Journal of Animal Science/vol.33, No.2; 2023/pp 137-145

The effect of apricot tree gum adding to Tris-base diluents on liquid semen storage and ewe pregnancy rate

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Received: December 29, 2020 Accepted: April 16, 2021

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Journal of Animal Science/vol.33 No.2/ 2023/pp 137-145
https://animalscience.tabrizu.ac.ir

DOI: 10.22034/AS.2022.42527.1587

Abstract

Successful artificial insemination requires successful sperm collection, evaluation and the addition of preservatives toincrease sperm shelf life. Despite many efforts and addition of protective and antioxidant substances, the quality of frozen-thawed sperm is low and the resulting fertility is not acceptable. For this reason, in present research, the cooling method has been used instead of freezing. The aim of this investigation was to evaluate the effect of apricot gum adding to Tris-base diluents on liquid ram semen storage and ewes pregnancy rate. Twenty-five ejaculates were collected from 5 Ghezel rams by artificial vagina in Khalatpushan research station. Average age of rams was 3 years. Semen samples were collected two times per week. After rapid transferring of samples to the laboratory they were evaluated for traits such as volume, wave motion, viability, morphology, total motility, progressive motility, pH and sperm concentration. Diluted semen was divided into four equal parts. One of parts contained only a diluent based on Tris (control group). Into the three treated parts, apricot Gum were added in concentrations of 100µl, 150µl, 200µl per ml diluted sperm (treated groups) and these cases were compared with the control part. In this investigation, it was observed that adding apricot gum at all concentrations of 100,150 and 200µl did not any significant effect on sperm quality up to the third day after cooling in Tris-based diluents ($P>0.05$), but after the third day of cooling significantly improved sperm quality parameters such as viability, total motility, progressive motility, morphology and membrane health ($P<0.05$). The differences among treatments parts with different apricot gum concentrations were not significant ($P>0.05$). In this study, 12 ewes were divided into 4 groups (n=3 ewes) and every group was artificially inseminated by cervical approach with one of diluted sperm parts. The number of pregnancy in ewes inseminated with 0 (control part), 100, 150 and 200µl apricot gum concentrations parts were, 1, 2, 2 and 3 respectively .In general, the results of this study showed that the addition of Apricot Gum to Tris-based diluents increases the quality in short time storage of ram liquid semen.

Keywords: Apricot gum, Artificial insemination, Liquid semen, Natural antioxidant

Introduction

Artificial insemination is a method of breeding in which semen is obtained from the male and introduced into the female reproductive tract by means of instruments (Evans and Maxwell, 1987). An artificial insemination is probably the most important single technique devised to facilitate the genetic improvement of animal (Salmon and Maxwell, 2000). Although artificial insemination has gained widespread acceptance in the dairy cattle industries of most developed countries, it has not yet received such universal acceptance in the sheep and goats breeding industries (Evans and Maxwell, 1987) .Artificial insemination with frozen-thawed ram semen has not been widely adopted for sheep mainly due to the very poor fertility obtained after cervical insemination compared with pregnancy rate after cervical insemination with fresh semen or that obtained with laparoscopic intrauterine insemination (Soltanpur and Moghaddam, 2014). Pregnancy rate derived of artificial insemination in small ruminants still beyond the acceptable values (Zeitoun, 2017). Cooled semen has been used for many years as an important tool for the conservation, dissemination and transport of genetic sheep genetics (Evans and Maxwell, 1987). Progress in the use of artificial insemination is related to search for substances with the potential ability to improve the fertilizing capacity of spermatozoa (Spalekova, 2014). Enhanced pathological ROS generation in living organisms may be caused by several mechanisms like: ionizing radiation, bio activation of xenobiotics, inflammatory cells, increased cellular metabolism, aggregation of oxidases and oxygenases and loss of antioxidant capacity (Sanocka and Kurpisz, 2004). Spermatozoon like all cells living under aerobic conditions, constantly faces the oxygen paradox; oxygen is required for life, but oxidative metabolism of biological molecules can be potentially toxic because of the formation of highly reactive oxygen species (ROS) that can modify cell functions or viability (Aitken and Clarkson, 1987). In recent years plant–derived polymers have evoked remarkable attention in various industries due to their diverse applications as food emulsifiers, stabilizers and thickeners, pharmaceuticals, cosmetics, textiles and in art (Nussinovitch, 1997). The Rosaceae family, Prunusgenus, consist of peach, plum, cherry and almond trees, all of which can produce exudates gums. Vegetable gums, i.e., those gums obtained from plants, are solids consisting of mixtures of polysaccharides (carbohydrates) which are either water-soluble or absorb water and swell up to from a gel or jelly when placed in water. They are insoluble in oils or organic solvents such as hydrocarbons, ether and alcohol. Polysaccharides are polymers of sugars and play important roles in energy storage, signal transduction and as structural components in all living organisms. In recent years polysaccharides have drawn much attention due to their beneficial properties of anti-aging, antioxidant, anticancer, anti-inflammatory and immune-modulatory activities. The aim of this investigation was to assess the effect of apricot gum adding to tris-based diluents on liquid ram semen storage and ewe pregnancy rate. Apricot gum mixtures are often complex and on hydrolysis yield simple sugars such as arabinose, galactose, mannose and glucuronic acid (Simas, 2008; Simas-Tosin, 2009). Chemical analysis of apricot gum was performed in (2012) by the Liuveras-Tenoria. They found total sugars (60%), galactose (43%), mannose (4%), arabinose (44%), xylose (7%) and ramnose (1%) in apricot gum (Liuveras, 2012). One of the major sugars in apricot gum is arabinose (44%), which has been identified in many studies as a red ox athenicoxidant. The aim of this study was to investigate the effect of adding apricot gum as a natural antioxidant to diluent based on teaching in the maintenance of liquid semen in rams and the fertility of inseminated ewes.

Materials and method

This study was conducted in the Khalatposhan research station of Tabriz University, East Azarbayjan Province, Iran. Semen was collected by artificial vagina twice a week during the breeding season. A total number of 25 ejaculates were collected from 5 Ghezel rams. Average age of rams was 3 years. Immediately after collection, each ejaculate was immersed into a water bath maintained at 37 ℃ prior to evaluation. The semen samples were evaluated for volume, wave motion, sperm concentration, membrane integrity, PH, total motility, progressive motility and viability by routine examinations. The volume of ejaculate by graduated tube (Shamsuddin, 2000). To evaluate the wave motion, a drop of undiluted semen was placed on a pre-warmed slide 37℃ without a corerslip and examined under phase contrast microscope (100×) (Nikon, Eelpse, E200, Japan). Fresh semen's samples have been studied in terms of volume, concentration, total motility, viability and morphology and only samples with over 2.5 billion sperm and a progressive motility of over 70% were used for dilution. Semen samples was diluted with Tris (2.71g), citric acid(1g), fructose (1.4g), penicillin (100,000IU) and streptomycin (100mg) in 100 ml distilled water. Then 73 ml of this solution was mixed egg yolk (20ml). One gram of solid apricot gum was dissolved in 10ml sterile distilled water to prepare the liquid apricot tree gum. After diluting the semen samples in a ratio of 1 to 6, it was divided into four equal parts and three parts were added to 100, 150 and 200 µl/ml of apricot tree gum diluting liquid, respectively, and one item (control group) no material was added. Diluted semen samples were poured into 2ml micro tubes and placed in a container containing 37Ċ water in the refrigerator for 90 min to slowly reach 5Ċ. Semen samples were evaluated every three days for 36 days. In this study, 12 ewes were artificially inseminated by cervical approach and all three ewes were inseminated with one treatment. Sperm used for artificial insemination were stored for 24 hours. The artificial inseminated ewes in this experiment were 4 years old. Data were analysed using the Glm and mixed procedures of SAS 9.2 software.

Results

The collected fresh sperm samples were characterized in terms of their volume, concentration, percentage of viability, progressive motility and total motility and wave motion to assess their suitability for cooling (table1).

Table 1. Descriptive statistics of ram sperm parameters before dilution

Parameter	Number of samples	Mean	Minimum	Maximum	Standard deviation
Volume(ml)	25	0.97	0.70	1.70	0.22
Concentration ($\times 10^{9}$)	25	4.76	2.91	6.54	1.10
PH	25	7.00	7.00	7.00	0
Wave motion (1-5)	25	4.95	4.00	5.00	0.22
Total motility (%)	25	96.20	91.00	98.00	1.70
Progressive motility (%)	25	94.20	89.00	97.00	1.88
Viability (%)	25	98.06	94.80	100.00	1.14
Abnormality (%)	25	3.31	2.10	5.61	0.86

Effect of apricot tree gum and storage time on sperm traits in liquid storage

Effect of apricot tree gum adding and storage time on sperm viability has been shown in table 2.

On the first and third days after cooling, the difference among the 100, 150 and 200µl treatments of apricot gum on sperm viability percentage was not significant ($P<0.05$), but on days 6 to 18 after cooling ,all three treatments had a significant different in sperm viability percentage compared to the control group ($P<0.05$). In this experiment, it was observed that adding apricot gum to the

diluents improves the viability rate of sperm compared to the control group.

Table2. The effect of apricot tree gum on sperm viability in during storage (%)

Day	Control	Apricot tree gum 100µl/ml	Apricot tree gum 150µl/ml	Apricot tree gum 200µl/ml
1	96.40±1.21	97.12±1.26	96.28±1.57	97.28±1.23
3	89.20±8.62	94.68±1.61	93.74±3.41	93.82±3.74
6	79.48±11.88 [b]	91.67±3.85 [a]	89.46±4.54 [a]	91.34±4.44 [a]
9	64.52±14.93 [b]	88.33±3.58 [a]	85.91±5.84 [a]	87.78±6.41 [a]
12	48.89±15.81 [b]	83.21±8.13 [a]	80.49±9.56 [a]	84.88±7.49 [a]
15	30.84±15.81 [b]	76.67±8.96 [a]	73.50±7.82 [a]	81.43±7.45 [a]
18	18.50±6.25 b[b]	69.83±12.23 [a]	69.56±12.15 [a]	75.95±7.94 [a]
21	§	64.42±11.82	61.36±11.90	69.59±9.22
24	§	57.35±9.86	55.59±11.83	62.38±10.23
27	§	50.92±7.84	47.58±11.70	53/08±11.88
30	§	44.45±9.47	41.86±11.16	45.58±10.89
33	§	33.13±7.72	30.20±7.61	32.16±13.06
36	§	23.85±6.83	20.55±8.37	20.09±10.43

Least squares means with different letters in each row show significantly different (P<0.05),§ -Dead sperm

As shown in table3, treated groups 100, 150 and 200µl of apricot gum did not difference significantly from the control group until day12 after cooling, but from day 15 to 36 the difference between these treatments and the control group was significant (P<0.05).

Table 3. The effect of apricot tree gum on sperm abnormality in during storage (%)

Day	Control	Apricot tree gum 100µl/ml	Apricot tree gum 150µl/ml	Apricot tree gum 200µl/ml
1	3.74±0.82	3.99±1.00	4.10±1.02	4.19±1.02
3	4.62±0.94	4.58±1.16	4.87±1.10	4.85±0.93
6	5.60±1.19	5.39±1.14	5.48±1.24	5.87±1.10
9	6.57±1.56	5.94±1.15	6.24±1.42	6.88±1.26
12	8.01±2.10	6.54±1.17	6.86±1.44	7.72±1.19
15	9.88±2.33 [b]	7.17±1.13 [a]	7.63±1.46 [a]	8.45±1.41 [a]
18	11.70±2.41[b]	7.90±1.04 [a]	8.38±1.61 [a]	9.17±1.63 [a]
21	§	8.65±1.09	9.03±1.52	9.94±1.72
24	§	9.29±1.34	9.80±1.57	10.57±1.63
27	§	10.28±1.37	10.41±1.57	11.16±1.74
30	§	11.13±1.22	11.25±1.54	12.03±1.81
33	§	12.19±1.31	12.11±1.73	12.98±1.69
36	§	13.13±1.42	13.13±1.84	13.98±1.99

Least squares mean with different letters in each row show significantly different (P<0.05), §-Dead sperm

Effect of apricot tree gum adding and storage time on sperm total motility has been shown in table 4. Sperm motility from day 1 to 3 in treated groups100, 150and 200µl was not significant compared to the control group, but from day 6 to 36, the difference between control group and gum treatments was significant (P<0.05).

Table 4. The effect of apricot tree gum on sperm total motility in during storage (%)

Day	Control	Apricot tree gum 100µl/ml	Apricot tree gum 150µl/ml	Apricot tree gum 200µl/ml
1	93.55±1.90	94.25±1.61	94.15±1.89	94.95±1.50
3	85.45±9.04	91.60±2.06	90.45±3.41	90.45±4.24
6	73.20±12.99[b]	87.30±4.34 [a]	84.85±5.64 [a]	86.50±5.94[a]
9	58.35±15.38 [b]	83.75±4.95 [a]	80.85±7.02 [a]	82.00±6.54 [a]
12	42.85±14.52 [b]	77.05±9.40 [a]	75.45±10.28 [a]	78.35±8.42 [a]
15	26.35±9.66 [b]	70.45±10.19 [a]	71.30±10.90[a]	74.35±8.62 [a]
18	14.90±5.30 [b]	63.20±11.60[a]	62.90±13.00 [a]	68.50±9.19 [a]
21	§	58.60±11.06	56.50±12.21	63.05±10.06
24	§	51.95±9.43	49.40±12.34	55.45±10.22
27	§	44.20±8.78	40.80±12.01	44.60±10.78
30	§	36.70±8.44	35.00±11.38	36.00±10.79
33	§	26.10±6.95	23.80±6.75	24.65±9.69
36	§	17.80±5.61	15.25±6.38	14.45±6.54

Least squares mean with different letters in each row show significantly different (P<0.05), §-Dead sperm

Effect of apricot tree gum adding and storage time on sperm progressive motility has been shown in table 5. The difference in of progressive sperm between the control group and the treatments with apricot gum on the first and third days was not significant, but from the sixth to the thirty-sixth day, the difference between the control group and the treated groups with of apricot gum was significant (P<0.05).

Table 5. The effect of apricot tree gum on sperm progressive motility in during storage (%)

Day	Control	Apricot tree gum 100µl/ml	Apricot tree gum 150µl/ml	Apricot tree gum 200µl/ml
1	91.30±2.12	92.17±2.27	91.70±2.38	91.25±2.22
3	80.80±10.45	87.90±4.11	85.80±5.60	84.45±6.82
6	73.20±12.99 [b]	82.25±5.42 [a]	79.40±7.20 [a]	77.55±8.75 [a]
9	46.35±16.39 [b]	77.45±8.22 [a]	75.80±7.61 [a]	73.15±8.22 [a]
12	28.85±12.46 [b]	68.75±10.98 [a]	66.40±12.56 [a]	67.85±10.05 [a]
15	15.45±5.92 [b]	59.95±9.75 [a]	61.45±11.32 [a]	60.60±10.01 [a]
18	8.10±3.38 [b]	49.40±10.98 [a]	49.05±13.23 [a]	50.70±10.93 [a]
21	§	41.95±12.41	41.60±13.16	41.95±13.05
24	§	35.70±10.38	35.80±11.09	35.65±12.23
27	§	28.45±8.23	29.60±9.24	27.10±9/07
30	§	23.85±6.83	22.60±7.32	20.85±8.08
33	§	22.55±6.95	14.55±5.46	12.85±4.74
36	§	15.50±5.04	8.00±3.68	5.95±2.99

Least squares mean with different letters in each row show significantly different (P<0.05), §-Dead sperm

Table 6 shows the effect of apricot tree gum adding and storage time on sperm membrane integrity. On the first and third day after cooling, any significant difference was not observed between treatments with different levels of apricot gum and control group (P>0.05) .On the sixth day after cooling, there was a significant difference between the treatment of 100µl of apricot gum and the control group (P<0.05) .There was no significant difference between treatment with 150 and 200µl levels of apricot gum and control group (P>0.05). From day 9 to 36, there was a significant difference between treatments with all three levels of 100, 150 and 200 µl of apricot gum and control group (P<0.05). From day 1 to 36, any significant differences were observed between treatments with different levels of 100, 150 and 200 µl of apricot gum (P>0.05).

Table 6. The effect of apricot tree gum on sperm membrane integrity in during storage (%).

Day	Control	Apricot tree gum 100µl/ml	Apricot tree gum 150µl/ml	Apricot tree gum 200µl/ml
1	91.61±2.48	92.92±2.11	92.94±2.55	93.32±1.86
3	82.49±11.59	88.88±3.08	88.69±3.91	88.09±4.78
6	70.67±12.84^{b}	84.65±4.88^{a}	82.36±6.77^{ab}	82.29±7.38^{ab}
9	55.32±15.52^{b}	80.68±5.52^{a}	77.62±7.34^{a}	77.30±8.17^{a}
12	38.84±14.41^{b}	74.03±10.30^{a}	71.36±11.56^{a}	72.85±9.88^{a}
15	20.66±9.71^{b}	67.01±10.28^{a}	67.05±10.58^{a}	68.58±9.77^{a}
18	10.37±5.20^{b}	63.33±11.71	58.78±14.11	62.67±10.43
21	§	54.20±10.56	44.90±12.53	57.34±10.19
24	§	47.63±9.47	52.30±14.11	49.38±11.09
27	§	40.15±7.17	37.01±11.64	40.68±11.87
30	§	33.90±8.44	31.35±11.13	31.77±10.92
33	§	22.68±7.24	20.61±6.81	20.26±10.32
36	§	13.84±5.49	9.90±5.62	8.62±5.51

Least squares means with different letters in each row show significantly different (P<0.05),§-Dead sperm

Effect of apricot tree gum on ewe's pregnancy rate

Twelve ewes were inseminated cervical by semen treated by apricot tree gum to evaluation of its effect. The results are given in table7.

Table 7. The effect of apricot tree gum on ewes' pregnancy.

Used treatment	Number of inseminated ewes	Number of pregnant ewes
Control	3	1
100µl	3	2
150µl	3	2
200µl	3	3

*Due to the low number of inseminated ewes, significant effect not was reported in this trait

Discussion

Mammalian sperm cells present highly specific lipidic composition, high content of polyunsaturated fatty acids, plasmalogenes and sphingomyelines. This unusual structure of sperm membrane is responsible for its flexibility and the functional ability of sperm cells (Sanocka and Kurpisz, 2004). However, spermatozoa's lipids are the main substrates for per oxidation, what may provoke server functional disorder of sperm (Aitken, 1989). A reason for higher, pathological lipid per oxidation of sperm membranes can be un balanced oxidative stress (Sanocka and Kurpisz, 2004). The spermatozoon like all cells living under aerobic conditions, constantly faces the oxygen paradox; oxygen is required for life, but oxidative metabolism of biological molecules can be potentially toxic because of the formation of highly reactive oxygen species (ROS) that can modify cell functions or viability (Aitken and Clarkson, 1987). Reactive oxygen species leak from mitochondria in to the cytoplasm, they cause cellular damage by oxidizing a variety of biologically important molecules including DNA, proteins, lipids and carbohydrates (Przekwas, 2003). Also, oxidative stress by an imbalance of oxidants and antioxidants in favour of the former and is capable of inflicting injury on membrane lipids, proteins and nucleic acid (Toyokuni, 1999). Endogenous antioxidant is sufficient to prevent free radicals that are produced in the normal state in the cells but an increase in free radical production leads to oxidative damage (Bunker, 1992). Natural polysaccharides and their conjugates have been widely used in food and medicine for long time. Numerous biological and pharmalogical favourable effects of natural polysaccharides have been extensively studies invitro and in animal models in vivo and more kinds of natural polysaccharides have been tested and even applied in therapies (Wang and Fong, 2004; Liu, 2016). It was demonstrated that some natural polysaccharides were effective at preventing oxidative damage in living organism this could be a potential resource of novel antioxidants (Tsiapali, 2001). It is well described that the reducing capacities of

polysaccharides is related to their sulfation rate molecular weight, glycosidic linkages, hydroxyl groups but also carboxylic groups of uronic acids (Hentati, 2018). It was previously reported that a direct correlation between the antioxidant activity and the reducing power of polysaccharides (El-shaurbagy GA, 2014). Indeed, this antioxidant activity is explained by the presence of many free hydroxyl groups in the structure Chemical analysis of apricot gum was performed in (2012) by the LIuveras-Tenoria. They found total sugars (60%), galactose (43%), mannose (4%), arabinose (44%), xylose (7%) and ramnose (1%) in apricot gum (LIuveras, 2012). of polysaccharides. In addition, some papers reported that the presence of arabinos in the structure of polysaccharides could reduce the production of hydroxyl radicals by chelation of pro-oxidant ions (Lefish, 2018). In a study conducted to investigate the effect of adding xanthan gum to horse sperm diluents, the researchers reported that adding xanthan gum to the diluents reduces sperm motility compared to the control group, but in other sperm quality parameters, they did not see a significant difference with the control group, which is inconsistent with the findings of our research (Gheller, 2019). In another experiment to investigate the effect of substituting Gum diluents on increased the storage time of semen better than the control group and in Arabic for egg yolk in ram sperm diluents, researchers reported that substituting Gum Arabic for egg yolk increased the belief of artificial inseminated ewes which is consistent with the results of artificial insemination of sheep in our study (Zeitoun, 2017). This study showed that adding apricot tree gum to the Tris-base treatments improved the quality traits of sperm in short time storage and this study was conducted for the first time.

Conclusion

This experiment showed that adding all three concentrations of 100, 150, 200 µl of apricot gum improved the quality parameters of liquid semen and increase their shelf life.

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DOI: 10.22034/AS.2022.42527.1587

اثر افزودن صمغ درخت زرد آلو به رقیق کننده بر پایه ی تریس در نگهداری اسپرم مایع و نرخ باروری در میش‌های تلقیح شده

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تاریخ دریافت: ۱۳۹۹/۸/۷ تاریخ پذیرش:۱۴۰۰/۱/۲۶

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چکیده

زمینه ی مطالعاتی: ضرورت ذخیره‌سازی اسپرم برای تلقیح مصنوعی و عدم موفقیت روش انجماد اسپرم قوچ، اهمیت بهینه‌سازی روش سردسازی اسپرم را اثبات می‌کند. **هدف:** این مطالعه برای بررسی اثر افزودن صمغ درخت زرد آلو به رقیق‌کننده بر پایه تریس در نگهداری اسپرم مایع و باروری میش‌های تلقیح شده انجام شد. **روش کار:** در این مطالعه ۲۵ انزال از ۵ قوچ نژاد قزل به وسیله‌ی واژن مصنوعی جمع‌آوری شد. اسپرم‌گیری به صورت دوبار در هفته انجام شد و نمونه‌ها از لحاظ صفات: حجم، حرکت موجی، زنده‌مانی، مورفولوژِی، تحرک کل، حرکت پیش رونده،pH وغلظت ارزیابی شدند. بعد از رقیق‌سازی اسپرم به نسبت ۱ به ٦، اسپرم رقیق شده به چهار قسمت مساوی تقسیم شده و به سه قسمت آن به ترتیب غلظت های ۱۰۰، ۱۵۰ و ۲۰۰ میکرولیتر صمغ درخت زردآلو اضافه شد و به یک قسمت آن (تیمارشاهد) هیچ ماده ای اضافه نشد و فقط حاوی رقیق‌کننده پایه بود. نمونه‌های اسپرم به مدت ۳٦ روز در دمای ۵ درجه سانتی گراد در یخچال نگهداری شده و به فاصله زمانی سه روزه مورد بررسی قرار گرفتند. **نتایج:** افزودن صمغ درخت زردآلو در هر سه سطح ۱۰۰، ۱۵۰ و ۲۰۰ میکرولیتر تا روز سوم بعد از سردسازی تاثیر معنی داری بر روی کیفیت اسپرم نداشت (p>۰/۰۵). اما بعد از روزسوم سردسازی باعث بهبود معنی‌دار پارامترهای کیفیتی اسپرم شد. (p<۰/۰۵) . اما بین خود تیمارهای دارای سطوح مختلف صمغ درخت زردآلو تفاوت معنی‌داری مشاهده نشد (P>۰/۰۵). در این مطالعه ۱۲ رآس میش با استفاده از تیمارهای مختلف دارای صمغ و تیمار شاهد تلقیح شده بود که تیمار شاهد ۱ رآس، ۱۰۰ و ۱۵۰µl صمغ ۲ رآس و ۲۰۰µl صمغ ۳ رآس باروری داشتند. **نتیجه گیری:** نتایج این تحقیق نشان داد که افزودن صمغ درخت زردآلو به رقیق کننده بر پایه تریس، باعث افزایش کیفیت و ماندگاری اسپرم مایع قوچ شد.

واژگان کلیدی: آنتی اکسیدان طبیعی، اسپرم مایع، تلقیح مصنوعی، صمغ زردآلو