml aqueous extract of *Prunus cerasus* (G1) and based on the present data it provides a good opportunity to improve sperm quality. However, high concentration at 2 mg /ml and 4 mg /ml of *Prunus cerasus* G4 or G5 did not improve post-thawed semen quality. This was might due to the effect of disaccharide that found in small amount in the extract of dates. Since, a study on epididymal spermatozoa of red deer found that mono-saccharides had positive effect than di-saccharides or the tri-saccharide (raffinose) when supplementing diluents for cryopreservation (Fernández-Santos *et al.,* 2007). In addition, the negative effect of higher concentration of AED or SED at dose of 2 g / 100 ml was concomitant with previous studies where high concentration of disaccharide have been used to limit their dosages effect on post-thawed sperm quality (Molinia *et al.,* 1994).

Conclusion

From the previous results we conclude that the black currant (*Prunus cerasus*) extracts had a remarkable effect on semen general and progressive motility of the rams on 0.25 and 0.5 gm/ml during cooling with good longevity effect, and it should be used in artificial insemination programs of sheep.

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Availability of data and material: The data that support the findings of this study are available from the corresponding author, upon reasonable request.

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