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Protective Role of Vitamin C Against Anti-Sperm Antibodies Production in Rams Subjected to Intensive Semen Collection

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Abstract: The present investigation was aimed to study the possible protective role of vitamin c against anti-sperm antibodies (ASA) production in rams under intensive semen collection programme. Thirty adult (1.5 - 2.0 years old) healthy rams were used. Rams were tested for the presence of anti-sperm antibodies either in blood or in seminal plasma by ELISA technique.Two rams were excluded for the presence of (ASA) in their blood and semen.The remained 28 rams were raised on a balanced commercial productive ration and ad libitum drinking water.Rams were divided into 3 groups; the control group (G1/8 rams), the treated group (G2/10 rams) that were given 20 mg vitamin c / kg body weight/ day dissolved in drinking water and the untreated rams (G3/ 10 rams) did not received vitamin c. The experimental time lasted for 8 weeks. Rams of control group were subjected for semen collection twice / week, while rams of the G2&G3 were subjected for semen collection once daily / 5 successive days /week and considered as intensive semen collection groups. At the end of 8th week, semen was subjected for routine analysis. Seminal plasma and blood sera were separated and tested for ASA by ELISA technique. Results revealed that 3 rams (30%) of the untreated intensive group were exhibited a higher (ASA) titer in their blood or seminal plasma (1/80 and 1/40, respectively). Semen analysis showed a significant decrease in ejaculate volume,sperm count and sperm motility %with adequate sperm agglutination in rams of untreated group. Vitamin c supplementation inhibits ASA production and normalized sperm parameters. Conclusively, vitamin c protected rams under intensive semen collection against ASA production and could maintains normal spermogram.

Key words: Anti-Sperm Antibodies · Rams · Vitamin C · Intensive Semen Collection

INTRODUCTION

The development and functions of the reproductive system are controlled by complex neuroendocrine mechanisms. The immune system plays a key role under physiological conditions and in reproductive disorders. It was reported that sperms and their precursors exhibit quite strong antigenic traits. A wide spectrum of antigenic structures is being expressed in the testis during spermatogenesis towards which, ontogenetically, autotolerance mechanisms are inactive. Protection against autoimmunity is provided by the hemotesticular barrier composed predominantly of Sertoli cells isolating the tubular content from the vasculature and limited lymphatic drainage of the testis [1].

Antisperm antibodies formation can be induced primarily during infectious and noninfectious inflammations, or by obstruction of testicular efferent duct. The incidence of ASA was also induced by an accident [2], very low temperature [3], cryptorchism [4], vasectomy [5] and by excessive male exploitation [6].

The occurrence of ASA is being connected with infertility in humans,laboratory and farm animals[2,7,8]. Negative effect of ASA on sperm function during in vitro fertilization was demonstrated byTasdemir *et al*. [9], Kim *et al*. [10] and Lombardo *et al*. [11]. The prevalence of antisperm antibodies in blood sera of boars and sows was reported byFayemi *et al.* [3] in bulls and in boars by Zral *et al*. [12]. Most of the unexplained questions concern the

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etiology and pathogenic mechanisms of functional changes in sperms and the subsequent subfertility induced by antibodies [13].

The objective of the present study was to determine the level of antisperm antibodies (ASA) in the blood sera and seminal plasma of rams subjected to intensive semen collection with or without vitamin c supplementation and to assess the relationship between the occurrence of ASA in sera and certain semen parameters.

MATERIALS AND METHODS

Experimental Design and Semen Collection: Thirty Egyptian Rahmani rams with clinically normal genitalia were used.The animals were 1.5 - 2.0 years old at the beginning of the study and weighed 45.0 ± 2.0 kg. They were housed outdoors in sheltered dry lots at Al-Saffaa sheep farm, Al-Badrashin, Giza, Egypt. All the animals grazed on Trifolium alexanderinum (green barssem) during winter and green corn (darrahwa) in summer as well as daily balanced ration consisted of a concentrate mixture adequate in protein, energy, vitamins and minerals to meet the nutrient requirements of sheep according to NRC [14] and had free access to fresh water. A general management schedule for disease prevention and hoof trimming was applied.All rams were trained for semen collection.

Firstly, all rams were tested for the presence of ASA, either in blood sera or seminal plasma. Detection of ASA was carried out by an enzyme immuno analytical method (ELISA) at Physiology Department, Faculty of Veterinary Medicine, Cairo University as described previously by Zral *et al*. [12] with some modifications. Ram spermatozoa were obtained by ejaculate centrifugation at 1200g/5 minutes. Washed 3 times in 0.005M phosphate buffered saline and resuspended at a concentration of 1 x 10^6 cells / ml. Sonicated 20 strikes with sonicator (Model W 380, Heat systems Ultrasonic Inc.). Wells of microplates (Becton Dickinson) were coated with sperm antign (50 ul /well). The antigen was fixed to the plates with 0.5% gluteraldehyde (50ul/well) overnight at 4°C, then washed and incubated at 4°C with 3% bovine serum albumin (BSA) (50ul/well) overnight. Excess BSA was removed by washing with PBS-Tween 20 mixture.The microplates were incubated with test sera (or seminal plasma samples) (50 ul/well) for 2 hrs at 37°C.The plates were washed and incubated at 37°C with 50 ul/well anti-sheep IgG {dilution 1:3000 (5 ul / 15 ml PBS) - VACSERA / EGYPT } for 30 minutes. Then washed and incubated at room temperature with alkaline phosphatase substrate buffer for 45 minutes. Stopping solution of NaOH (1 N) was added at room temperature for 15 minutes. All washings were done three times with PBS-Tween 20 mixture solution.

The developed colour intensity was measured using a spectrophotometer iEMS Reader MF (Lab systems, Finland) at wave length 492 nm and evaluation was done by a computer program Genesis. As positive reactions were considered in which the absorbance values were more than two-fold higher compared with the negative control. Dilution of 1:40 and higher was considered as a positive finding.

Two rams were excluded for the presence of (ASA) in their blood.The remained 28 rams were raised on a balanced commercial productive ration and ad libitum drinking water.They were divided into 3 groups; the control group (G1 / 8 rams), were subjected for semen collection twice / week. The vitamin c - treated group (G2/ 10 rams) were given 20 mg vitamin c / kg live weight/ day / 8 weeks,dissolved in drinking water and the untreated group (G3 / 10 rams) did not received vitamin c. Rams of treated and untreated groups were subjected for semen collection once daily / 5 successive days /week (intensive semen collection groups).

Semen was collected by using artificial vagina and restrained anestrous teaser ewe and examined for ejaculate volume, sperm count, individual sperm motility %, life sperm %, abnormal sperm % and sperm agglutination according to Evans and Maxwell [15].The experimental time lasted for 8 weeks to cover a complete spermatogenic cycle.Only first ejaculate samples were used in this study.At the end of 8th weeks of experiment, spermatoanalysis,detection of ASA in sera and seminal plasma were performed.

Statistical Analysis: Significance of differences among the characteristics was assessed by $\chi 2$ test, and by using analysis of variance (ANOVA) test. Means were tested at least significant difference (LSD). All statistical analysis was performed using SPSS (v.15.0) software.Results were considered significant only at P <.05 or less.

RESULTS

Table (1) demonstrates that ~~in~~ rams of G3, 3 rams of 10 (30%) were exhibited ASA titre 1/80 in sera and 1/40 in seminal plasma that represents a significant change (P<.05) comparing with rams of G1 & G2.However, in rams of G2, ASA were not detected neither in sera nor in seminal plasma.

Table 1: Occurance of ASA in sera and seminal plasma of rams subjected to intensive semen collection with or without vitamin c supplementation

		Negative ASA		Positive ASA	
Groups	No. of Animals	No.	%	No.	%
Control / G1	8	8	100	0.00	0.00
Intensive/Treated*/ G2	10	10	100	0.00	0.00
Intensive/Untreated/G3	10	7	70	3	30
X^2 - test		P <.05		P <.05	

(*): vitamin c supplemented.

Table 2: Relationship between the presence of ASA in sera of rams and certain semen parameters.

	Parameters					
Animals	Ejaculate volume (ml)	Sperm conc. ($x10^9$/ml)	Individual Sperm motility (%)	Life sperms (%)	Abnormal sperms (%)	Sperm agglutination
ASA-Negative (Titre<1/40)	1.45±0.18*	4.80±0.48*	86.20±2.95*	86.90±2.02	6.60±0.98	Rare
ASA-Positive (Titre>1/40)	0.80±0.07*	3.10±0.45*	78.30±2.05*	83.60±1.96	5.80±0.42	Adequate

- Data are means ± SE.
- Means within the same column having the same astriks are significantly different at P <.05.

Table (2) shows a significant decreases in ejaculate volume, sperm count and individual sperm motility (P <.05) in rams with ASA - positive titre (> 1/40) as compared with ASA-negative rams (titre< 1/40).Adequate sperm agglutination was also detected in semen of ASA-positive rams.

DISCUSSION

The presence of antisperm antibodies in secretions of reproductive organs and blood is often associated with infertility in both humans and laboratory and farm animals [16]. The bonding of those antibodies to the surface sperm antigens, which are predominantly functional molecules such as receptors and enzymes, can induce enhanced sperm agglutination, modulates acrosome reaction [17], in hibits metabolic processes of sperms and thus decreases their motility and ability to penetrate into oocyte [17]. The present results revealed a higher incidence of ASA in rams subjected to intensive semen collection either in blood or in seminal plasma. The presence of ASA was associated with an adverse effects on ejaculate volume, sperm count and sperm motility with adequate sperm agglutination.The same results were obtained by several authors.The incidence of ASA was found to be significantly higher in sexually active mature breeding bullsthan in the candidate breeders [1]. A higher levels of ASA was observed in rabbits due to excessive and long-term exploitation [6].

Also. it was reported that ASA can be induced primarily during infectious and non infectious inflammations, or by obstruction of testicular efferent passage ways [18].The ASA was also induced after accidental and/or surgical injury of testicles, cryptorchism and exposure to very low temperature [19].

Many authors have proved that semen quality was influenced by ejaculatory frequency. In horses, Pickett *et al.* [20] recorded a low semen quality associated with a higher ejaculation rate.Also, repeated semen ejaculations in man was reported to affects the density and volume of semen[21].Other studies have shown that frequency of semen collection is a primary factor affecting sperm output when semen is collected repeatedly from horses [22] and goats [23]. Motility, percentage of live spermatozoa and percentage of normal spermatozoa were not affected by frequency of semen collection. Similar observations have been reported for incattle [24] and sheep [25]. In accordance, the obtained results agree well with such findings where the life and normal sperm percentages were not affected by the intensive semen collection in rams. A higher variability in ejaculate volume and sperm concentrations has also been reported in samples collected fromAlpacas [11] and camels [26].

Concerning the effects of ASA on semen parameters,it was reported that binding of ASA to surface antigens of sperms can result in sperm agglutination [10],this was also detected in the present study in case of intensive semen collection, in hibition of metabolic processes, reduction of motility and decrease of sperm velocity. Authors attributed the production of ASA during repeated ejaculation to the disturbance in the functional stability of the hemotesticular barrier, represented by dysconnections of Sertoli cells projections that might be led to exposure of sperm cell antigen to the

own immune system with higher proliferation of B and T lymphocytes and the drastic increase in the secretion of interleukin-2 [27].

In the present study,vitamin c supplementation was not associated with ASA production in rams subjected to intensive semen collection and normalize the sperm parameters.In consistence, Sen Gupta *et al*. [28] found that vitamin c is vital for the maintenance of mammalian spermatogenesis and counteracts the testicular oxidative stress induced by pro-oxidant exposure.Also, Ping-Chi *et al*. [29]observed that supplementation with vitamin c prevent loss of sperm motility and increased the capacity of oocyte penetration in rats.It has been reported earlier that the role of vitamin c, as a nutritive antioxidant, has only been appreciated lately, where it can neutralize the free radicals and react directly with the peroxide radicals, in addition to its important antioxidant function of regenerating the reduced GSH [30].

Patra *et al*. [31] found that vitamin c ameliorates the induced oxidative stress in the testis facilitating the restoration of testicular tissue morphology and function by suppressing testicular lipid peroxidation and restoring the levels of Glutathione-s-transferase (GST) and reduced glutathione (GSH) to normal physiological levels.Vitamin c has also been reported to protect the sperm from loss of motility. Vitamin C inhibits lipid peroxidation and protects against DNA damage andhold the body's cell together. Additionally, the basement membrane lining the capillaries, the intracellular cement holding together the endothelial cells, all require the presence of vitamin C for their formation and maintenance [32]. Basically, vitamin c appeared to be an important element maintaining normal testicular morphology and function through its direct cellular protective effects keeping the soundness of testicular cellular structure and the stability of hemotesticular barrier or indirect through its powerful antioxidant action.

Conclusively, it is advisable to use vitamin c as a diatary supplement during intensive semen collection programs to minimize the production of ASA maintaining a higher fertility rate of rams.

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