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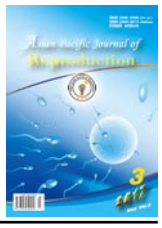

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Effect of *Diospyros kaki* enriched extender on cattle bull sperm parameters and conception rate

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ABSTRACT

Objective: To explore the effect of *Diospyros kaki* on cattle spermatozoa during chilling and cryopreservation. **Methods:** Five milliliter of blended Persimmon (*Diospyros kaki*) flesh was added to 45 mL TCF to obtain 10% stock solution. Kaki enriched extender (KEE) was prepared by adding to TCF in concentrations 0.0/5.0 mL (control, 0%), 0.5/4.5 mL (1%), 1/4 mL (2%), 1.5/3.5 mL (3%), 2.0/3.0 mL (4%), 2.5/2.5 mL (5%), 3.0/2.0 mL (6%), 3.5/1.5 mL (7%), 4.0/1.0 mL (8%), 4.5/0.5 mL (9%) and 5.0/0.0 mL (10%) to obtain a final volume 5 mL in each tube. Whole egg yolk was added to each tube to obtain KEE with 20% egg yolk (KEEY), all tubes were centrifuged to get rid of debris. Semen was added to the supernatants in other tubes. Extended semen was subjected to evaluation [motility, alive sperm and intact sperm membrane (HOST) %] in both chilled and cryopreserved semen. Conception rate was carried out. **Results:** Sperm motility was significantly (P<0.000 1) kept high after 11 d of chilling with the concentration 1%, 2%, 3%, 4%, 5% as compared to the control (41.67±1.67, 41.67±1.67, 40.00±0.00, 41.67±1.67 and 41.67±1.67, respectively) and also non-significantly kept high at the other concentrations up to 9 d of chilling. Addition of KEE had significantly (P<0.003 3) improved post thawing sperm motility % with the concentrations 1, 2, 3, 4, 5 and 6% as compared to the control (51.67±5.27, 55.00±3.16, 48.33±1.05, 45.00±3.96, 57.00±2.50, 55.00±5.00 and 43.33±5.11 respectively).While the other concentrations exhibit no effect. Addition of KEE maintained alive sperm%, abnormalities% and % of intact spermatozoa membranes (HOST%) as good as the control with all concentrations of kaki used in our study. The conception rate upon using frozen semen in insemination showed higher conception rate in concentrations of 2%, 4% and 6 % KEE in cattle. **Conclusion:** It could be concluded that some concentrations of *Diospyros kaki* improved bull semen quality post-chilling and post-freezing.

1. Introduction

Spermatozoa are the endpoint of male spermatogenesis and have particular anatomic and metabolic features. Nowadays, Sperm cryopreservation and storage are of a great demand for conserving the supergenetic origins of the males, the development of artificial reproductive technologies such as artificial insemination (AI) and *in vitro* fertilization (IVF)[1] are of a great interest. AI with frozen semen is essential in breeding and selection schedules contributing to increase production of domestic species. Nowadays, semen cryopreservation has many biotechnological applications. It can be used to solve infertility problems, life threatening diseases,

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preservation of semen and DNA from endangered species and conservation of biodiversity. The interaction of several factors (cooling rate, storage temperature, chemical ingredients of the extender, cryoprotectant concentration, over accumulation of oxygen free radicals, seminal plasma composition and hygienic control) are the key that affect the life-span of spermatozoa[2]. Cryopreservation of bovine semen often induce an additional source for reactive oxygen species (ROS) attack on sperm due to decreased activities of antioxidant enzymes and the sperm membrane become more susceptible to lipid peroxidation[3] which affect the membrane permeability[4]. Natural antioxidants exert a protective effect preserving the metabolic activity and cellular viability of cryopreserved bovine spermatozoa[5]. Nowadays, the use of herbal natural product has gained interest worldwide. Many of the herbs have been developed into herbal supplement which are claimed to assist in healthy life style. Among these herbs, is Persimmon (*Diospyros kaki*) fruit possessing high nutritive value and contains many biologically active substances including antioxidants, dietary fiber, triterpenoids and minerals[6-12]. Persimmons revealed considerable health and medicinal benefits, which are considered to be related to the various hydrophilic and lipophilic antioxidants including phenolic compounds, vitamin C and carotenoids, contained in the fruit[13]. Persimmon fruits contain abundance of nutrients and phytochemicals such as carbohydrates, organic acid, vitamins, tannins, polyphenols, dietary fiber, triterpenoids, and carotenoids, which contribute significantly to their taste, colour, nutritive and medicinal values[14-21].

No available literatures were found for interpreting the benefit for using those materials in preserving extended cattle and buffalo semen.

2. Materials and methods

2.1. Preparation of semen extender

Kaki enriched extender (KEE): 5 mL of blended Persimmon (*Diospyros kaki*) flesh was added to 45 mL TCF to obtain 10% stock solution. KEE was prepared by adding to TCF in concentrations 0.0/5.0 mL (control, 0%), 0.5/4.5 mL (1%), 1/4 mL (2%), 1.5/3.5 mL (3%), 2.0/3.0 mL (4%), 2.5/2.5 mL (5%), 3.0/2.0 mL (6%), 3.5/1.5 mL (7%), 4.0/1.0 mL (8%), 4.5/0.5 mL (9%) and 5.0/0.0 mL (10%) to obtain a final volume 5 mL in each tube. Whole egg yolk was added to each tube to obtain KEE with 20% egg yolk (KEEY), all tubes were centrifuged to get rid of debris. Supernatants were transferred to other tubes and then stored at -20 ℃ till used.

A preliminary study on the use of 10%-50% had given bad results so, the former design was introduced as the persimmon contains high amount of astringent tannin which may be the cause of those poor results.

2.2. Semen collection and initial evaluation

Three mature cattle bulls maintained at Semen Freezing Center, General Organization for Veterinary Services Ministry of Agriculture, Abbasia, Egypt, were used as semen donors. Ejaculates were collected using a bovine adapted artificial vagina at weekly intervals for 18 wk. Semen samples were initially evaluated for subjective sperm motility and sperm concentration. Ejaculates fulfilling minimum standard of sperm motility (70%) and sperm morphology pooled in order to have sufficient semen for a replicate and to eliminate the bull effect. The semen was given a holding time for 10 min at 37 ℃ in the water bath before dilution.

2.3. Semen processing

Semen samples were diluted with TCF extender and used as control and other aliquots of pooled semen samples were diluted with TCF extenders containing the different concentrations of kaki in order to provide concentration of 60 million sperm/mL. Extended semen was slowly cooled (approximately for 2 h) to 5 ℃ and equilibrated for 2 h. Semen was packed into 0.25 mL polyvinyl French straws. After equilibrium periods, the straws were horizontally placed on a rack and frozen in vapor 4 cm above liquid nitrogen for 10 min and were then dipped in liquid nitrogen. A fraction of extended semen from control and each concentration of the additives were kept at 5 ℃ for 7-10 d (chilling) and sperm motility was evaluated daily.

2.4. Assessment of semen quality parameters

The assessment was undertaken on after freeze thawing of bull spermatozoa. Also, sperm motility was evaluated for raw semen, 2 h after cooling and chilled semen daily up to 7-10 d. Frozen straws were thawed at 37 ℃ for 1 min. The parameters studied were subjective semen characteristics (motility, alive, abnormality and hypoosmotic swelling test (HOST) %)[22].

2.5. Conception rate

Conception rate no. of cows (n=145) were inseminated with KEEY bull semen. Another no. of cows was inseminated with bull semen diluted with TCFY (control group). Pregnancy was confirmed by rectal palpation 2 mo later after insemination. The inseminated cows were used via the cooperation in Beni-Suef Governorate. CR was calculated according to the equation:

$$CR = \frac{\text{No. of conceived cows}}{\text{Total no. of inseminated cows}} \times 100$$

2.6. Statistical analysis

Statistical analysis data were analyzed using the SPSS (2005)[23] computerized program v. 14.0 to calculate the analysis of variance

(ANOVA) for the different parameters between control and additives replications. Significant difference between means was calculated using Duncan test at $P<0.05$.

3. Results

3.1. Effect of Diospyros kaki enriched extender on cattle bull sperm motility during chilling

Sperm motility was significantly ($P<0.000\ 1$) kept high after 11 d of chilling with the concentration 1%, 2%, 3%, 4%, 5% as compared to the control (41.67±1.67, 41.67±1.67, 40.00±0.00, 41.67±1.67 and 41.67±1.67, respectively) and also non-significantly kept high at the other concentrations up to 9 d of chilling (Table 1).

3.2. Effect of Diospyros kaki enriched extender on post thawing cattle bull sperm characteristics

Addition of KEE had significantly ($P<0.003\ 3$) improved post thawing sperm motility% with the concentrations 1%, 2%, 3%, 4%, 5% and 6% as compared to the control (51.67±5.27, 55.00±3.16, 48.33±1.05, 45.00±3.96, 57.00±2.50, 55.00±5.00 and 43.33±5.11 respectively) while the other concentrations exhibit no effect. Addition of KEE maintained alive sperm%, abnormalities% and % of intact spermatozoa membranes (HOST %) as good as the control with all concentrations of kaki used in our study (Table 2).

The conception rate (Table 3) upon using frozen semen in insemination showed higher conception rate in concentrations of

Table 1
Effect of *Diospyros kaki* enriched extender on cattle bull sperm motility during chilling.

Treatment	2 h	Days										
		1	2	3	4	5	6	7	8	9	10	11
Control	91.67^{a}±1.67	91.67^{a}±1.67	86.67^{a}±3.33	86.67^{a}±3.33	85.00^{a}±2.89	75.00^{a}±7.64	71.67^{a}±6.01	68.33^{a}±4.41	63.33^{a}±3.33	55.00^{a}±7.64	45.00^{a}±10.41	25.00±5.77
1	91.67^{a}±1.67	90.00^{a}±0.00	90.00^{a}±0.00	86.67^{a}±1.67	85.00^{a}±2.89	83.33^{a}±4.41	78.33^{a}±1.67	71.67^{a}±1.67	65.00^{a}±2.89	55.00^{a}±5.00	46.67^{a}±10.14	41.67±1.67
2	91.67^{a}±1.67	90.00^{a}±0.00	88.33^{a}±1.67	86.67^{a}±1.67	85.00^{a}±2.89	85.00^{a}±5.00	78.33^{a}±1.67	71.67^{a}±3.33	66.67^{a}±4.41	55.00^{a}±5.00	46.67^{a}±10.14	41.67±1.67
3	91.67^{a}±1.67	90.00^{a}±0.00	88.33^{a}±1.67	88.33^{a}±1.67	86.67^{a}±3.33	85.00^{a}±5.00	75.00^{a}±.00	70.00^{a}±5.00	61.67^{a}±7.26	50.00^{a}±0.00	41.67^{a}±6.01	40.00±0.00
4	91.67^{a}±1.67	91.67^{a}±1.67	90.00^{a}±0.00	90.00^{a}±0.00	88.33^{a}±1.67	85.00^{a}±2.89	80.00^{a}±0.00	76.67^{a}±1.67	61.67^{a}±7.26	50.00^{a}±0.00	40.00^{a}±5.00	41.67±1.67
5	91.67^{a}±1.67	91.67^{a}±1.67	90.00^{a}±0.00	88.33^{a}±1.67	86.67^{a}±1.67	81.67^{a}±1.67	81.67^{a}±1.67	71.67^{a}±1.33	56.67^{a}±3.33	50.00^{a}±5.77	36.67^{a}±8.33	41.67±1.67
6	91.67^{a}±1.67	91.67^{a}±1.67	90.00^{a}±0.00	90.00^{a}±0.00	88.33^{a}±1.67	85.00^{a}±2.89	80.00^{a}±0.00	73.33^{a}±1.67	63.33^{a}±7.26	55.00^{a}±8.66	38.33^{a}±9.28	16.67±3.33
7	91.67^{a}±1.67	91.67^{a}±1.67	88.33^{a}±1.67	88.33^{a}±1.67	86.67^{a}±3.33	80.00^{a}±5.77	75.00^{a}±2.89	70.00^{a}±2.89	56.67^{a}±9.28	50.00^{a}±10.00	36.67^{a}±8.82	16.67±3.33
8	91.67^{a}±1.67	88.33^{a}±1.67	88.33^{a}±1.67	88.33^{a}±1.67	86.67^{a}±1.67	80.00^{a}±5.77	75.00^{a}±2.89	68.33^{a}±3.33	55.00^{a}±10.41	48.33^{a}±10.93	35.00^{a}±8.66	16.67±3.33
9	91.67^{a}±1.67	90.00^{a}±2.89	88.33^{a}±1.67	86.67^{a}±1.67	86.67^{a}±1.67	73.33^{a}±10.14	68.33^{a}±7.26	61.67^{a}±7.26	55.00^{a}±10.41	43.33^{a}±13.33	30.00^{a}±13.23	16.67±3.33
10	91.67^{a}±1.67	88.33^{a}±1.67	86.67^{a}±1.67	86.67^{a}±1.67	85.00^{a}±2.89	71.67^{a}±11.67	66.67^{a}±8.82	58.33^{a}±10.14	51.67^{a}±13.02	40.00^{a}±15.28	25.00^{a}±12.58	16.67±3.33
F-value	0.000 0	0.68	0.62	0.57	0.25	0.61	1.22	1.18	0.38	0.32	0.50	11.92
Sig.	1.000	0.731 4	0.780 9	0.823 1	0..986 6	0.790 2	0.334 4	0.353 3	0.940 1	0.966 7	0.872 5	0.000 1

Table 2
Effect of *Diospyros kaki* enriched extender on post thawing cattle bull sperm characteristics.

Treatment	Motile (%)	HOST (%)	Alive (%)	Abnormality (%)
Control	43.33^{bc}±5.11	81.33^{a}±3.33	90.67^{a}±3.38	16.00^{a}±1.53
1%	51.67^{ab}±5.27	82.33^{a}±2.19	89.67^{a}±3.28	15.00^{a}±0.58
2%	55.00^{ab}±3.16	88.67^{a}±1.67	84.67^{a}±1.20	17.33^{a}±2.91
3%	48.33^{abc}±1.05	88.00^{a}±2.89	80.67^{a}±5.93	17.33^{a}±0.58
4%	45.00^{abc}±3.96	86.67^{a}±2.33	80.33^{a}±0.33	18.67^{a}±1.76
5%	57.00^{a}±2.50	81.33^{a}±2.40	82.33^{a}±1.67	19.00^{a}±0.58
6%	55.00^{ab}±5.00	83.33^{a}±2.85	82.67^{a}±0.33	16.33^{a}±3.84
7%	37.50^{c}±3.35	88.33^{a}±0.67	83.33^{a}±3.84	16.33^{a}±0.88
8%	45.00^{bc}±2.24	82.00^{a}±1.15	81.00^{a}±2.65	17.00^{a}±1.53
9%	39.17^{c}±3.27	87.00^{a}±4.04	83.33^{a}±0.67	19.00^{a}±2.08
10%	43.33^{bc}±4.22	85.00^{a}±0.58	77.67^{a}±0.33	20.00^{a}±1.73
F-value	3.12	1.46	1.97	0.64
P<	0.003 3	0.219 6	0.088 7	0.768 3

Duncan $P<0.05$. Different letter superscripts indicate a significant difference between means within column using the multiple range Duncan's test at $P<0.05$.

2%, 4% and 6 % KEE in cattle.

Table 3
Effect of Diospyros kaki enriched extender on a field conception rate test in cattle.

Treatment	Conception rate (%)
Control	45.50
KEE 1%	50.00
KEE 2%	62.50
KEE 3%	45.45
KEE 4%	66.67
KEE 5%	40.60
KEE 6%	60.00
KEE 7%	25.00
KEE 8%	20.00
KEE 9%	42.86
KEE 10%	5.00

4. Discussion

Cryopreservation of sperm is of a great demand[1]. According to Gadea *et al.*[24], Uysal and Bucak[25] and Bucak *et al.*[26], minimizing the physical and chemical stresses of cooling, freezing and thawing of sperm cells and consequently improving viability and subsequent fertilizing capacity is achieved by including cryoprotectants in the semen extender. Cryopreservation causes wide-ranging physical, chemical and mechanical injures to sperm membranes of all mammalian species[27], which are attributed to temperature changes, alterations in the transition from the lipid phase, production of reactive oxygen species (ROS) and osmotic stress[5,28]. Moreover, the over accumulation of ROS causes a state of oxidative stress that involves structural damage of sperm membranes, fall of intracellular ATP levels causing decreasing in the viability and motility of cryopreserved sperm[29,30]. To decrease the harmful effects of ROS, seminal plasma possesses powerful source of ROS scavengers which offer protection for equine sperm, including enzymes such as superoxide dismutase, catalase, glutathione peroxidase, and small molecular antioxidants such as ascorbic acid and α-tocopherol [31,32].The herbal remedies contain antioxidants to counteract the deleterious action of reactive oxygen species (ROS).

The results of the present trials revealed that sperm motility of cattle semen was kept high up to 11 d of chilling in KEEY with the concentrations 1%, 2%, 3%, 4% and 5% as compared to the control. This means that semen with these concentrations could be used in AI up to 11 d of chilling. Also, with the other concentrations of KEEY semen could be used in AI up to 9 d of chilling. On the other hand, KEEY significantly improved post thawing sperm motility with the concentrations 1%, 2%, 3%, 4%, 5% and 6% and also maintained alive sperm%, abnormalities and % of intact spermatozoa membranes. Conversely, KEEY had improved the post thawing sperm motility with all concentrations (1%-9%) except the 10%. All the used concentrations (1%-10%) had maintained alive %, Abnormalities% and % of intact spermatozoa membranes (HOST %). Higher conception rate in cattle with concentrations 2% and 4% kaki (62.50% and 66.67% respectively) coincide with the good post-thawing sperm parameters with these concentrations (sperm motility 55.00±3.16 and 45.00±3.96 respectively, Host 88.67±1.67 and 86.67±2.33 respectively, alive 84.67±1.20 and 80.33±0.33 respectively, abnormalities 17.33±0.58 and 8.67±1.76, respectively). Higher conception rate with the 6% kaki concentration (60.00%) come in accordance with the higher post thawing sperm parameters with this concentration (sperm motility 55.00±5.00, Host 83.33±2.85, alive 82.67±0.33 and abnormalities 16.33±3.84).

Cryopreservation of bovine semen often exert an additional source of reactive oxygen (ROS) attack and lipid peroxidation[3] which affects the membrane permeability[4]. The increased ROS level is due to the decreased activities of antioxidant enzymes. Natural antioxidants protect and preserve the metabolic activity and cellular viability of cryopreserved bovine spermatozoa[5]. *Diospyros kaki* is rich in strong antioxidants as represented by high contents of carotenoids[11,33], flavonoids and polyphenols[34–36], organic acids and vitamins[11,37], carbohydrates[11,38] and minerals[39]. Olayemi *et al.*[40] and Aljady *et al.*[41] recorded that phenolic acids and flavonoids have strong antioxidant capacity and improved semen quality of preserved semen. Flavonoids are excellent scavengers of free radicals and the number of hydroxyl group on the phenyl ring enhances the antioxidant capacity of polyphenolic molecule[42,43]. Kaki is rich in flavonoids which have strong antioxidants activity and suppressing the production of malondialdehyde and nitric oxide and inhibit apoptosis[35]. El-Sheshtawy *et al.*[44, 45] conclude that pollen grains and honey improved preservability of bull semen due to its high contents of antioxidants flavonoids, vitamins and organic acids. It could be concluded that natural additives to semen extenders (moringa, kaki and silymarin) improved semen preservability in both chilled and frozen semen.

Conflict of interest statement

The authors hereby certify that they have no conflict of interest.

References

[1] Medeiros CM, Forell F, Oliveira AT, Rodrigues JL. Current status of sperm cryopreservation: Why isn't better. *Theriogenology* 2002; **57**(1): 327-344.

[2] Barbas JP, Mascarenhas RD. Cryopreservation of domestic animal sperm cells. *Cell Tissue Bank* 2009; **10**(1): 49-62.

[3] El-Sisy GA, El-Nattat WS, El-Sheshtawy RI. Buffalo semen quality, antioxidants andperoxidation during chilling and cryopreservation. *Online J Vet Res* 2007; **11**: 55-61.

[4] Awda BJ, Mackenzie-Bell M, Buhr MM. Reactive oxygen species and boar sperm function. *Biol Reprod* 2009; **81**(3): 553-561.

[5] Câmara DR, Mello-Pinto MMC, Pinto IC, Brasil OO, Nunes JF, Guerr MMP. Effects of reduced glutathione and catalase on the kinematics and membrane functionality of sperm during liquid storage of ram semen. *Small Rumin Res* 2011; **100**(1): 44-49.

[6] Gorinstein S, Zemser M, Zachwieja Z, Weisz M, Folta M, Barton H, et al. Comparative contents of dietary fiber, total phenolics, and minerals in persimmons and apples. *J Agric Food Chem* 2001; **49**(2): 952-957.

[7] Chen XN, Fan JF, Yue X, Wu XR, Li LT. Radical scavenging activity and phenolic compounds in persimmon (*Diospyros kaki* L. cv. Mopan). *J Food Sci* 2008; **73**(1): C24-C28.

[8] Park Y-S, Leontowicz H, Leontowicz M, Namiesnik J, Jesion I, Gorinstein. Nutraceutical value of persimmon (*Diospyros kaki* Thunb) and its influence on some indices of atherosclerosis in an experiment on rats fed cholesterol containing diet. *Adv Hort Sci* 2008; **22**(4): 250-254.

[9] Akter MS, Eun JB. Characterization of insoluble fibers prepared from the peel of ripe soft persimmon (*Diospyros kaki* L. cv. Daebong). *Food Sci Biotechnol* 2009; **18**: 1545-1547.

[10]Veberic R, Jurhar J, Mikulic-Petkovsek M, Stampar F, Schmitzer V. Comparative study of primary and secondary metabolites in 11 cultivars of persimmon fruit (*Diospyros kaki* L.). *Food Chem* 2010; **119**(2): 477-483.

[11]Zhou C, Sheng Y, Zhao D, Wang Z, Tao J. Variation of oleanolic and ursolic acid in the flesh of persimmon fruit among different cultivars. *Molecules* 2010; **15**(9): 6580-6587.

[12]Dembitsky VM, Poovarodom S, Leontowicz H, Leontowicz M, Trakhtenberg S, Gorinstein S, et al. The multiple nutrition properties of some exotic fruits: Biological activity and active metabolites. *Food Res Int* 2011; **44**(7): 1671-1701.

[13]George AP, Redpath S. Health and medicinal benefits of persimmon fruit: A review. *Adv Hort Sci* 2008; **22**(4): 244-249.

[14]Celik A, Ercisli S. Persimmon cv. Hachiya (*Diospyros kaki* Thunb.) fruit: Some physical, chemical and nutritional properties. *Int J Food Sci Nutr* 2008; **59**(7-8): 599-606.

[15]Del Bubba M, Giordani E, Pippucci L, Cincinelli A, Checchini L, Galvan P. Changes in tannins, ascorbic acid and sugar contents in astringent persimmons during on-tree growth and ripening and in response to different postharvest treatments. *J Food Compos Anal* 2009; **22**(7-8): 668-677.

[16]Ebert G, Gross J. Carotenoid changes in the peel of ripening persimmon (*Diospyros kaki*) cv Triumph. *Phytochemistry* 1985; **24**(1): 29-32.

[17]Gorinstein S. Comparative content of total polyphenols and dietary fiber in tropical fruits and persimmon. *J Nutr Biochem* 1999; **10**(6): 367-371.

[18]Liu YJ, Xie JL. Ursolate compounds from fruit of *Diospyros kaki* L. f. (in Chinese). *J Plant Resour Environ* 2001; **10**: 1-3.

[19]Ma Q, Qin T, Zhang Q. Analysis of ingredient in persimmon frost (in Chinese). *Food Res Dev* 2005; **26**: 143-145.

[20]Matsuo T, Ito S. The chemical structure of Kaki-tannin from immature fruit of the persimmon (*Diospyros kaki* L.) (in Japanese). *Agr Biol Chem* 1978; **42**: 1637-1643.

[21]Yuan B, Xu HL, Leng SP. Content and chemical composition of carotenoids in persimmon fruit (in Chinese). *Chin Agr Sci Bull* 2006; **22**: 277-280.

[22]Salisbury GW, Van Demark NL, Lodge JR. Semen evaluation: In "*Physiology of Reproduction and Artificial Insemination of Cattle*." 2nd ed. Sanfrancisco: W.H. Freeman & Compagny; 1978. pp. 400-427.

[23]SPSS. SPSS v.14.0 for Windows Evaluation Version Release. 14.0.0. 2005.

[24]Gadea J, Gumbo D, Novass C, Zquezf AZ, Grullol A, Gardo GC. Supplementation of the dilution medium after thawing with reduced glutathione improves function and the *in vitro* fertilizing ability of frozen-thawed bull spermatozoa. *Int J Androl* 200; **31**(1): 40-49.

[25]Uysal O, Bucak MN. Effects of oxidized glutathione, bovine serum albumin, cysteine and lycopene on the quality of frozen-thawed ram semen. *Acta Vet Brno* 2007; **76**: 383-390.

[26]Bucak MN, Atessahin A, Yuce A. Effect of anti-oxidants and oxidative stressparameters on ram semen after the freeze-thawing process. *Small Rum Res* 2008; **75**(2-3): 128-134.

[27]Watson PF. The causes of reduced fertility with cryopreserved semen. *Anim Reprod Sci* 2000; **60–61**: 481-492.

[28]Ortega Ferrusola C, González Fernández L, Morrell JM, Salazar Sandoval C, Macías García B, Rodríguez-Martinez H, et al. Lipid peroxidation, assessed with BODIPY-C11, increases after cryopreservation of stallion spermatozoa, is stallion dependent and is related to apoptotic-like changes. *Reproduction* 2009; **138**(1): 55-63

[29]Baumber J, Ball BA, Gravence CG, Medina V, Davies-Morel MC. The effect of reactive oxygen species on equine sperm motility, viability, acrosomal integrity, mitochondrial membrane potential, and membrane lipid peroxidation. *J Androl* 2000; **21**(6): 895-902.

[30]Agarwal A, Prahakaran SA, Said TM. Prevention of oxidative stress injury to sperm. *J Androl* 2005; **26**(6): 654-660.

[31]Aitken RJ, Baker MA. Oxidative stress and male reproductive biology. *Reprod Fertil Dev* 2004; **16**(5): 581-588.

[32]Sikka SC. Role of oxidative stress and antioxidants in andrology and assisted reproductive technology. *J Androl* 2004; **25**(1): 5-18.

[33]Zaghdoudi K, Pontvianne S, Framboisier X, Achard M, Kudaibergenova R, Trabelsi MA, et al. Accelerated solvent extraction of carotenoids from: Tunisian Kaki (*Diospyros kaki* L.), peach (*Prunus persica* L.) and apricot (*Prunus armeniaca* L.). *Food Chem* 2015; **184**: 131-139.

[34]Denev P, Yordanov A. Total polyphenol, proanthocyanidin and flavonoid content, carbohydrate composition and antioxidant activity of persimmon (*Diospyros kaki* L.) fruit in relation to cultivar and maturity stage. *Bulg J Agric Sci* 2013; **19**(5): 981-988.

[35]Sun L, Zhang J, Fang K, Ding Y, Zhang L, Zhang Y. Flavonoids from persimmon (*Diospyros kaki*) leaves (FPL) attenuate H_2O_2-induced apoptosis in MC3T3-E1 cells via the NF-κB-pathway. *Food Funct* 2014; **5**(3): 471-479.

[36]Xie C, Xie Z, Xu X, Yang D. Persimmon (*Diospyros kaki* L.) leaves: A review on traditional uses, phytochemistry and pharmacological properties. *J Ethnopharmacol* 2015; **163**: 229-240.

[37]Karakasova L, Babanovska-Milenkovska F, Lazov M, Karakasov B, Stojanova M. Quality properties of solar dried persimmon (*Diospyros kaki*). *J Hygienic Eng Design* 2013; **4**: 54-59.

[38]Baltacioglu H, Artik N. Study of postharvest changes in the chemical composition of persimmon by HPLC. *Turk J Agric For* 2013; **37**(5): 568-574.

[39]Marqués AM, Domingo A, Cervera ML, de la Guardia M. Mineral profile of kaki fruits (*Diospyros kaki* L.). *Food Chem* 2015; **172**: 291-297.

[40]Olayemi F, Adedayo R, Muhummad R, Bamishaiye E. The nutritional quality of three varieties of zobo (*Hibiscus sabadariffa*) subjected to the same preparation condition. *Plant Food Hum Nutr* 2011; **6**: 10-15.

[41]Aljady AM, Kamaruddin MY, Jamal AM, Mohd Yassin MY. Biochemical study on the efficacy of Malaysian honey in infected wounds: An animal model. *Med J Islam Acad Sci* 2000; **13**(3): 125-132.

[42]Wettasinghe M, Shahidi. Scavenging of reactive-oxygen species and DPPH free radicals by extracts of borage and evening primrose meals. *Food Chem* 2000; **70**: 17-26.

[43]LeBlanc BW, Davis OK, Boue S, DeLucca A, Deeby T. Antioxidant activity of Sonoran Desert bee pollen. *Food Chem* 2009; **115**(4): 1299-1305.

[44]El-Sheshtawy RI, El-Nattat WS, Ali AH, Sabra HA. The Effect of Phoenix dactylifera pollen grains tris-infusion on semen preservability of local bull breeds. *Glob Vet* 2014; **13**(5): 728-732.

[45]El-Sheshtawy RI, El-Nattat WS, Sabra HA, Ali AH. Effect of honey solution on semen preservability of local breeds of cattle bulls. *Wld Appl Sci J* 2014; **32**(10): 2076-2078.