Inhibition of fertility in mice by passive immunization with antibodies to isolated zonae pellucidae

A. G. Sacco

*Wayne State University School of Medicine, Department of Gynecology and Obstetrics,
The C. S. Mott Center for Human Growth and Development, 275 East Hancock Avenue,
Detroit, Michigan 48201, U.S.A.*

Summary. A chromatographically purified preparation of gamma globulin produced against isolated zonae pellucidae of mice was used to immunize mice. A single 2.5 mg dose totally inhibited fertility for a minimum of 11 days.

Introduction

The zona pellucida contains a specific antigen(s) currently being investigated for immunocontraceptive purposes (Shivers, 1977). The present report is a further assessment of the effect on fertility of passive immunization with antibodies against the zona. It differs significantly from previous reports by utilizing chromatographically purified gamma globulin prepared from antisera produced against isolated zonae and a passive immunization regimen involving spontaneous matings.

Materials and Methods

Eggs were obtained from Sch:ARS(CF1)f outbred mice following established procedures for inducing superovulation. Mechanically isolated zonae were prepared as previously described (Sacco & Palm, 1977). The antiserum against the zonae was produced in 2 rabbits which received 2588 and 3200 zonae respectively (Vaitukaitis, Robbins, Nieschag & Ross, 1971).

Anti-zona activity was tested by observing the antibody-produced precipitate on the zona surface and/or by the indirect fluorescent antibody technique following published procedures (Sacco, 1977a, b). Tissue specificity was verified by immunodiffusion methods involving 17 mouse tissues (Sacco, 1977a, b) and by immunofluorescent reaction against mouse cornea and cartilage (Sacco & Shivers, 1973).

Antiserum (30 ml volumes) was rendered specific to mouse zonae by absorption with freeze-dried mouse plasma (30 mg/ml) and spleen (0.4 g/ml). The effectiveness of the absorptions was verified by double diffusion analyses. Gamma globulin fractions of absorbed antiserum were prepared by DEAE cellulose chromatography (Fahey & Terry, 1973). Only purified gamma globulin fractions were used for passive immunization.

Passive immunizations were initially carried out on hormonally primed mice to determine the correct gamma globulin dose for spontaneous matings. Single injections of gamma globulin were given intraperitoneally 28–30 h after PMSG. Doses tested ranged from 0.5 to 3.0 mg/mouse. Females injected with saline (8.5 g NaCl/l) served as controls. Immunized mice were mated after the hCG injection and examined for copulatory plugs the next morning. All mice were killed 24 h later and their reproductive tracts were flushed. All eggs and embryos were observed for the zona precipitate and those not demonstrating this layer were tested by the fluorescent antibody method. For some mice, ovarian eggs were similarly examined.

A second group of mice were allowed to mate spontaneously after passive immunization with 2.5 mg gamma globulin/mouse. Controls consisted of mice injected with saline (8.5 g NaCl/l), preimmune gamma globulin, and preimmune gamma globulin previously 'absorbed' with plasma and spleen. Mice were injected at random stages of the oestrous cycle and were checked daily for copulatory plugs. Most mice were killed at Days 16–18 of gestation and fetuses and/or implantation sites were noted but the pregnancies in 6 gamma globulin-injected mice were allowed to go to term and the mice remated.

Results

All bleedings produced a dense precipitation layer on the mouse zona but the antiserum was not monospecific for zona since immunodiffusion studies demonstrated single precipitin bands between the antiserum and mouse brain, duodenum, liver, ovary, plasma, spleen and serum. An absorption regimen involving plasma and spleen was required to obtain monospecific anti-zona serum. Purified gamma globulin preparations of this absorbed antiserum (Pl. 1, Fig. 1) produced the zona precipitate to a titre of 32 (125 µg protein/ml) and bound to the zona, as determined by fluorescent techniques, to a titre of 256 (15.6 µg protein/ml).

Table 1 summarizes the results of passive immunization of mice with various doses of anti-zona gamma globulin. Antibody was detected by fluorescence (Pl. 1, Fig. 2) on all zonae regardless of dose given. Findings concerning the presence and intensity of the precipitation layer varied. Zonae from mice immunized with 0.5 mg gamma globulin never possessed the precipitate. For mice immunized with higher doses, results varied, from the precipitate being completely absent to being present as a dense layer. Varied responses concerning precipitate

PLATE I

Fig. 1. Unfertilized mouse egg treated with chromatographically purified plasma-spleen absorbed anti-zona pellucida gamma globulin (2 mg/ml). Note dense precipitate on outer surface of zona (arrow). $\times 1230$.

Fig. 2. Isolated zonae of eggs obtained from mated mouse passively immunized with 2.0 mg anti-zona gamma globulin and tested approximately 66 h later for antibody binding by the indirect fluorescent antibody technique. Saline-injected mice served as controls (C). Strong to moderate fluorescence was present although no precipitate was observed on zonae from this mouse. $\times 320$.

Fig. 3. Unfertilized eggs from mated mouse passively immunized with 2.0 mg anti-zona gamma globulin which demonstrate variations in intensity of the zona precipitate. Two-cell embryos are from control mouse injected with saline and do not possess a precipitate on the zona. Thick arrow = dense precipitate, thin arrow = moderate precipitate, double arrows = faint precipitate. $\times 310$.

Fig. 4. Reproductive tracts of mated mice passively immunized with 2.5 mg purified plasma-spleen absorbed anti-zona gamma globulin (right) and 2.5 mg preimmune gamma globulin (left) and killed on Day 18 of gestation. Control mouse (left) had 12 normal fetuses while antibody-immunized mouse showed no evidence of pregnancy.

Fig. 5. Ovarian eggs obtained at Day 18 of gestation from anti-zona gamma globulin-immunized mouse demonstrating non-uniform appearance of precipitate on zona. Arrows indicate regions where precipitate is present. Compare to precipitate on zona in Fig. 1. Control ovarian egg (lower right) is from spontaneously mated mouse immunized with preimmune gamma globulin. $\times 1160$.

Fig. 6. Ovarian eggs obtained at Day 18 of gestation from anti-zona gamma globulin-immunized mouse and tested for antibody binding to zona by the indirect fluorescent antibody method. Note variations in intensity of fluorescence among zonae. Zonae on eggs from control mice (C) immunized with preimmune gamma globulin did not fluoresce. $\times 320$.

PLATE 1

(Facing p. 534)

Table 1. Passive immunization of mice with various doses of purified anti-zona gamma globulin and effect on fertilization

Dosage (mg)	No. of mice immunized	Eggs examined				
		From all mice		From mated mice		
		Total	Ppt. layer	Total	Ppt. layer	Fertilized (%)
0	8	384	—	299	—	257/299 (86)
0.5	2	44	—	0	N.A.	N.A.
1	3	112	— to +++	7	—	7/7 (100)
1.5	6	335	— to +++	184	— to +++	51/184 (28)
2	18	779	— to +++	392	— to +++	0/392 (0)
2.5	2	78	+ to +++	72	+++	0/72 (0)
3	1	24	— to +++	0	N.A.	N.A.
Totals	40	1756		954		

N.A. = not applicable; Ppt. = precipitation (— = none; + = faint; ++ = moderate; +++ = dense).

intensity were frequently observed on zonae obtained from the same female, regardless of the dose received (Pl. 1, Fig. 3).

Eggs from mice immunized with 1 mg doses were fertilizable (Table 1) although fluorescent methods demonstrated antibody bound to zona. Immunization with 1.5 mg doses produced an inhibitory effect, as evidenced by a 28% fertilization rate compared with 86% for the controls. Fertilized eggs from females immunized with 1 or 1.5 mg anti-zona gamma globulin did not possess the zona precipitate. Doses of 2 mg or more completely inhibited fertility although some eggs from these mice did not possess the zona precipitate.

Table 2 summarizes fertility results after passive immunization with 2.5 mg anti-zona gamma globulin. Complete inhibition of fertility was obtained (Pl. 1, Fig. 4) in contrast to controls of which 100% immunized with saline and preimmune serum absorbed with plasma and spleen, and 75% immunized with unabsorbed preimmune serum became pregnant. Average litter sizes for control groups were 12.4, 11.8 and 11.7 fetuses respectively.

Ovarian eggs obtained at Days 16–18 of gestation from antibody-injected and spontaneously mated mice possessed the zona precipitate. However, it no longer appeared uniform in density (Pl. 1, Fig. 5) and the intensity of fluorescence also varied between zonae (Pl. 1, Fig. 6).

Table 2. Inhibition of fertility in spontaneously mating mice following passive immunization with anti-zona pellucida gamma globulin

Treatment	No. of mice	Dose (mg)	Days between immunization and mating (mean)	No. of mice pregnant/no. immunized	Mean litter size
Saline	10	0	1–6 (2.9)	10/10	12.4
Preimmune serum	20	2.5	1–15 (3.7)	15/20	11.7
Preimmune serum (absorbed with plasma and spleen)	5	2.5	1–6 (3.5)	5/5	11.8
Antiserum (absorbed with plasma and spleen)	28	2.5	1–11 (3.6)	0/28	0*

* Determined at Days 16–18 of gestation for 22/28 mice. Remaining 6 mice were allowed to give birth (see Table 3).

Table 3. Return of fertility in mice after a single injection of 2.5 mg anti-zona pellucida gamma globulin

Mouse	1st pregnancy		2nd pregnancy	
	Days between immunization and mating	No. of young	Days between immunization and mating	No. of young
1	1	0	27	0
2	1	0	33	0
3	1	0	24	9
4	3	0	24	10
5	4	0	36	10
6	5	0	41	12

Zonae from ovarian eggs from hormonally primed mice all possessed uniformly dense zona precipitates and strong fluorescence.

Table 3 demonstrates that the infertility produced by immunization with 2.5 mg anti-zona gamma globulin is temporary. From 6 immunized mice which failed to produce offspring following the first successful mating, 4 became pregnant and delivered normal offspring after a second mating.

Discussion

At some doses, purified anti-zona gamma globulin totally inhibits fertility in the mouse (Tables 1 and 2), thus confirming earlier reports (Jilek & Pavlok, 1975; Tsunoda & Sugie, 1977) in which less purified reagents were used.

The infertility is reversible (Table 3) with a single 2.5 mg dose being 100% effective for at least 11 days (Table 2) and, perhaps in some instances, as long as 33 days (Table 3). This duration of total infertility is comparable to that reported by others (Oikawa & Yanagimachi, 1975; Yanagimachi, Winkelhake & Nicolson, 1976) for the hamster.

The observations concerning variations in presence and intensity of the antibody-produced zona precipitate, on zonae obtained from the same mouse (Pl. 1, Fig. 3) and after immunization with various doses of purified gamma globulin (Table 1), indicate that a visible zona precipitate need not be present for inhibition of fertilization to occur. Likewise, these findings also show that the mere association of zona antibody to zona, as detected by fluorescence, does not automatically imply that the zona is impenetrable to spermatozoa and the egg cannot be fertilized. The critical concentration of zona gamma globulin necessary to inhibit fertilization therefore appears to be at a level detectable by fluorescence but below that required to produce a visible precipitate.

Ovarian eggs obtained from hormonally primed mice and observed 2–3 days after passive immunization possessed uniformly dense zona precipitates while those obtained from spontaneously mated mice and observed 18–28 days following immunization possessed an irregular precipitate, thus suggesting a gradual breakdown in the zona precipitate. While it is generally believed that the return of fertility in immunized animals is due to maturation, ovulation and fertilization of eggs with unaffected zonae, this gradual breakdown in the precipitate may also play some role in restoring fertility.

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