Global Veterinaria 12 (6): 840-849, 2014
ISSN 1992-6197

DOI: 10.5829/idosi.gv.2014.12.06.1146

Effect of Adding Protein High Viscosity (Gelatin) in Tris Extender on Semen Conservation Status, Fertility Rates, Antioxidant Status and Sex Ratio of Rabbits

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Abstract: Twenty sexually mature bucks and sixty-four does of V-line (VL) rabbits' strain (in two experiments) were used to investigate the effect of adding protein high viscosity (gelatin) to Tris-extender on rabbits' semen quality during conservation and the fertility ratio. The first experiment, protein high viscosity (gelatin) were added to the semen extender at levels, 0.0, 0.7, 1.0 or 1.4g/100 ml, followed by either incubated at 37°C for 4 hours or stored at 5°C for 48 hours. In the experiment 2, does were artificially inseminated by tested semen extender to determine the effect of gelatin adding both on rate of fertility and sex ratio. Results showed that; the addition of gelatin into semen extender had a significant positive effect on decreasing both the abnormal and dead spermatozoa and increasing the sperm progressive motility. Moreover, the ability of spermatozoa to storage at 5°C for 48 hours was prolonged especially with 1.0g and 1.4g gelatin/100 ml semen extender. Whereas, the high gelatin concentration (1.4 g gelatin/100 ml semen extender) gave the best results during either incubation semen extender at 37°C for four hours or storage it at 5°C for 48 hours. The results showed that the fertility rate, total number of born and the number of live and weaning offspring were significantly higher of females that were artificially inseminated with gelatin semen extender than those were artificially inseminated with the non-gelatin semen and the highest values were obtained with 1.0g gelatin group. Accordingly, the proportion of male to female kits (sex ratio) was significantly increased in gelatin treatment groups compared to the non-gelatin treatment group. In conclusion, diluting rabbits' semen with Tris-extender, which contains protein height viscosity (gelatin), appeared to be a successful method for improved rabbits' semen quality during conservation and in the same time increases fertilizing ability, which had direct impact on change the proportion of male to female ratio (sex ratio).

Key words: Antioxidant · Fertilizing ability · Gelatin · Rabbit semen · Sex ratio · Storage time

INTRODUCTION

As in many domestic species, artificial insemination in rabbits is currently performed with sperm stored in liquid state for short periods of time [1]. Sperm stored in liquid state have a highly fertilizing ability, which allow increasing reproductive performance of inseminated does [2]. The biggest problem to exploiting the stored rabbits' semen was the damage of sperm membrane structure during conservation, which led to decrease the motile sperm count [3]. Moreover, artificial insemination is usually performed using cooled semen that was stored for short periods [4] and high fertility rates and prolificacy were obtained [5]. Otherwise, Fertility rate by using semen stored at room temperature or cooled at 5°C was higher than that used frozen semen [6]. On the other hand, the solid storage (gelatin) at 15.8°C improves the survival and *in vitro* penetration capacity of sheep spermatozoa [7]. To enhance the sperm motility and increase its ability to fertilization during cooling fresh semen and storing it up to five days, gelatin has been added to extenders in different species such as rabbit [4]. Indeed, the use of

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gelatin (high viscosity protein) may exert a beneficial effect through: (1) avoid sperm cell precipitation and consequently reduce changes in medium conditions or composition, (2) immobilizing spermatozoa and thus reducing the metabolic demands of motion to preserve their fertilization potential [4, 8]. Reactive oxygen species (ROS) are produced by dead spermatozoa in semen and play an important role in diluted sperm survival [9]. In this medium that contains sperm sediment cells, pH may be lower and concentration of some toxic metabolic products may be higher [10]. The destructive effects of reactive oxygen species (ROS) in seminal plasma were measured through the stress enzymes activities such as superoxide dismutase (SOD), catalase (CAT), glutathione reductase (GSH) and glutathione peroxidase, GSH-Px [11]. Moreover, sperm membrane has a large content of unsaturated fatty acids and lacks cytoplasmic component and which in presence of ROS lead to lipid peroxidation and thus impaired cell function and decreased sperm motility [12]. Use semen extender to change sex ratio has been done and dependent on many morphological and velocity grade of sperm. The molecular weight of X chromosome is larger than the Y chromosome because it contains more nucleotides of DNA. It might be expected that differences in DNA mass between X and Y chromosome–bearing sperm would influence swimming velocity [13]. The Y-chromosome bearing spermatozoa are faster, when the spermatozoa arrive at the oviduct; it may be makes the relative majority of the Y-chromosome bearing spermatozoa can develop around the ovum [14].

The aim of this study was to evaluate the effect of adding protein high viscosity (gelatin) to semen extender during either conservation semen at refrigeration temperature (4°C) for 48 hours or incubation it at 37 °C for 4 hours on semen quality, fertility, antioxidant status and sex ratio.

MATERIALS AND METHODS

Animal Husbandry, Semen Collection and Treatments: Twenty mature bucks (with average 12 months age and 3kg body weight) and sixty-four does of V-line (VL) rabbits' strain were used in two experiments. All rabbits were housed in a naturally ventilated building and kept in individual wire galvanized cages (60 × 55 × 40 cm). Cages of the does were equipped with an internal nest-box. Rabbits were fed *ad libitum* with a commercial pellet diet containing 18% crude protein, 13% crude fiber and 2670 kcal digestible energy/kg diet for female and that was 16.3% crude protein, 13.3% crude fiber, 2.5% fat and 2600 kcal/kg digestible energy for males. All the experimental animals were healthy and clinically free from internal and external parasites and kept under the same managerial and hygienic conditions.

The First Experiment: Semen was collected artificially (from the twenty mature bucks) twice a week for ten successive weeks by using an artificial vagina. Gel plug was removed immediately after collection. Semen ejaculates were individually evaluated microscopically and the ejaculates which showed active progressive motility percentages (over 70%) were pooled and used. Semen was added to Tris-buffer extender, so that the dilution rate was 1semen: 3extender. The Final concentration rate was 80–100 x 10^6 spermatozoa/ml then divided into four portions. The first portion was served as a control, while, the protein high viscosity (gelatin) was added to the other three portions at levels 0.7, 1.0 and 1.4 g/100 ml extender, respectively as follows:

- Tris-based extender (control)
- Tris-based extender + 0.7g gelatin/100 ml extender
- Tris-based extender + 1.0g gelatin/100 ml extender.
- Tris-based extender + 1.4g gelatin/100 ml extender.

Each of control and tested extender were incubated at 37°C for up to 4 hours or stored at 5°C (refrigerator temperature) for up to 48 hours. Extender was prepared by dissolving 3.025 g of Tris (hydroxymethyl aminomethane), 1.50 g of citric acid anhydrous and 1.25 g of D (+) glucose and held in a water bath at 37°C. Percentages of sperm motility, abnormal and dead spermatozoa were estimated according to Viudes de Castro *et al.* [15]. The storage ability was calculated according to Seleem [16].

The Second Experiment: Sixty four V-line (VL) rabbit does (16 per each extender level) were randomly assigned for each testing extender. Does were artificially inseminated with the control and the other three tested extenders. Artificial insemination was carried out twice to obtain two letters from each female. All extenders were prepared and incubated at 37°C for 4 hours before artificially insemination. About 48 hours before artificial insemination, multiparous does were subcutaneousl─y injected with 75 IU of serum pregnant mare (PMSG) (Folligon, Intervet, Holland) to induce ovulation. Does were injected. with 0.8 mg of gonadotropin-releasing hormone analogue (Buserelin, Suprefact®, Hoechst-Roussel, Germany) at the time of insemination according to Boussin [17]. Rabbit does were divided into 4 equal

experimental groups and inseminated artificially with semen extenders as previously described. The insemination procedure was approached as described by Adams [18]. Fertility rate, kindling rate, total number of born and live and weaning kits were recorded. The sex of offspring was determined by the shape of the urogenital region on the day of birth according to Bodnár [19].

Assessment of Oxidative Stress Markers: Malondialdehyde (MDA) levels were determined according to Yagi [20]. Glutathione peroxidase (GPx) activity assayed using the method of Chiu *et al.* [21]. Superoxide dismutase (SOD) levels were determined kinetically according Misra and Fridovich [22]. Catalase (CAT) activity was estimated by the method of Aebi [23].

Statistical Analysis: All data were subjected to analysis of variance according to the statistical analysis system described by SAS [24]. The differences means among groups were tested by using Duncan's multiple rang test [25].

RESULTS AND DISCUSSION

Effect of Protein High Viscosity (Gelatin) and Storage Time on Semen Efficacy: The effect of various gelatin concentrations on sperm motility, sperm abnormality and dead sperm is illustrated in Tables 1 and 2. Adding gelatin as a protein high viscosity at any testing levels (0.7, 1.0 and 1.4 g gelatin/100 ml extender) resulted in a significant increase ($P<0.05$) in progressive sperm motility compared to the control group and this effect was obtained in both extender groups that were incubated at 37°C for 4 hours or that were stored for 48 hours at 4-6°C. As well, there were a significant reduction ($P<0.05$) in abnormal and dead sperm compared to the control (non-gelatin group), regardless the incubation or storage time. Add gelatin to semen extender with 1.4 g gelatin/100 ml extender had a significant impact on increase the sperm progressive motility and decrease the abnormal and dead sperm compared to the low and medium gelatin levels, which was showed during both the incubation and conservation periods. The percentage of progressive motility of spermatozoa were significantly decreased ($P<0.05$), while, abnormal and dead sperm percentages ($P<0.05$) were significantly increased with elevating storage or incubation time, regardless of gelatin supplementation levels.

Figures 1 and 2 showed the interaction effects between incubation or conservation periods and gelatin levels. Results showed that the progressive motility of spermatozoa was significantly declined ($P\leq0.05$), meanwhile, both of the abnormal and dead spermatozoa was significantly increased ($P\leq0.05$) with increasing incubation or conservation periods, while, the addition of different levels of gelatin to extender conducted to reduce the harmful effect of incubation or conservation periods on semen measurements and the best results were obtained with 1.0 and 1.4g gelatin levels. Moreover, the interaction effect between gelatin levels and the time of incubation or storage revealed that the control recorded the highest decreasing of progressive motility and the highest increasing in abnormal and dead sperm percentages compared to the gelatin groups. The beneficial effects of gelatin addition on the sperm characteristics in post-diluted and equilibrated semen indicated that supplementing Tris-buffer extender of rabbits' semen with 2% gelatin was effective in reducing spermatozoa metabolism and movement in post-diluted and equilibrated semen, because gelatin increases the viscosity of the extender, which affects the motility of spermatozoa [26]. Decreasing sperm metabolism probably reduces lactic acid generation in extended semen and it is known that low pH value of seminal plasma kill the spermatozoa [27]. The 2% gelatin addition to extender of fresh semen increased storage period of rabbit semen up to five days [4, 10]. Also, rabbit semen could be preserved for 48 and 72 h at 5°C with satisfied and acceptable sperm quality in terms of motility, livability, abnormality and acrosome integrity by adding 2% gelatin to Tris-buffer extender [28]. The successful storage of rabbit spermatozoa appears to be related to reversibly reducing their motility (increase the viscosity) and metabolic activity, thereby, prolonging their fertile life [6]. Adding gelatin to semen extender may be tended to hold sperm inactive and assists in keeping the particles of the egg yolk and the sperm in suspension, supplies extra nutrients and retards general contamination (bacteria and molds) during storage [29]. Moreover, comparison different concentrations of gelatin (0.5, 1, 2 and 4%) in soya milk extender for the storage of liquid ram semen concluding that the best results were obtained with a concentration of 0.5% [30]. It is thought that gelatin prevents sedimentation of live and dead cells at the bottom and thereby enabling spermatozoa to be more uniformly distributed, minimizing detrimental effects of pH and toxic metabolic products. The use of gelatin

Fig. 1 Fig. 2

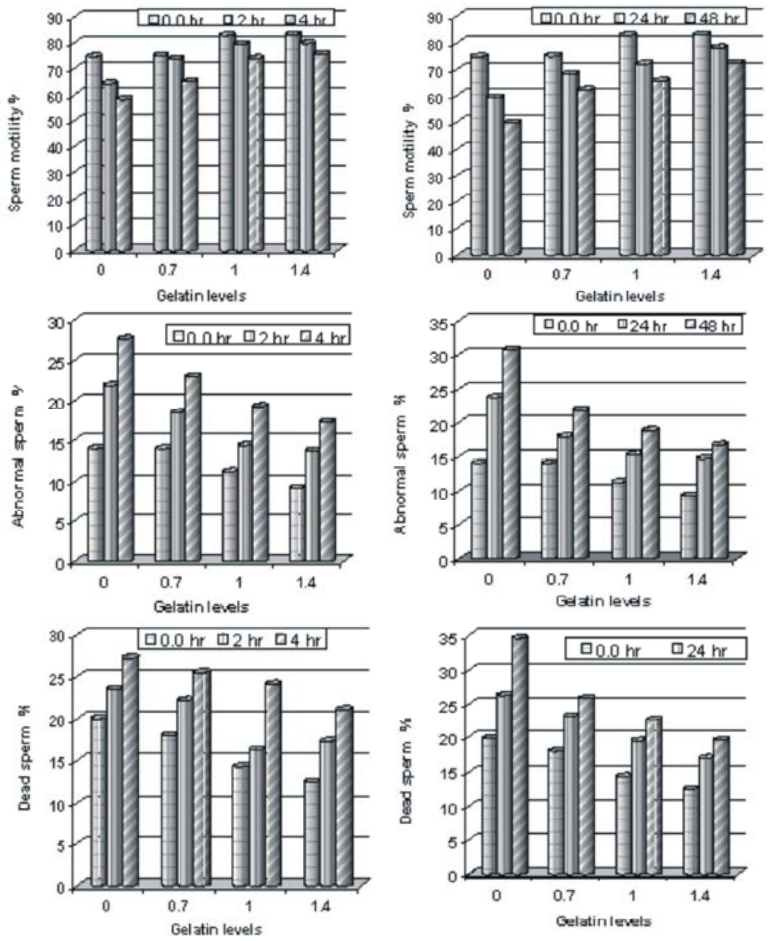

Fig. 1 and 2: The interaction effects between gelatin supplementation with different levels in semen rabbit extenders and incubation periods (Fig. 1) and conservation periods (Fig. 2) on the sperm progressive motility, abnormal sperm and dead sperm compared to the control (without gelatin)

Table 1: Effect of adding different levels of gelatin (protein high viscosity) in semen extender and incubation period at 37ºC on rabbits sperm characteristics

	Sperm characteristics (%)		
Item	Progressive motility %	Abnormal sperm %	Dead sperm%
Gelatin supplementation			
Control	65.8±1.36^{c}	21.2±0.98^{a}	23.6±1.20^{a}
Gelatin (0.7 g/100ml extender)	71.3±1.06^{b}	18.5±0.64^{b}	21.9±0.84^{a}
Gelatin (1.00 g/100ml extender)	78.7±1.26^{a}	15.0±0.64^{c}	18.2±0.79^{b}
Gelatin (1.4 g /100ml extender)	79.4±0.91^{a}	13.4±0.63^{d}	16.9±0.79^{b}
Incubation time (h)			
0	78.8±1.06^{a}	12.1±0.54^{c}	16.2±0.81^{c}
2	74.3±1.06^{b}	17.1±0.61^{b}	19.8±0.65^{b}
4	68.3±1.31^{c}	21.8±0.82^{a}	24.5±0.92^{a}

^{a,b,c} Means denoted within the same column for each effect with different superscripts are significantly different at ($P \leq 0.05$)

(high viscosity protein) may exert a beneficial effect through: (1) avoid sperm cell precipitation and consequently reduce changes in medium conditions or composition, (2) immobilizing spermatozoa and thus reducing the metabolic demands of motion to preserving their fertilization potential [4, 8].

Table 2: Effect of adding different levels of gelatin (protein high viscosity) in semen extender and conservation period at 5ºC on rabbits sperm characteristics.

	Sperm characteristics (%)		
Item	Progressive motility	Abnormal sperm	Dead sperm
Gelatin supplementation			
Control (0.0 gelatin)	61.2±1.36^{d}	22.8±0.98^{a}	26.9±1.20^{a}
Gelatin (0.7 g/100ml extender)	68.6±1.06^{c}	17.9±0.64^{b}	22.4±0.84^{b}
Gelatin (1.00 g/100ml extender)	73.4±1.26^{b}	15.1±0.64^{c}	18.8±0.79^{c}
Gelatin (1.4 g /100ml extender)	77.8±0.91^{a}	13.5±0.63^{d}	16.4±0.79^{d}
Cooling time (h)			
0	78.8±1.06^{a}	12.1±0.54^{c}	16.2±0.81^{c}
24	69.4±1.06^{b}	17.9±0.61^{b}	21.5±0.65 ^{b}
48	62.5±1.31^{c}	22.0±0.82^{a}	25.7±0.92^{a}

^{a,b,c} Means denoted within the same column for each effect with different superscripts are significantly different at ($P \leq 0.05$)

Table 3: Effect of adding different levels of gelatin (protein high viscosity) in semen extender and incubation periods at 37°C during on Malondialdehyde (MAD), glutathione peroxidase (GPx), superoxide dismutase (SOD) and catalase (CAT) enzymes activities

	Antioxidant status			
Item	MAD U/ml	GPx U/ml	SOD U/ml	CAT U/ml
Gelatin supplementation				
Control	0.111±0.013^{a}	2.25±0.213^{c}	2.25±0.213^{c}	25.64±1.444^{c}
Gelatin (0.7 g/100ml extender)	0.096±0.008^{ab}	5.33±0.376^{b}	3.24±0.424^{bc}	27.10±1.219^{b}
Gelatin (1.00 g/100ml extender)	0.076±0.007^{bc}	5.23±0.493^{b}	4.86±0.481^{ab}	26.63±1.255^{b}
Gelatin (1.4 g /100ml extender)	0.061±0.003^{c}	6.75±0.634^{a}	5.23±0.493^{a}	33.85±0.933^{a}
Incubation time (h)				
0	0.077±0.006^{b}	4.64± 0.517^{b}	5.53± 0.253^{a}	30.98± 0.918^{a}
4	0.096±0.009^{A}	6.053±0.677^{A}	3.601±0.208^{B}	26.63±1.078^{B}

Means within the same row (a, b& c) or the same column (A, B& C) bearing different letter superscripts are different significantly ($P \leq 0.05$)

Table 4: Effect of adding different levels of gelatin (protein high viscosity) in semen extender and conservation periods at 5°C during on Malondialdehyde (MAD), glutathione peroxidase (GPx), superoxide dismutase (SOD) and catalase (CAT) enzymes activities

	Antioxidant status			
Item	MAD U/ml	GPx U/ml	SOD U/ml	CAT U/ml
Gelatin supplementation				
Control	0.131±0.013^{a}	2.77±0.213^{c}	3.33±0.510^{b}	23.05±1.444^{c}
Gelatin (0.7 g/100ml extender)	0.104±0.008^{b}	6.09±0.376^{b}	3.45±0.424^{b}	25.15±1.219^{bc}
Gelatin (1.00 g/100ml extender)	0.078±0.007^{c}	7.08±0.634^{ab}	3.95±0.481^{ab}	26.86±1.255^{b}
Gelatin (1.4 g /100ml extender)	0.065±0.003^{c}	8.15±0.068^{a}	4.27±0.493^{a}	32.80±0.933^{a}
Incubation time (h)				
0	0.077±0.006^{b}	4.64± 0.517^{b}	5.53± 0.253^{a}	30.98± 0.918^{a}
48	0.112±0.012^{a}	7.40±0.676^{a}	1.96±0.115^{b}	22.96±1.295^{c}

Means within the same row (a, b& c) or the same column (A, B& C) bearing different letter superscripts are different significantly ($P \leq 0.05$)

Oxidative Stress Markers Status: Data of Tables 3 and 4 showed the effect of addition different levels of gelatin to rabbit semen extender and the effect of incubation and conservation periods on the lipid peroxidation (Malondialdehyde concentration (MAD)) and glutathione peroxidase (GSH-Px), superoxide dismutase (SOD) and catalase (CAT) enzymes activities. Results showed that increasing gelatin supplementation to rabbits' semen extender resulted in a gradually and significantly decrease ($P \leq 0.05$) the overall mean of MAD concentration and this effect was gelatin levels-dependent manner, regardless the incubation or conservation time. On the other hand, the MAD concentration in rabbits' semen extender was increased significantly ($P \leq 0.05$) with increase the incubation or storage period. Glutathione peroxidase (GSH-Px) enzyme activity of rabbit semen extender was increased by increasing gelatin level and this effect was significant ($P \leq 0.05$), regardless the incubation or storage time. Moreover, the results revealed that the highest gelatin levels (1.4g/100 ml extender) had the highest Glutathione peroxidase value, which was significant ($P \leq 0.05$) compared to the low and medium levels.

Fig. 3 Fig. 4

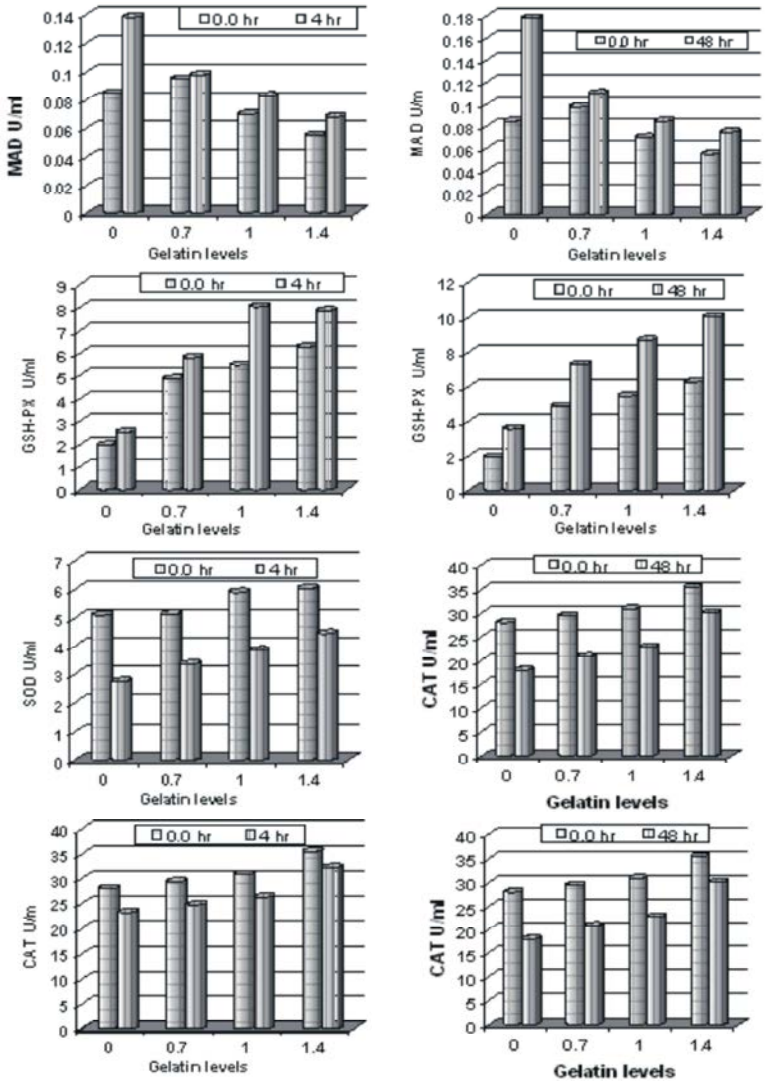

Fig. 3 and 4: The interaction effects between gelatin supplementation with different levels in semen rabbit extenders and incubation periods (Fig. 3) and conservation periods (Fig. 4) on the Malondialdehyde concentration (MAD), glutathione peroxidase (GSH-Px) and superoxide dismutase (SOD) and catalase (CAT) enzymes activities compared to the control (without gelatin)

On the other hand, the MAD and Glutathione peroxidase values in rabbits' semen extender was increased significantly ($P \leq 0.05$) with increase the incubation or storage period. Superoxide dismutase (SOD) and catalase (CAT) enzymes activities of rabbits' semen extender, which contains different levels of gelatin (0.7, 1.0 and 1.4 g/100 ml extender) showed a significant increased ($P \leq 0.05$) with increasing gelatin level, regardless the incubation or conservation time. The highest SOD and CAT means were recorded with the highest level of gelatin (1.4 g/100 ml extender). Whereas, increasing incubation or conservation periods of treated rabbits' semen extender resulted in a significant reduction ($P \leq 0.05$) in both SOD and CAT means, regardless of gelatin supplementation.

Figures 3 and 4 showed the interaction effects between incubation periods (for 4 hours) or conservation periods (for 48 hours) and gelatin supplementation levels on some anti-stress enzymes activities. Results showed that MAD production (as an indicator of lipid peroxide rate) due to sperm activity was increased in the control group with increasing incubation or conservation time compared to the other tested groups, while, MAD

concentration in semen extender was decreased with increasing gelatin concentration in semen extender and this effect was significantly ($P \leq 0.05$). In addition, glutathione peroxidase means in the semen extender, which contains different levels of gelatin were significantly ($P \leq 0.05$) higher with increasing incubation or conservation periods and the 1.0 and 1.4 g gelatin levels had the highest glutathione peroxidase values after 48 hours of conservation. The interaction effect between incubation or conservation periods and gelatin concentration levels in the semen extender showed a significant ($P \leq 0.05$) reduction in the SOD values with increasing incubation or conservation period and this decrease was highly significant ($P \leq 0.01$) in the control group compared to the other tested groups. Also, the highest gelatin level (1.4 g gelatin/100 ml extender) was less affected with increasing conservation period. The interaction effects between conservation periods and gelatin levels in semen extender on the catalase enzyme activity had the same trend of SOD. Whereas, the control group was the highest affected with increasing the incubation or conservation period, while the high gelatin level was the lowest affected. In this study, it was clear that supplementation of gelatin to the semen extender improved rabbits' semen quality variables. The extender supplemented with 1.0 and 1.4 gelatin resulted in the greatest sperm motility and decreased abnormality and dead sperm. Moreover, the extender supplemented with gelatin significantly enhanced GSH-Px, CAT and SOD activity compared with the control group. The current findings were in agreement with many studies and indicated favorable effects of gelatin on incubation and cooling viability of rabbit spermatozoa.

It could explain the role of these enzymes on sperm functions by understanding the job carried out by these enzymes. From the previous studies, the mammalian sperm membrane is particularly rich in unsaturated fatty acids (PUFA). This renders the sperm very susceptible to lipid peroxidation (LPO), which occurs as a result of the oxidation of the membrane lipids by partially reduced oxygen molecules, such as superoxide, hydrogen peroxide and hydroxyl radicals. Lipid peroxidation of the sperm membrane ultimately leads to the impairment of sperm function due to the attacks by reactive oxygen species (ROS), which affect and reduce the sperm motility and fertility [31]. It is worth noting that although analysis of sperm motility and morphology could indicate a normal sample, a decreased capacity of the antioxidative enzyme protective system could result in infertility due to premature degradation of the sperm membrane by lipid peroxidation [32]. The mammalian sperm antioxidant system predominantly includes SOD, GSH, GSHPx and CAT activities to restrict lipid peroxidation [33]. SOD is an important component of the enzymatic antioxidant system and it has a positive effect of SOD activity on the sperm membrane integrity after the cryopreservation process [34]. The potential role of CAT, mainly resulting from the formation of H_2O_2, is in the control of oxidative stress in cells [35]. The arrest of free-radical propagation by GSH peroxidases seems to be the main protection against peroxidation and cell death in mammalian semen [36]. Glutathione peroxidase plays an important role in the elimination of hydrogen peroxide [37]. In the present study, the extender supplemented with gelatin elevated the antioxidant capacities and therefore improved the motility and decreased of abnormal and dead sperm after incubation and cooling. The elevation of GSH-Px, SOD and CAT, activity was indicative of improved antioxidant capacity, which was similar to studies on the freezing of ram semen [12]

Effect of Gelatin Addition on Fertility, Prolificacy Traits and Sex Ratio: Results in Table 5 showed the fertility and prolificacy status after artificial insemination with semen extender supplemented with different levels of gelatin. Add gelatin at different tested levels to rabbits' semen extender increased the number of fertile does (fertility rate) to reach 75, 88 and 84% compared to control group (69%). Moreover, kindling rate improved (72, 84 and 81%) compared with that of the control group (59%). Add gelatin boosted total born, live and weaning kits compared with non-gelatin group and the medium gelatin level (1.0g gelatin /100ml in diluted semen) had the highest significant number of total and live born and total weaning kids (8.9, 8.3 and 7.5),respectively. These results are in agreement with the observation that a significant ($p \leq 0.05$) increased in kindling rate and litter size for APRI does inseminated with fresh semen supplemented with 2% gelatin to tris-buffer extender compared with control does [37]. Also, the fertilizing ability of spermatozoa is influenced by a combination factors including motility, viability and an ability to undergo capacitation and the acrosome reaction in the female reproductive tract [38]. Moreover, the solid storage of rabbit spermatozoa in a gelatin-supplemented extender (1.4 g/100ml) preserved their fertilizing potential for 72 hours [4]. Addition of gelatin might help to increase the viscosity in the AI-does limiting the back flow of semen at deposition and might

Table 5: Effect of adding different levels of gelatin (protein high viscosity) in semen extender on the reproductive parameters of female rabbits fertilized artificially with tested extender

Gelatin levels (g/100ml)	No. of does	Fertility rate%	Kindling rate %	Total born (SM±SE)
Control	16*2	69%	59%	7.7±0.43^{b}
0.7	16*2	75%	72%	8.6±0.41^{ab}
1.0	16*2	88%	84%	8.9±0.26^{a}
1.4	16*2	84%	81%	8.5±0.25^{ab}

Means within the same column (a & b) bearing different letter superscripts are different significantly ($P \leq 0.05$)

Table 6: Effect of adding different levels of gelatin (protein high viscosity) in semen extender on sex ratio of female rabbits fertilized artificially with tested extender

	Gelatin levels (g/100ml extender)			
Items	Control	0.7	1.0	1.4
Total of live born at birth	6.5±1.14 ^{b}	7.9±0.37^{a}	8.3±0.26^{a}	8.1±0.20^{a}
Total of born at weaning	6.1±0.34^{b}	6.9±0.35^{ab}	7.5±0.31^{a}	6.6±0.19^{b}
Total of Male	3.0±0.18^{c}	4.3±0.21^{b}	5.7±0.19^{a}	3.9±0.47^{b}
Total of Female	3.1±0.25^{a}	2.6±0.14^{b}	1.8±0.15^{c}	2.7±0.08^{b}
Ratio Male/Female	49.1±1.94^{c}	61.4±0.43^{b}	76.0±1.30^{a}	58.6±0.45^{b}

Means within the same row (a, b & c) bearing different letter superscripts are different significantly ($P \leq 0.05$)

thus be beneficial at a vaginal deposition of liquid ram semen [39]. On the contrary, gelatin addition did not improve sperm motility and viability after thawing in rabbit [8].

Results in Table 6 showed that sex ratio was significantly altered according to gelatin supplementation in diluted semen extender. The 1.0 g gelatin supplementation had the highest percentage changes of sex ratio (76%) compared to the other two gelatin levels (61.4 and 58.6%, respectively) or the non-gelatin addition (49.1%). The ability of preselect the sex of offspring of agricultural animals would have a significant impact on the genetics and economics of livestock production. The economic impact in this result is respectable to the preferable increase number of male instead of female kids in commercial farms. There are numerous reports of sex ratio variation in mammals in relation to factors such as food availability and competition for resources [39]. Another aspect of artificial insemination in animals is the use of sex sorted spermatozoa. Separation of the X and Y bearing sperm is desirable in animals as one sex has significantly more value than the other in certain species. It has first been established for spermatids are a difference in DNA content between the mammalian X-chromosome-bearing spermatozoa and the Y- chromosome [40]. Since, DNA content measurements have been used to identify the sex-chromosome bearing sperm populations with good accuracy in semen from at least 23 mammalian species [41] and offspring have been produced from sexed sperm of at least seven species, including rabbits [13]. The use semen extender to change sex ratio has been done by many researchers, used the caffeine administration to dilute rabbit semen and study the effect that on its progeny [19]. Who, found that the caffeine caused a statistically significant alteration (about 15%) in sex ratio of the offspring in favour of the males. Who, explained that caffeine was able to modify the sex ratio due to its natural difference between the velocities of the X- and Y-chromosome bearing spermatozoa [42] and Y-chromosome bearing spermatozoa are faster and may be the caffeine can increase the difference in velocity.

In our study, add gelatin to the extender maybe resulted in increase the viscosity, which reduced the velocity of X-chromosome compared to the Y-chromosome. The natural difference in velocities of the X- and Y-chromosome due to that X-chromosome has molecular weight heavier than Y-chromosome In the same respect, addition of gelatin might also help to increase the viscosity in the AI-dose limiting the back flow of semen at deposition and might thus be beneficial at a vaginal deposition of liquid ram semen [30].

CONCLUSION

Adding gelatin to rabbit semen extender led to enhance sperm characteristics and improved fertility and prolificacy. Moreover, it had significant effect on sex ratio after artificial insemination.

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