The volume fractions of the structural constituents (expressed as a percentage of the fibre volume) and the surface density of the SR (expressed as $\mu m^2/\mu m^3$ of fibre volume) of the retractor unguis muscle and extensor tibiae muscle of *Vespula vulgaris* (L)

		Retractor unguis	Extensor tibiae
Volume fractions	Myofibrils	47.4	64.2
	SR*	34.5	22.1
	Sarcoplasm	16.3	10.6
	Nuclei	1.8	2.2
	Mitochondria	-	0.9
Surface density	SR*	21.4	14.7

* The figures for the SR also indicate those for the T system which, except at the dyads, is very difficult to distinguish from the SR. The volume occupied by the T system is, however, extremely small (< 1%).

tate and lead citrate and examined in a Siemens 102 electron microscope.

Morphometric analysis of the components of the myofibres was made using a multipurpose test system[7] in which the sampling grid contained 84 lines and 168 points. The analysis was carried out on random selected prints taken from transverse sections at a final magnification of 20,000. For each muscle 2500–3000 points were counted. The volume fractions of each of the components and the surface density of the SR were calculated from the standard formulae[5,7].

Results. The arrangement of the components of the myofibres in the wasp leg muscles conforms to that of the tubular pattern common in arthropod muscle[8]. The nuclei are arranged in a central core passing down the middle of the fibres. The myofibrils are mostly strap-like and radiate out from the central core. The SR is arranged in single, double or triple layers between the myofibrils. Part of the SR is differentiated into electron-dense plaques which form dyads with the T system at the A band-I band junction region.

An unusual feature of the wasp retractor unguis muscle described here is the large accumulation of SR, surrounding the central nuclei, and immediately inside the sarcolemma and distal to the myofibrils (figures 1 and 2). The elements of the SR found in these large accumulations are continuous with the elements running between the myofibrils. This unusual large development of the SR in the retractor unguis muscle was not seen in the adjacent extensor tibiae muscle where the fine structure was that of normal tubular muscle. The results of the morphometric analyses of these 2 muscles are shown in the table. Another unusual feature of the retractor unguis is that the mitochondrial content is extremely low. Mitochondria are present (figure 1) but extremely rare and did not feature in the morphometric analysis of the muscle.

Discussion. The values obtained for the retractor unguis for the relative volume and surface areas of the SR are considerably higher than other published figures for insect leg muscles. In a careful analysis of tonic and phasic fibres in the locust leg, Cochrane et al.[5] obtained volume fractions of 1.1%, 6.8% and 19% and surface densities of 1.0 $\mu m^2/\mu m^3$, 2.9 $\mu m^2/\mu m^3$ and 11.9 $\mu m^2/\mu m^3$ for the tonic extensor tibiae, phasic extensor tibiae and phasic retractor unguis respectively. Measurement of these features in the jumping muscle of the flea gave figures of 18% and 16.0 $\mu m^2/\mu m^3$ respectively[4]. Why the figures for the wasp leg reported here should be so much higher and why the SR is organized in such an unusual way is unclear. One might attempt to look for an answer in the behaviour or locomotory habits found in wasps but not in other insects examined to date. One specialized behavioural feature of wasps is that they are able to carry relatively heavy prey such as caterpillars when flying. It is likely that the retractor unguis which operates the tarsal claw is involved in holding the load during flight. However, that would be a tonic function and the fine structure is not that of a typical tonic muscle where the SR content is normally low. A characteristic of phasic muscles is that they have a relatively high mitochondrial content[2,3] so on this criterion this muscle does not have a typical phasic structure either. An explanation of the unusual structure of the muscle clearly awaits more knowledge of its normal function in the wasp. In the meantime it remains an interesting but unexplained observation.

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Possible presence of autoantibodies to zona pellucida in infertile women[1]

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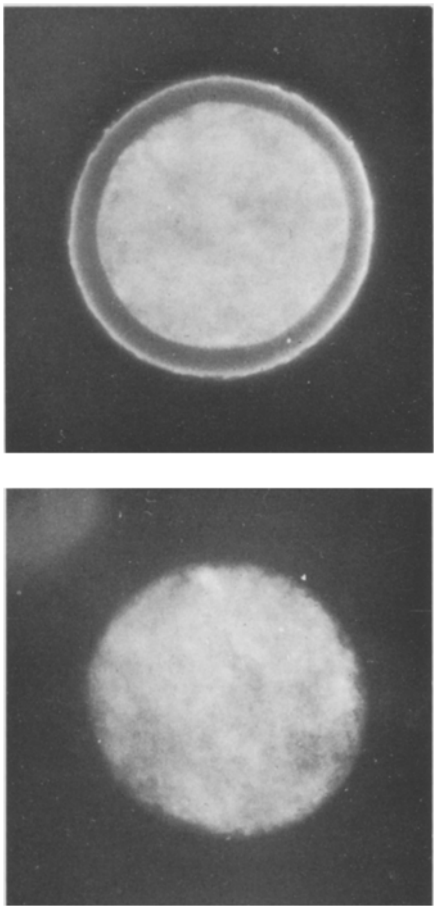
Summary. Of 52 serum samples from infertile women tested against isolated porcine oocytes by immunofluorescence, 8 produced intense reactions in zonae pellucidae, while only one of 52 sera from control subjects showed the reaction. Autoantibodies to the zona may be present in these women.

Following the discovery of an ovary-specific antigen(s) located in the zona pellucida in hamsters[2] and in rabbits[3], many attempts have been made to investigate the immunobiological effects of antibodies to the zona on the reproductive process, for the purpose of immunological regulation of fertility[4]. It has been reported that the human zona pellucida contains at least one antigen specific to the human ovary[5], and that a common antigen is shared by human and porcine zonae[6]. These facts provide the theoretical basis for detection of antibodies to the human zona using porcine zonae as a target. In the present communication, an attempt was undertaken to examine whether or not the antibodies to porcine zonae are present in sera of infertile women as a potential etiological factor in the infertility.

A total of 104 blood samples was taken from 52 infertile patients, including 29 with unexplained sterility, 17 with ovulatory failure, 5 with known etiology of tubal or sperm



Indirect immunofluorescent reactions observed on the outer surface of the porcine oocyte. Serum from an infertile woman gave a strong positive reaction (a), while control serum a negative reaction (b). Both sera were absorbed sequentially with human red blood cells of AB group and with porcine red blood cells.

origin and 1 with habitual abortion, and from 52 control subjects including 19 nonpregnant and 16 pregnant women, and 17 healthy adult men. Separated sera were stored at −20 °C until use. Each serum was absorbed sequentially with human AB and porcine erythrocytes. It was found in a preliminary experiment that fluorescence disappeared or diminished considerably in intensity after the absorption, and this was confirmed to be due to removal of iso- or heterohaemagglutinin in test sera, because control sera of any of the AB0 blood groups agglutinated porcine erythrocytes even after absorption of the sera with human AB erythrocytes with concomitant reduction in fluorescence intensity. Serial absorption with human AB and porcine erythrocytes eliminated the agglutination reaction. Absorption was performed with 10% formalin-fixed cells twice for 1 h at room temperature in 1:1 mixture of cells and serum which had been diluted with an equal volume of 0.01 M phosphate buffered saline, pH 7.4 (PBS). Follicular oocytes were obtained by mincing fresh porcine ovaries and filtering successively through meshes of 200 μm and 100 μm pore size (NIP Superscreen, NBC Industries Co., Tokyo). The filtrate was suspended in 0.01 M Tris-HCl buffered saline, pH 7.4, and ova with cumulus cells were collected under a stereomicroscope (Olympus SZ-II). Cumulus cells were removered by using 0.01% (w/v) sodium citrate solution in PBS as described previously[6]. Cumulus-denuded oocytes were washed with several changes of PBS by transfer with a micropipette and used as targets for the subsequent procedure of antibody detection.

Each serum was inactivated by holding at 56 °C for 30 min, centrifuged, and the supernatant was used as an absorbed serum. For the indirect immunofluorescence, rabbit antiserum to human IgG, IgA, IgM, kappa and lambda labelled with fluorescein isothiocyanate (FITC), purchased from Dakopatta A/S (Copenhagen, Denmark), was found to have a molar F/P (fluorescence/protein) ratio of 1.86 by our measurements. By direct staining it was found necessary to dilute the original conjugate 80 times with PBS to eliminate nonspecific staining. Several oocytes were incubated with the absorbed serum for 30 min at room temperature and washed with several changes of PBS, then the oocytes were subjected to a second incubation with the diluted conjugate for 10 min. The ova were washed several times with PBS and the reaction was observed under a fluorescent microscope (Olympus Type BH-REL).

The fluorescence was graded as strong (ǂ), weak (+), faint (±) and no reaction (−) by its intensity on the outer surface of zonae (table). When unabsorbed sera were used, 26.9% of infertile sera gave strong positive reactions, as did a considerably high proportion (17.3%) of control sera. However, when absorbed sera were used only one of 52 control samples (1.9%) gave a strong positive reaction, while an appreciably higher incidence (15.4%) was observed in infertile sera (figure). Of the 9 positive individuals, 8 (age 29–35) had long-standing infertility for 2–8 years, since marriage or since the last conception in 2 cases, one of whom had experienced absortion once and the other full term delivery once. The remaining one (23 years) is unmarried.

If blood group antigens are present on the surface of human or porcine oocytes, as has been demonstrated in the human sperm[7], hetero- and isoagglutinins in the test sera will interfere with the specific reaction between autoantibodies and porcine ova when whole serum is used, because human agglutinins related to AB0 blood groups have been found to belong to both IgM and IgG immunoglobulin

Incidence in immunofluorescent reactions of sera from infertile women against porcine zonae pellucidae

	No. of sera tested	Unabsorbed sera			Sera absorbed with human and porcine erythrocytes		
		ǂ	+ ~ −	Percent of ǂ	ǂ	+ ~ −	Percent of ǂ
Sterile women	52	14	38	26.9	8	44	15.4
Unexplained	29	8	21	27.6	5	24	22.7
Anovulation	17	5	12	29.4	3	14	17.6
Known etiology	5	0	5	0	0	5	0
Habitual absorption	1	1	0	100.0	0	1	0
Control subjects	52	9	43	17.3	1	51	1.9
Nonpregnant	19	7	12	36.8	1	18	5.3
Pregnant	16	2	14	12.5	0	16	0
Normal men	17	0	17	0	0	17	0

Intensity of fluorescence is graded as strong (ǂ), weak (+), faint (±) and no (−) reactions.

classes[8]. Elimination of these agglutinins was achieved in this study by absorption with human AB and porcine erythrocytes, as evidenced by the extinction of haemagglutination reaction with simultaneous reduction in intensity of fluorescence. Thus, fluorescence seen after absorption will signify the presence of specific antibodies to the zona antigen in sera showing a strong positive reaction.

Although a causal relationship between the presence of autoantibodies and infertility in these women cannot be established at the moment, the facts that heteroantibodies to zonae of rodents can block fertilization in vitro[9-11] or in vivo[10,12] temporarily by passive transfer of the immune sera may suggest an involvement of the detected autoantibodies as an etiological factor in the development of infertility in these women. However, the question why autoantibodies are present in particular women remains unsolved, because all the women should have been equally exposed to the zona antigen and hence equally sensitized to it. One explanation is that the women with the autoantibody could be much more prone to autosensitization to the zona antigen than women without the antibody, and constant antigenic stimulation evoked through the physiological processes of atresia or ovulation could produce a higher titer of the antibody sufficient to develop infertility. This concept encourages us to utilize the zona antigen for immunological control of conception, because active immunization with this material could sensitize fertile women to produce sufficient antibody to inhibit fertilization. Further rigorous research is required to realize this system for human fertility control.

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Long-term depression of two primary immune responses induced by a single dose of 5-(3,3-dimethyl-1-triazeno)-imidazole-4-carboxamide (DTIC)[1]

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Summary. 2 primary immune responses (anti-SRBC antibody response and allograft rejection) have been tested in mice at various time intervals after single doses of either DTIC or cyclophosphamide. The DTIC-induced immunodepression proved to be extremely long-lasting, being still detectable after 2 months.

Among the most recent antitumor agents, 5-(3,3-dimethyl-1-triazeno)-imidazole-4-carboxamide (DTIC) has been the subject of extensive studies, recently reviewed[2], particularly since it was found to be highly active in mediating immunogenic changes of experimental murine lymphomas[3-8].

Preliminary observations in our laboratory showed unusual effects of DTIC on the immune responses. Therefore experiments were carried out to investigate how the immunodepressive activity of the drug compares to that of cyclophosphamide (Cy), a well-known alkylating agent widely employed as an experimental immunodepressant. The data demonstrate that a single dose of DTIC produced marked depression of both allograft and humoral antibody responses. The inhibitory effects were far more long-lasting than those induced by an equitoxic dose of Cy.

Materials and methods. 2 primary immune responses were tested in mice at various times after injection of single doses of either DTIC or Cy. The humoral antibody production was assayed against sheep red blood cells (SRBC) and the allograft response was measured against a mouse lymphoma line, which was transplanted across a histocompatibility barrier as strong as the entire H-2 complex. The humoral antibody production was assessed according to the method described by Jerne et al.[9], and results were expressed as number of primary hemolytic plaque-forming cells (PFC)/spleen. Mortality data, i.e. median survival time (MST) and number of dead mice over the total number of animals injected (D/T), have been used to measure the extent of allograft response in mice challenged with an allogeneic lymphoma.

Results. 2 4-month-old hybrid (DBA/2×BALB/c)F_1 ($CD2F_1$) mice of both sexes were treated i.p. with either DTIC 240 mg/kg or Cy 200 mg/kg. SRBC were administered 1 or 60 days later and the animals were then tested for their capability of producing humoral antibodies. Tests were performed on day 2, 4 and 7 after SRBC administration. The left side of the figure shows that both DTIC and Cy, given 1 day before SRBC injection, completely abrogated the humoral antibody production as measured by the number of PFC/spleen. When the drugs were administered 60 days before the anigenic stimulus (right side of the figure), only treatment with DTIC caused complete suppression of the antibody response. In a similar experimental design, the allograft response was tested in $CD2F_1$ (H-2^d/H-2^d) mice pretreated with either DTIC 240 mg/kg or Cy 200 mg/kg and then challenged i.v. with 10^6 allogeneic

Strain	Drug	Day	MST	D/T	Range
C57Bl/10	-	-	8	7/7	7-9
$CD2F_1$	-	-	-	0/6	-
$CD2F_1$	Cy	− 1	8	8/8	8-12
$CD2F_1$	DTIC	− 1	8	8/8	8-11
$CD2F_1$	Cy	−60	-	0/6	-
$CD2F_1$	DTIC	−60	10	6/6	9-10

Depression of the allograft response by equitoxic doses of 5-(3,3-dimethyl-1-triazeno)-imidazole-4-carboxamide (DTIC) and Cyclophosphamide (Cy) in mice challenged with the allogeneic lymphoma L5MF-22. MST, median survival time in days; D/T, dead over the total number of animals injected.