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1-EFFECTS OF ANTIBODIES- ANTIGEN ON RABBITS FERTILITY

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ABSTRACT

The present study was carried out at Sakha Research Station , Kafr El – Sheikh Governorate, belonging to Animal Production Institute (APRI), Agricultural Research Center, Ministry of Agriculture from September to December (2005).

Eighty New Zealand White rabbits (70 does and 10 bucks) and 10 Californian bucks (CL) were randomly selected at 7 months old. Thirty were immunized with bovine serum albumin (BSA), classified later into high (HR) and low response (LR) responded groups. Ten does from each group were selected at random. They formed with the remaining un-immunized (40) does six equal groups. The Clor antibodies sera immunized with BSA to obtain immune sera (IS) or antibodies sera (Abs), used for NZW buck semen treatments. Such treatments were as follows: 1- Control un-immunized semen (Culm), 2-Semen + Abs, 3- Semen + Egg-Yolk-Tris extender (YET) + Abs, 4- Semen + (YET) + caffeine + Abs, and 5- Semen + YET). The HR and LR and LR groups were artifically inseminated with the control un-immunized semen. The four un-immunized does groups were subjected to A.I. using semen from treatments 2, 3, 4, 5, respectively. Insemination was conducted after Receptal injection for does to induce ovulation. Seventy-two hours after insemination does were dissected, and then fertilization rate and embryos numbers were detected.

Sperm motility percentage was significantly higher ($P<0.01$) for control un-immunized semen then semen having antibodies. Fertilization rate and total embryos number survival embryos were the greatest for un-immunized does inseminated with semen free of antibodies ($P<0.05$). caffeine addition to the immunized semen improved the fertilization rate and embryos survival compared with semen having antibodies.

It could be concluded that immunized rabbits with antigens resulted in some reproductive failures, may due to the block of binding of antibodies to these antigens, which inhibits gamete function and compromise fertilization. The significant increase of embryonic mortality in response Key words: Antibodies, antigen, sperm motility, Fertility, embryonic mortality, rabbits. To the in vitro immunization of rabbit semen was quit similar to the in vivo immunzation of rabbit does.

INTRODUCTION

Some cases of infertility considered have found to have immunologic basis (Virpillat italic, 1995). The spermatozoa of many animals are highly antigenic cells and when the state of immune tolerance is disrupted, auto-immunization in males or iso-immunization in females can occur. The possibility that such antibodies may adversely affect the fertility of the immunized animals should be considered. Chiu and Chamley (2002) postulated that the significant antibody-mediated infertility is still unclear and

complicated by the finding of anti-sperm antibodies in the fertile and infertile couples.

Undoubtedly, reproduction starts with unison of gametes contributed by bucks and does partners. The spermatozoa have antigens on their surface that are unique,tissue-specific immunogenic and accessible to antibodies. Binding of the antibodies to these antigens can inhibit the gametes function and compromise fertilization (Rajesh, 1996).

The present study aimed to declare the importance of the antigen-antibody reactions as a possible cause of infertility and / or sub-fertility in rabbits with special reference to the effect of treatment of buck semen with antibodies in this concept.

MATERIALS AND METHODS

The present study was carried out at Sakha Research Station , Kafr El – Sheikh Governorate, belonging to Animal Production Institute (APRI), Agricultural Research Center, Ministry of Agriculture from September to December (2005).

Eighty New Zealand white rabbits (NZW) (70 does body weighted on the average 3500 ± 0.20 g and 10 bucks with body weight of 3350 ± 0.120 g) in addition to, 10 Californian bucks all were selected at 7 months old. Thirty does were immunized with bovine serum albumin (BSA), in order to produce antibodies against (BSA) and were classified into two groups, high response groups (HR) and low response groups (LR). From each group 10 does were selected. Whenever, 10 does and 10 bucks were left without immunization and kept as control groups. Californian bucks were also immunized with BSA to obtain immune sera (IS) or antibodies sera (Ab_s S), for treated semen of NZW rabbit bucks.

Animal Hosing and Feeding :

Rabbits were separately housed in individual galvanized metal cages. Each rabbit received a concentrated commercial diet ad – libitum . The diet was contained 17% crude protein , 2.13% crude fat and 12.91% crude fiber. The ingredients were 34.50 Berseem hay, 12.40 Wheat bran, 17.50 Soybean meal (44%), 30.0 Barely, 3.0 Molasses, 1.0 Daicalsium phosphate, 0.70 Limestone, 0.34 Salt, 0.30 Premix[1], 0.06 DI-Methionine, 0.10 Antitoxins, 0.10 Anti fungal. Fresh water was available continuously via automatic nipples .

Immunization :

The BSA was obtained as a crystal lyophilized powder packags 10 g, Fluka Biochemika and Freund's complete adjuvant 10 ml , Sigma , it purchased through Chema. Science Co. Kafr EL-Sheikh Governorate.

Thirty does received 5 doses of 4 mg emulsified of BSA antigen , precipitated with 4mg Complete Freund's adjuvant (CFA) in a volume of 0.5ml .The first injection was given intravenously (i.v) and the others S.C. with 5 days intervals. Individual blood samples were obtained from the

marginal ear vein, 8 days after the last inoculation. The immunized rabbits were classified into high (HR) and low (LR) responded groups. Ten does from each group was selected at random. They formed with the remaining Un-immunized (40) NZW does six equal experimental groups. The cL bucks were immunized with BSA to obtain immune sera (IS) or antibodies sera (abs), which were used for immunization of NZW buck semen treat ment.

Antibody assay :

The individual serum antibody response measured by passive hemagglutination with rabbit erythrocytes coupled with BSA antigen . The coupling was made by treating the erythrocytes with glutaraldhyde according to the method of Avrameas italic .(1969) .

The titration was made in microplates 0.025 ml of doubling serum dilution in buffered sagroups was added to 0.025 ml of a suspension containing 10^8 sensitized erythrocytes / ml. The reaction was scored after 24 hr at 37^0C . The antibody titer was expressed as log 2 of the highest serum dilution given positive agglutination .

So, the responder does were selected and classified into the two groups: (HR) and (LR).

The CL bucks rabbits were inoculated BSA and 8 days after the last inoculation, the blood samples were collected from the marginal ear vein, cinterfugated at 3000 rpm for 15 minutes after blood clotting to obtain immune sera (IS), which was incubated for 30 minutes at 37^0C to get ride of the complement and well mixed with serum from unimmunized bucks .

Preparation of semen and extender :

Semen was collected individually from the 10 NZW bucks by means of artificial vagina to determine the initial motility to be used in artificial insemination (AI). Only ejaculates had more than 70 % initial motility, were used.

Semen extender :

According to many competitive studies have adopted for A.T in rabbits using different citrate based extenders (Miller italic,1969 and Shavalova ,1975) and it was the best extender used by (Khalifa italic , 2002).

In addition, the beneficial effect of caffeine (El – Menoufy italic,1986 , Fattouh and Abdou , 1991 and El–Kalawy,2002) and its inhibitory action on phosphodiestrase enzyme with consequent accumulation of cyclic nucleotides (especially cAMP) within the sperm cells (Tash and Means, 1982), therefore, the selected extender consisted of 1.5 g citric acid, 1.25 g fructose, 3.028 g tris (hydroxyl methyl amino methane) , 20 ml. egg yolk and was completed with distilled water to 100 ml. Caffeine was added at the concentration of 3mM/ml semen. One ml of antibiotic containing 30.000 IU, streptopencid 10.000 IU, penicillin G , 50 mg streptomycin sulfate was added .

Semen with immune sera (IS)in vitro

Some of the pooled semen was mixed with the immune sera (IS) and some of it was left untreated with (IS). Semen was treated with antibodies according to Kiddy italic., (1985) with some modifications . The fresh rabbit semen was diluted into sperm concentration of approximately 20 $x10^6$ sperms per ml. Aliguat of 0.05 ml of the diluted semen was added to 0.20 ml of serum in 10 x 75 mm test tubes and mixed thoroughly. The tubes were incubated in a water bath at 37^0 C.

Rabbit insemination :

The semen ejaculates were diluted with the extender at rate of 1 : 4 (semen: extender) to contain about 10 millions motile spermatozoa / ml. In the same time, one dose of 10 ml. The ml of receptal (synthetic GnRH was injected per doe before 10-13 hours of artificial insemination in order to stimulate the ovulation according to (Hafez 1970 , and Adams 1972).

The two immunized does groups (HR and LR) were artificially inseminated with untreated semen (semen + extender) mean while the four unimmunized doe groups were inseminated artificially with: semen + antibodies, 2- semen + extender + antibodies, 3- Semen + extender + caffeine + antibodies, 4- Semen + extender, respectively.

Embryos detection :

The inseminated does were scarified and dissected after 72 hours from insemination and then fertilization rate and survival number of embryos were detected.

Statistical analysis :

Data were statistically analyzed according to SPSS program (SPSS for windows, computer program., 1993). Snedecor and Cochran (1967) Percentage values were transformed to arcsine values before analyzed . Dancan's Multiple Range tests , (Duncan , 1955) was used for testing significance of the differences between the means .

RESULTS AND DISCUSSION

Results of immunization :

Table (1) illustrates that mean values of antibody titers against BSA for doe rabbits in primary immunization decreased from 6.50 after 7 days post immunization to 1.2 after 21 days in high responded group (HR) versus 2 . 88 to 0.60 in low responded group (LR), respectively.

Table (1): **Primary and secondary antibody titers against BSA of high and low responded NZW does after 7,14 and 21 days post immunization.**

Days after Immunization	No. of Does	Primary titers		Secondary titers	
		HR	LR	HR	LR
7	10	6.50	2.88	9.63	3.12
14	10	5.29	1.17	6.24	1.44
21	10	1.20	0.60	2.40	0.78

The corresponding values for the secondary antibodies titers decreased from 9.63 to 2.40 in HR group versus 3.12 to 0.78 in (LR) group, respectively. It is worthy mentioning that the average primary antibodies titer against BSA for the Californian bucks was 4.80 after 7 days postimmunization.

These finding were in agreement with the results reported by Sant italic. (1983) who demonstrated that the differences between, high and low responded groups were respected to the level of humoral immune response and as a resulting of cell mediated immune response (CMI) which BSA as antigen stimulates differentiation of both B and T cells into plasma cells and memory cells.

Also , Ferreira italic. (1986), Wilkie and Mallard. (1999). Eyal Klipper italic. (2000) and Chen-Fusheng italic. (2002) they emphasized that BSA induced a large modification in responsiveness in the high and low responded groups at the selection limit .

Examination of semen motility

Table (2) shows that the percentage of sperm motility was (72.8+3.7%) significantly higher ($p < 0.01$) in the normal semen than semen treated with antibodies (40.8 ± 3.4). This difference may be due to the effect of antibodies as a block to the sperm movement based upon the degree of agglutination either head – head or tail – tail, which was lower (35.7%) in the normal semen than semen treated with antibodies (79.3%).

Table (2):Properties normal untreated and antibodies-treated (immunized) NZW buck semen.

Semen treatment	No. of Bucks	Agglutination type, % (head-head vs. tail-tail)	Motility
Normal, (T1)	10	35.7	72.8 ±3.7
Immunized, (T2)	10	79.3	40.8 ±3.4
T1 - T2	-	-43.6*	32.*

* The differences were significant, (P <0.05).

These results are in coincidence with findings of Smith italic (1984) and Kiddy italic., (1985) who reported that sperm agglutination head specific and tail specific antigens of rabbit sperms are most important in stimulating the production of specific agglutinins and has the ability to block the sperm movement and reduce the sperm motility percentage.

Fertilization rate and Embryo conditions :

Table (3) represents the average of fertilization rate and embryo conditions (survival or death). In this respect, there were significantly differences ($p< 0.05$) between the treat immunized and control groups. The fertilization rate of the HR and LR NZW does, inseminated with the NZW normal semen was 60 and 65% and percentage of survive embryos was 60% (3/5) and 66.7% (4/6), respectively. The differences were mot significant ($x2 = 0.614$). The fertilization reates for the unimmernized NZW does were 10.0,

35.0, 80.0 and 90.0%, when the does were artificially inseminated anted with NZW semen treated with either antibodies, EYT extender + antibodies, EYT. Extender + caffeine + antibodies or EYT extender, respectively, (Table 3). Percentage of Survival embryos was 0.0, 40.0, 83.3 and 85.7% and total number of embryos was 1, 5, 6 and 7, respectively , Table 3.

Table (3): **Fertilization rate and number of embryos of NZW does (10 does/group) artificially inseminated with different NZW treated semen.**

Semen treatments	Does group	Fertilization rate, %	NO. Embryos		
			Survive		Total
			NO.	%	
Untreated semen	HR	60	3^{c}	60.0	5
Untreated semen	LR	65	4^{c}	66.7	6
Semen + Abs	UID	10	0	0.0	1
Semen + Ext + Abs	UID	35	2^{c}	40.0	5
Semen + Ext +C+ Abs	UID	80	5^{b}	83.3	6
Semen + Ext	UID	90	6^{a}	85.7	7

$^{a, b, c}$ **Values in the same column with different superscripts significantly differ (P<0.05)**

Ext.: Semen was extended with egg yolk tris extender at rate 1:4.

Abs: Antibodies, NSE: normal untreated semen

C: caffeine UID: un-immunzed does

It cleary appears that unimmunized NZW does inseminated with semen + EYT extender with caffeine + antibodies had higher fertilization rate (80%) and lower of embryonic mortality (16.7%) composed to the same treatment but free of caffeine (35%fertilization rate and 60% mortality). This means that addition of caffeine had significant beneficial effect on fertilization rate and embryos survival $x2= 22.06$ ($P<0.01$). This may be due to stimulate on sperm motility and acts as inhibitor of phosphodiestrase enzyme, with a consequent accumulation of cyclic nucleotides (e.g. cAMP) within the sperm cells (Tash and Means , 1982). Semen treatment with (antibodies sera), caused a decrease in the fertilizing ability of sperm due to the agglutination of the added antibodies, which may prevent fertilization. Insemination with control semen + EYT gave the greatest fertilization rate (90%) and the least embryonic mortality rate (14.3%).

These results are in coincidence with that of Kiddy *et al* .,(1984) that the agglutination of washed rabbit sperm in a potent antisera was significantly reduced, when the sperms were suspended in vaginal washings from normal female rabbits. Smith *et al.*(1984) declared that this phenomenon may result from the presence of antigens in the vagina with specificities in common with some or all of the sperm.

The obtained results indicated that the treatments of rabbit semen (in vitro) with IS caused a significant increase in the embryonic death approximately such as that observed for in vivo treated doe rabbits (which) may be due to the mechanism by which antibodies acting against some antigens. It may also result from mutations induced in the sperm and or ova

survive. Antigen has in general molecular configurations in common with the gene that cause it to be produced (Kiddy italic ., 1985 ; Smith italic., ., 1984)

Studying the effect of genes controlling antibodies by using PCR technique and gene mutation should attract the attention of investigators.

It could be concluded that rabbits immunized by antigens would cause some reproductive failures which may be due to the block of antibodies and genes controlling it. Such reproductive failures and the greater embryonic death could be reduced by using AI technique with extender containing Egg yolk tris with caffeine.

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١ – تأثير المستضدات والاجسام المضادة على الخصوبة فى الارانب

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ستخدم فى هذه الدراسة عدد تسعون أرنب (٧٠ أنثى ، ١٠ ذكور من نوع الارنب النيوزيلندى الابيض ، ١٠ ذكور من نوع الكاليفورنيا) وتم تقسيم الاناث الى خمسة مجاميع بالاضافة الى المجموعة المقارنة وقد تم تحصين عدد ٣٠ أنثى بمصل البيومين البقر وانتخب منهم المجموعة الاولى عالية المناعة والثانية منخفضة المناعة. وايضا تم تحصين ١٠ ذكور الكاليفورنيا بمصل البيومين البقر للحصول على الاجسام المضادة التى يتم استخدامها فى معاملة السائل المنوى للارانب من النوع النيوزيلندى الابيض. كانت المعاملات للسائل المنوى هى (سائل منوى + أجسام مضادة) ، (سائل منوى + مخفف ترس + أجسام مضادة) ، (سائل منوى + مخفف ترس بالكافين + أجسام مضادة) ، (سائل منوى + مخفف ترس). تم تجميع السائل المنوى من ١٠ ذكور النوع النيوزيلندى الابيض مع تقدير الحيوية المبدئية لاستخدامها فى التلقيح الصناعى حيث تم استخدام القذفات التى حققت نسبة ٧٠% حيوية مبدئية ثم ذبحت الاناث بعد ٧٢ ساعة من التلقيح الصناعى لمعرفة معدل الخصوبة وعدد الاجنة. واوضحت النتائج ان الحركة الجماعية كانت أعلى بدرجة معنوية ($P < 0.01$) فى السائل المنوى العادى (الكنترول) عن السائل المنوى المعامل بالاجسام المضادة وكانت معدل الخصوبة وعدد الاجنة الحية أعلى بدرجة معنوية ($P < 0.05$) فى المجموعة الكنترول عن المجاميع المعاملة.

أوضحت الدراسة ان تأثير معاملة السائل المنوى بالاجسام المضادة فى المعمل كانت مثل تأثير الاجسام المضادة المتكونة داخل الجسم على السائل المنوى فى كلا الحالتين كان لها تأثير معنوى على خفض الخصوبة وزيادة موت الاجنة. وللتغلب على تلك المشكلة يمكن استخدام التلقيح الصناعى بمخفف ترس المحتوى على كافين بتركيز ٣ ملى كافين/ مللى سائل منوى.