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Studies on Antigenicities of Sperm and Seminal Plasma, and Effects of Their Antibodies on Fertilization in Rabbit

Ⅱ. Effects of isoantibodies on rate of superovulation and fertilization

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家兎에 있어서 精子 및 精漿의 抗原性과 이의 抗體가 受精에 미치는 影響

Ⅱ. 抗體가 過排卵 및 受精率에 미치는 影響

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적 요

정자와 정장의 항체가 정상 및 면역가토의 과배란과 수정율에 미치는 영향을 알고자 시도된 실험결과는 다음과 같다.

1. 면역가토에서의 과배란처리에 따른 평균배란집은 정자와 정장처리구에서 각각 22.9와 25.3개로서 대조구의 41개보다 현저히 적었다($P<0.05$).
2. 자궁무게와 난포크기는 처리구간에 유의적 차이가 없었으며 그러나 정자 및 정장면역 가토에서의 수정율은 각각 62.8%와 58.0%로서 대조구의 91.4%보다 현저히 낮았다. ($P<0.05$).
3. 가토정액을 정자 또는 정장항혈청과 혼합하여 인공수정시 수정율이 각각 5.6%와 16.0%로서 대조구보다 현저히 낮았다($P<0.05$).

결론적으로 정자 또는 정장면역이 수정율에 현저하게 영향하는 것으로 나타났다.

Introduction

Many of earlier investigators have shown that the decrease in fertility resulted from immunization with sperm or testicular materials into experimental animals and the role of sperm antigens had been reviewed numerous times (Tyler, 1961; Behrman and Menge, 1973; Beer and Billingham, 1976; Metz, 1979). It has been demonstrated that the isoimmunization of guinea pig (Katsh, 1959), mice (Edwards, 1964), rabbit (Menge, 1970, 1971), cattle (Menge, 1967) and man (Jones et al., 1973) reduces male or female fertility. Also, recent studies on *in vitro* fertilization have indicated that the autoantigens specific to sperm are involved in essentially all steps of prefertilization and fertilization (Tung, 1983).

O'Rand and Metz (1976) and O'Rand (1977) indicated that the intrinsic plasma membrane antigens of sperm are important in antifertility action and an extract from sperm membrane proteins reduces significantly fertility in the isoimmunized female rabbits. Metz (1979) demostrated that the antihyaluronidase antibodies were clearly shown to inhibit cumulus dispersion of eggs and fertilization *in vitro* in the rabbits. A similar failure to reduce *in vitro*

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fertilization rates had also reported in the immunized sheep by Morton (1977). Goldberg (1974) reported that the female rabbits immunized with sperm enzyme LDH-X showed a 67% reduction in fertility compared to the non-immunized rabbits and binding of anti-LDH-X to sperm surface was presumably responsible for the reduced fertility *in vitro*. On the other hand, Katsh (1959), Menge (1970) and Metz et al. (1972) reported that the iso-and auto-antigens of seminal plasma appeared to have no significant effect on fertilization. Russo and Metz (1974) stated that in laboratory antimals the antibodies to seminal plasma did not immobilize sperm and reduced fertility. However, in human Isogima et al. (1974) demonstrated that the antibodies against seminal components occurred in sera of a number of sterile women and these reports imply that the presence of antibody within female genital tract is very important and the antigen sources from sperm are model antigen for infertility. Secretory IgA and IgG class antibodies against the rabbit sperm were isolated from uterine fluid by Menge et al. (1974) and this antibody was capable of inhibiting *in vivo* and *in vitro* fertilization. Goldberg (1974) reported that the antibodies against rabbit sperm specific LDH-X have been detected in uterine fluid. Bratanov (1972) indicated in the cattle with the sperm isoantibody titers of above 1:512 in serum, the antibodies as a cause of immune infertility were detected in uterine secretions. Beer and Billingham (1976) demonstrated that the specific intrauterine sensitization to cellular antigens in an inbred rodent line led to increased the litter size when the litter was sired by the males in the line used for sensitization. Almlid (1981) reported that the nonspecific cellular antigens added to semen at the time of antificial insemination were of value in increasing litter size in gilts and Murray et al. (1983) also found that the reproductive efficiency in gilts was improved by intrauterine treatment with boar semen before mating. Menge (1970) stated that the sera from female mammals isoimmunized with sperm have been shown to agglutinate sperm, kill or immobilize them in the presence of complement compounds. McLaren (1964) found that the reduction of fertilization rate in the sperm-immunized female was apparently caused by the failure of sperm to reach the site of fertilization. Metz and Anika (1970) reported that the hetero antibody fractions could prevent the inseminated sperm from passing cervix. Waldman and Ganguly (1974) claimed that the antibodies inhibiting sperm migration present in cervix-vaginal secretions. Killiam and Amann (1973) observed the sperm-precipitating antibodies in cervical secretions and serum of intracervically immunized cow with frozen sperm. Menge and Protzman (1967) also reported that the addition of antisperm or antitestis serum to the rabbit sperm before antificially inseminating them into the female decreased fertility, while the antisemen serum agglutinated sperm but did not reduce their fertility. Erickson et al. (1975) found that the rabbit antiserum to mouse LDH-X did reduce in the *in vitro* fertilization rate of mice. Okano et al. (1978) reported that the ova collected from the rabbits inseminated with semen mixed in dilution of sperm isoantiserum turned out to be unfertilized, while 50% rabbits inseminated with seminal plasma isoantiserum had normal fertilized ova and the rest had unfertilized ova. Moore (1981) demonstrated that preincubating rabbit epididymal sperm with antiserum to specific glycoprotein resulted in a significant reduction in their fertilizing ability compared

with the control incubated with preimmune serum. Tung (1983) suggested that the antisperm antiserum in guinea pig can prevent cumulus dispersion of eggs, block capacitation of sperm, and interfere with the attachment of sperm to zona pellucida.

This experiment was conducted to invistizate the effects of sperm and seminal plasma isoantibodies on rate of superovulation and fertilization in immunized rabbits and the fertilization rates of rabbits antificially inseminated with semen mixed on dilution of sperm and seminal plasma isoantiserum.

Materials and Methods

1. Animals

Twenty-six nulliparous New Zealand White rabbits weighing 3.0 to 3.5 kg were used in this experiment. Ten immunized and 4 normal does of them were used to compare the response of superovulation and rate of fertilization between the immunized and control does. The rest normal does were used to investigate the effect of antificial insemination with semen-antiserum mixture on the rate of fertilization.

2. Preparation of antigens and immunization

Semen collected with the aid of an artificial vagina were pooled. The seminal plasma was removed by refrigerated centrifugation at 10,000 rpm for 30 min and the sperm were sonicated five times by an intermittent sonification and by a cell ultrasonic homogenizer. For preparation of antigens the sperm and seminal plasma was diluted with PBS, respectively and then mixed with Freund's complete adjuvant for the first two injections and with Freund's incomplete adjuvant for over third injections. Immunization did according to the same methods with the previous report. Antigen dilution was administrated in at least 10 intradermal sites on each animal's back.

3. Superovulation

At 2 months after immunization, the spperovulation for immunized and normal does was induced by subcutaneous injection of 40 IU per day of PMSG for 5 days and 0.1 mg of estrodiol-17β was intramuscularly administrated together with the last injection of PMSG. After 48hr the does were mated twice at 5 min intervals with 2 different bucks and injected immediately with 300 IU of HCG intravenously.

4. Artificial insemination

The pooled semen of fertile bucks was mixed about 50 million sperm per ml with the antiserum against sperm or seminal plasma; the titer of which was 1:256 and 1:512, respectively. All does were inseminated intravaginally with 0.5 to 0.8 ml semen-antiserum mixture. They were mated twice at 5 min intervals with a vasectomized buck just after AI.

5. Ova collection and fertilization evaluation

Normal or superovulated does were slaughtered 48hr after AI or natural mating. The oviduct and uterus was removed from the reproductive tract and then was flushed with 5 ml saline for ova collection. The recovery rate of ova were calculated from the ratio of ovulation points and number of ova recovered. The fertilization was judged to have occurred if two or more approximately symmetrical cleavage showed under a stereoscopic microscopy.

Results

1. Superovulation and fertilization in immunized rabbits

Tables 1 and 2 show the response of superovulation, collection rates of ova and changes of ovaries due to the administration of PMSG and HCG into the sperm-and seminal plasma-treated animals. The mean values of ovulation points in the treated groups were significantly lower with 22.9 and 25.3 for sperm and seminal plasma, compared with 41.0 of the control animals ($P<0.05$). The mean numbers of ova collected were very lower with 20.0 and 18.8 for sperm and seminal plasma groups, respectively, compared with 35.0 for the control group, while the recovery rates of ova were a little lower for the immunized groups than 84.9% for the control group without any significant difference. The ovaries removed from both of the immunized groups were lighter in weight when compared with the control group. The results, however, did not show any significant differences between them. The average number of normal follicles in size had no difference among the groups, whereas haemorrhagic follicles with more than 2mm in diameter varied individually among the three groups. As a result, the ovaries and follicles didn't have any differences in weight and number between the treated and control animals. The recovery rates of ova by superovulation for the immunized rabbits turned out to be 71 to 85%, which was low compared with the normal rabbits.

Table 3 explains the fertilized numbers and the degree of cleavage examined in the immunized groups slaughtered 48hr after coitus. The mean rate of fertilization in the sperm-treated animals was 62.8%. Two of the 6 immunized animals had no fertilized ova, whereas a rabbit showed 100% fertilized ova. The mean of fertilization rates in the seminal plasma-treated animals was 58.0%, and one of the 4 animals and 100% fertilized ova; on the other hand, a rabbit had only 4% fertilized ova. These fertilization rates, therefore, were significantly

Table 1. Effects of superovulation on number of ovulation points and recovery rate of ova in immunized rabbits

Immunization	Replication	No. of ovulation points (A)	No. of ova recovered (B)	Recovery rate of ova (B/A, %)
Control	4	*41.0±2.9[a]	35.0±3.5[a]	84.9± 3.2
Sperm	6	22.9±5.8[b]	20.0±5.5[b]	70.9± 4.9
Seminal plasma	4	25.3±4.6[b]	18.8±5.0[b]	73.3±11.6

*Values are mean±SE.

a, b Means with a different superscript within each column differ ($P<0.05$).

Table 2. Effects of superovulation on ovarian weight and number of visible follicles in immunized does

Immunization	Replication	Ovarian weight (R+L) (g)	No. of follicles (>2mm in diamter)	
			Normal	Hemorrhagic
Control	4	*1.5±0.3	5.3±2.1	14.8±1.9
Sperm	6	1.3±0.2	4.7±1.4	10.2±3.4
Seminal plasma	4	2.0±0.2	11.4±4.0	14.5±3.2

*Values are mean±SE.

Table 3. Effects of immunization on fertilization rate in superovulated does

Immunization	Doe No.	No. of recovered ova	No. of unfertilized and abnormal ova	No. of fertilized ova						Fertilization rate (%)
				4-cell	8-cell	16-cell	32-cell	Morula	Total	
Control	1	37	3	6	7	–	1	–	34	88.8
	2	43	10	–	–	–	3	30	33	76.7
	3	35	–	3	21	1	1	–	35	100
	4	35	–	4	15	13	3	–	35	100
	Total or mean	150	13	13	63	14	8	30	128	*91.4±2.8[a]
Sperm	1	2	–	–	1	1	–	–	2	100
	2	40	16	1	9	8	6	–	24	60.0
	3	23	12	–	7	4	–	–	11	47.8
	4	12	4	3	3	2	–	–	9	69.2
	5	16	16	–	–	–	–	–	–	0
	6	9	–	–	3	1	5	–	9	0
	Total or mean	103	48	4	24	16	11	–	55	62.8±15.3[b]
Seminal plasma	1	25	24	–	–	–	1	–	1	4.0
	2	29	16	–	–	–	2	11	13	44.8
	3	8	–	–	3	–	5	–	8	100
	4	12	2	–	–	–	6	4	10	83.3
	Total or mean	74	42	–	3	–	14	15	32	58.0±21.4[b]

*Values are mean±SE.

a, b Means with a different superscript differ ($P<0.05$)

low compared with 91.4% of the control group ($P<0.05$). There was, however, no sgnificant difference between the immunized animals. According to the results, the effect of superovulation in the immunized animals appeared 56 to 60% of that for the control group. It was impossible through this experiment to decide whether the causes for reduction of superovulation in the immunized groups were due to immunization of sperm and seminal plasma, or due to subcutaneous injections of them for a long time.

2. Fertilization in normal rabbits artificially inseminated with semen-antiserum mixture

Tables 4, 5 and 6, show the recovery rates of ova, appearance of ovaries and fertilization rates in the normal rabbits artificially inseminated with the semen diluted in sperm or seminal plasma antisera. The mean number of ovulation points and recovery rates of ova were 9.6 and 90.9%, respectively, in the experimental groups. The recovery rate of ova increased by 7% compared with that of the superovulated animals. The average weight of left and right ovaries was 0.49 gm, being 32% of that of the superovulated rabbits. For the control group inseminated with a 1:1 dilution of semen and normal rabbit serum, the fertilization rate was 100%, while for the rabbits inseminated with the same dilution of semen and sperm antiserum, one of the four rabbits produced 12.5% fertilization rate and the rest had all unfertilized ova. In case of the rabbits inseminated with the same

Table 4. Ovulation points and recovery rates of ova in normal does artificially inseminated

Treatment	Replication	No. of ovulation points (A)	No. of recovered ova (B)	Recovery rate of ova (B/A, %)
Control	4	*9.8±1.1	8.8±0.8	91.0±5.9
Sperm antiserum	4	10.3±1.0	7.8±0.5	88.6±4.6
Seminal plasma antiserum	4	10.3±1.0	9.5±0.8	93.1±2.4
Mean		9.6±0.8	8.7±0.8	90.0±4.2

*Values are mean±SE.

Table 5. Ovarian weights and numbers of visible follicles in normal does artificially inseminated with sperm or seminal plasma antisera

Treatment	Replication	Overian weight (R+L) (g)	No. of follicles (>2mm in diameter)	
			Normal	Hemorrhagic
Control	4	*0.47±0.1	6	2
Sperm antiserum	4	0.54±0.1	0	3
Seminal plasma antiserum	4	0.47±0.1	5	5

*Values are mean±SE.

Table 6. Fertilization rates in does artificially inseminated with a mixture of semen and sperm or seminal plasma antisera

Mixture	Replication	No. of recovered ova	No. of unfertilized and abnormal ova (A)	No. of fertilized ova (B)	Fertilizztion rate (B/A, %)
Semen+control serum	4	*8.8±0.9	0	8.8±0.9	100[a]
Semen+sperm antiserum	4	7.8±0.5	7.5±0.5	0.3±0.3	5.6±2.6[b]
Semen+seminal plasma antiserum	4	9.5±0.9	8.0±1.1	1.5±0.7	16.0±7.4[c]

*Values are mean±SE.

a, b, c Means with a different superscript differ ($P<0.05$).

dilution of semen or seminal plasma antiserm, one of the four rabbits had unfertilized ova and the rest had a range of 8.4 to 33.3% fertilization rates. The mean rates of fertilization between the rabbits inseminated with the semen diluted in antisera showed significant difference between two groups, the fertilization rates being lower in sperm antiserum ($P<0.05$).

Discussion

The results of reduction of fertilization rates into 62.8% and 58.0% for both immunized groups when compared to the control group coincided well with earlier those obtained after immunization of sperm and testical materials (Menge, 1967, 1970, 1971; Edwards,

1964) as well as the reduction in fertility following autommunization with sperm (Behrman and Menge, 1973). The fertilization rates obtained in the rabbits immunized with seminal plasma were not very different from those reported by Katsh (1959) and Metz et al. (1972), but made a difference in the point that antibodies to seminal plasma do not reduce fertility (Russo and Metz, 1974; Menge, 1970). It seems that such a reduction in fertility in the immunized animals was caused by the failure of sperm to reach the site of fertilization because of the immobilization and death of spermatozoa, or agglutination. The possibility that these results may have been attributed to the happening of immobilization and agglutination of sperm within the female tract is based on the reports by Symons and Herbert (1971) and Menge et al. (1974), in which they confirmed the existence of antibodies within the tract.

In addition, Bratanov (1972) found that a few sterile bovine with antibodies in high titers also had antibodies present in the uterine secretions. And Waldman and Ganguly (1974) reported that the antibodies responsible for interference with sperm transport appeared in the cervico-vaginal secretions. Almlid (1981) and Murray et al. (1983) were able to bring up the increase in litter size by the removal of antibodies in the female tract.

These results indicate that the dilution of semen in an immune serum induces a remarkable reduction in fertilization rates as reported by Menge and Protzman (1967) about decreased fertility resulting from the injection of seminal plasma. Low fertilization rates in the inseminated animals, especially with sperm antiserum treated semen strongly support an earlier report by Thibault and Dauzier (1960) about the lack of fertilizing ability of rabbit spermatozoa after being agglutinated and then free.

According to Vaerman and Ferin (1974), available data clearly favour the presence of IgA, IgG and IgM plasma cells in the tissues of the human cervix, and the local production of IgA there. Antibodies that have been detected in the cervical secretions include sperm immobilizing forms. The evidence for such immunocompetence in the endometrium and oviduct mucosa is really no better than suggestive at the present time. The morphology of the cervical mucosa was such as would be appropriate for secretary IgA production; the uterine endometrium was less so, while to vaginal mucosa looked an unlikely milieu, because it lacked glands and its epithelium was stratified instead of columnar.

The development of an immune state within the female tract may not be based only upon the local production of antibody but can derive from systemic antibody. Such a transfer from the circulation to the female tract would depend upon permeability to serum proteins, which in general is very low but varies with stage of cycle. The higher levels of IgA, IgG, and IgM noted by Chandra et al. (1974) in women wearing an intrauterine contraceptive device were presumable owing to increased tissue permeability. Interesting alternative routes could be via the peritoneal fluid (Sokolovskaya and Reshetnikova, 1969) and the follicular fluid (Edwards 1974), both of which have quite high levels of immunoglobulins. From these sources antibodies can pass directly into the oviduct and thus exert influence at the site of fertilization.

The agglutination of spermatozoa appears to be directly blocked by substances in female tract secretions. Smith (1949) found that the addition of vaginal washings from normal oes-

trus rabbits inhibited the sperm agglutination otherwise produced by relatively powerful antisera. Spermatozoa removed from such media by centrifugation and suspended in a fresh artificial diluent were found to agglutinate as well as those suspended freshly.

Summary

Effects of sperm and seminal plasma isoantibodies upon the rate of superovulation and fertilization were studied in both normal and immunized rabbits. The results obstained were summarized as follows:

1. On examination of the superovulation in the immunized animals, the average number of ovulation points was 22.1 and 25.3 for sperm-treated animals and for seminal plasma-treated animals, respectively. As compared to the control group of 41.0 in number, the immunized groups showed statistical significance in ovulation ($P<0.05$).

2. In ovary weight and follicle's size there were no significant differences among the three groups, whereas sperm-and seminal plasma-treated groups had an average rate of fertilization of 62.8% and 58.0%, respectively, in remarkable contrast to the control group of 91.4% ($P<0.05$).

3. When the animals were inseminated with a mixture of semen plus sperm or seminal plasma antisera, a sharp reduction of fertilization was observed with 5.6% and 16.0% as compared to the control group ($P<0.05$). Consequently, immunization with either sperm or seminal plasma had a substantial effect on fertilization.

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