Reproduction in rats and mice isoimmunized with homogenates of ovary or testis with epididymis, or sperm suspensions

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Summary. Female rats and mice were injected with homologous spermatozoa without adjuvant, or with homogenates of ovary or testis+epididymis in adjuvant. Ovulation was not inhibited and the numbers of eggs released were normal. Fertilization rates were significantly reduced in the mouse by all tissues injected and in the rat by injection of ovarian homogenate only. Fracture of and a weak precipitate on the zona pellucida of the eggs were observed in the mouse but not in the rat after injection of ovarian homogenates. The number of pregnancies and litter sizes were reduced following the first and second mating after treatment in the mouse but not in the rat. When eggs with or without follicular cells were examined *in vitro*, fertilizability was very low if the donor had been treated with ovarian homogenate, better with testis+epididymis homogenate and best after treatment with sperm suspension. Failure of fertilization appeared to be the major cause of infertility after such treatments.

Introduction

Injection of females with homologous spermatozoa or testis homogenates reduces fertility in rabbits (Behrman & Nakayama, 1965; Menge, 1968; Bell, 1969a), guinea-pigs (Isojima, Graham & Graham, 1959; Katsh, 1959; Behrman, Otani, Porter & Nakayama, 1963) and mice (Edwards, 1964; McLaren, 1964, 1966; Bell, 1969b; Bell & McLaren, 1970; Erickson, 1973). In contrast to the numerous studies of the antigenic properties of testis and spermatozoa, relatively few studies have been made on the antigenic composition of ovaries and eggs (Shivers, 1974). This study was undertaken to examine whether the fertility of female rats and mice is affected by injection of homologous spermatozoa or of homogenates of the ovary or testis+epididymis, and whether the effect is on ovulation, fertilization or pregnancy.

Materials and Methods

Mature female rats (190–250 g) were of the CD strain from Sprague–Dawley stock and female albino Swiss mice (23–30 g) were of the CD-1 strain.

Mouse spermatozoa were recovered from the cauda epididymidis of mature mice and suspended in warm phosphate-buffered saline (PBS). For immunization, 0.5 ml sperm suspension ($2.5\text{--}16.0 \times 10^6$ cells) was injected i.p. and each female received 3 injections/week for 7 weeks (20 injections). Control animals received PBS only.

Mouse testis+epididymis in cold PBS (1:1) was homogenized and the homogenate centrifuged at 8500 g for 15 min. The supernatant fluid was diluted to a protein concentration of 40 mg/ml and was emulsified with an equal volume of Freund's complete adjuvant (FCA). The emulsion (1 ml) was injected subscapularly and the second and third injections were given 14 and 36 days later. Thirteen to 15 days after the third injection, three booster injections consisting of 0.5 ml supernatant fluid (no adjuvant; protein concentration: 19.6 mg/ml) were given every other day. The concentration of

protein was determined by the method of Gornall, Bardawill & David (1949). Control animals received PBS+FCA or PBS alone.

The homogenates of rat and mouse ovaries and rat testis+epididymis were prepared in the same way but the supernatant fluid was diluted to a protein concentration of 26.5–30 mg/ml, 8.4–19.8 mg/ml and 30–50 mg/ml, respectively. The injection procedures were similar except that 0.5 ml was the volume injected for mouse ovary homogenates. The second and third injections were given 12–26 days and 14–25 days later. The three booster injections were given 14–29 days after the third injection.

Vaginal smears were examined daily. Rats were usually paired 13–34 days after the last injection and mice 10–26 days. After parturition, or at least 22 days after the first mating, most females were mated again. Some females were killed 1 or 2 days after mating and eggs recovered from the oviducts to examine fertilization. Some mice were induced to superovulate by PMSG and HCG treatment; the eggs were recovered and tested for fertilization *in vitro* (Tsunoda & Chang, 1975a, b).

Results

In the rat, only the ovarian homogenate affected the number of pregnancies and the number of offspring but the reduction was not significant (Table 1). All the treatments affected conception and litter size in mice (Table 1) but some recovery occurred at the second pregnancy.

Table 1. Effect on pregnancy and litter size of rats and mice injected with homogenates of testis+epididymis or ovary (with Freund's complete adjuvant: FCA) or with sperm suspensions

Injection	First pregnancy			Second pregnancy		
	No. of females mated	No. pregnant (%)	Litter size (Mean $\pm$ S.D.)	No. of females mated	No. pregnant (%)	Litter size (Mean $\pm$ S.D.)
Rat						
Testis + epididymis	11	11 (100)	11.2 $\pm$ 5.1	8	7 (88)	11.5 $\pm$ 5.6
Ovary	7	5 (71)	8.4 $\pm$ 6.6	5	4 (80)	9.2 $\pm$ 8.0
Saline (+ FCA)	5	5 (100)	13.2 $\pm$ 3.6	4	4 (100)	12.5 $\pm$ 1.9
Mouse						
Sperm suspension	16	2 (13)**	1.4 $\pm$ 4.0**	15	10 (67)	3.7 $\pm$ 4.2*
Saline	8	6 (75)	7.4 $\pm$ 4.9	7	6 (86)	6.1 $\pm$ 4.1
Testis + epididymis	13	8 (62)	4.1 $\pm$ 4.9*	13	7 (54)	4.3 $\pm$ 5.1*
Ovary	8	1† (13)**	0.9 $\pm$ 2.5**	5	1† (20)**	1.4 $\pm$ 3.1**
Saline (+ FCA)	8	7 (88)	7.3 $\pm$ 4.1	8	7 (88)	7.8 $\pm$ 4.0

Significantly different from controls (Student's *t* test): **P*<0.05; ***P*<0.001.

† Same mouse.

The zona pellucida of many of the unfertilized eggs recovered from mice treated with ovary homogenates (Table 2) was often broken and a weak precipitate was observed on the outer surface.

None of the treatments had any effect on the response of mice induced to superovulate. An average of 12–26 eggs was produced/treatment group (range 5–39/female, *N*=36). Some of the eggs had a broken zona pellucida.

The number of eggs with cumulus cells that were fertilized *in vitro* was reduced by all three treatments (Table 3). Nearly all the eggs recovered from the ovary-treated mice had precipitate on the zona; those from the only fertile female treated with ovary homogenate did not have a precipitate but the number fertilized was slightly decreased. After removal of follicular cells, fertilization was low in the control eggs and was not reduced further by injection of sperm suspensions.

Table 2. The effect on fertilization rate of eggs from rats and mice injected with homogenates of testis+epididymis or ovary (with Freund's complete adjuvant: FCA) or with sperm suspensions

Injection	No. of females mated	No. of females with fertilized eggs (%)	No. of eggs recovered	No. of eggs fertilized (%)
Rat				
Testis + epididymis	7	6 (86)	63	55 (87)
Ovary	12	9 (75)**	115	69 (60)**
Saline (+ FCA)	11	11 (100)	124	113 (91)
Mice				
Sperm suspensions	7	3 (43)**	72	14 (19)**
Saline	5	4 (80)	60	37 (62)
Testis + epididymis	5	3 (60)*	72	39 (54)*
Ovary	3	0 (0)**	36	0 (0)**
Saline (+ FCA)	5	5 (100)	62	48 (77)

Significantly different from control (χ^2 test): * $P < 0.01$; ** $P < 0.001$.**Table 3.** Fertilizability of eggs from mice injected with an homogenate of testis+epididymis or ovary or with sperm suspension

Injection	No. of females used	Freund's complete adjuvant	No. of eggs		
			Examined	Penetrated (%)	Undergoing fertilization (%)
Eggs in cumulus					
Sperm suspension	4	—	91	71 (78)	66 (73)*
Saline	3	—	30	30 (100)	30 (100)
Testis + epididymis	3	+	52	26 (50)	25 (48)**
Ovary	2	+	48 (2)‡	2 (4)	2 (4)**
	1†	+	19	15 (79)	14 (74)
Saline	3	+	50	49 (98)	47 (94)
Denuded eggs					
Sperm suspension	6	—	85	37 (44)	31 (36)
Testis + epididymis	5	+	73	13 (18)	10 (14)**
Ovary	4	+	5 (17)‡	3 (60)	2 (40)
Saline	4	— and +	39	23 (59)	19 (49)

Significantly different from control (χ^2 test): * $P < 0.01$; ** $P < 0.001$.

† This female was induced to superovulate after pregnancy.

‡ Number of eggs with broken zona pellucida.

Discussion

Infertility induced in females by injection of spermatozoa has been attributed to one or more sites of action in the reproductive tract (see Metz, 1973, for review). McLaren (1964) found fewer spermatozoa in the ampullae of immunized mice, and considered that the effect of immunization may have been to prevent spermatozoa from reaching the site of fertilization. Menge & Lieberman (1974) suggested that uterine secretions were altered by immunization of rabbits with semen. In the present study the fertilization rate was not affected in the rat by either treatment but was significantly reduced in the mouse, more by spermatozoa than by testis+epididymis homogenate. These findings confirm those of the breeding results presented in Table 1, and indicate that the low fertility was due to inhibition of fertilization. Menge (1968) also found a depression of fertilization rate and embryonic survival in rabbits immunized with semen and testis. The low fertilizability of mouse eggs *in vitro* accords with the results of Russo & Metz (1974) for eggs from rabbits isoimmunized with spermatozoa.

The precipitate on some of the mouse eggs resembles that described by Shivers, Dudkiewicz, Franklin & Fussell (1972) and Jilek & Pavlok (1975). Tsunoda & Chang (1976) considered that the precipitate was correlated with the failure of fertilization *in vitro* and *in vivo* in the mouse and hamster but not in the rat. The present results for the mouse confirm this observation.

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