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## **Enzyme therapy- a method of immune therapy for women with a history of immunologically-induced habitual abortion**

Prof. Dr. F.-W. DITTMAR

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### ORIGINAL ARTICLE

Enzyme therapy -a method of immune therapy for women with a history of immunologically-induced habitual abortion

Dittmar, F.-W.

Department Gynecology and Obstetrics (Senior physician: Prof. Dr. F.- W. Dittmar) of the Starnberg District Hospital, Academic Teaching Hospital of Ludwig-Maximilians University, Munich, Germany

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Enzyme therapy, immune therapy, habitual abortion

### Abstract

Background: For women with a history of immunologically-induced habitual abortion, only a few therapies are currently available which are also known to be associated with a limited rate of success and substantial side effects.

Objective:

The goal of this investigation was to evaluate the benefits of a systemic enzyme therapy in pregnant women with a history of habitual abortion based upon the course of pregnancy and delivery, and also including the "fetal outcome".

Materials and methods:

144 pregnant women with immunologically-induced abortion received a gestagen preparation and an enzyme combination preparation, and were observed until the time of delivery. The standard data concerning pregnancy, birth and the child were collected and evaluated.

Results:

114 of the 144 enzyme-treated women demonstrated an inconspicuous course of pregnancy up to the birth of their 114 healthy children. The enzyme therapy was seen to cause no unpleasant side effects.

## Conclusions:

For immunologically-induced habitual abortion, enzyme therapy is seen to be an effective form of immune therapy. The maternal immune system is stabilized by the enzymes administered, thereby affording relief during the full-term development of a pregnancy. The further clarification of the molecular mechanisms of action of these agents is the subject of current research projects. Enzyme therapy can primarily be recommended for women who have suffered several abortions, in spite of the fact that they had received the common forms of immune therapy, and who are consequently looking for a therapeutic alternative. According to experiences made to date, enzyme therapy is considered to be very successful for women with a history of habitual abortion and can be reflected in their completed pregnancies. Furthermore, it is tolerated well and also seen to be quite economical.

## Introduction

Habitual abortion is defined as being the occurrence of three or more spontaneous abortions seen to develop subsequently and without the occurrence of an interim pregnancy which has been carried out for longer than the 28th week of pregnancy. The terms recurrent spontaneous abortion (HSA) or recurrent miscarriage (RM) are also used for designating habitual abortions in countries where English is spoken. With regard to the gestational period, one can distinguish between an early habitual abortion, which occurs up to the end of the 12th week of pregnancy, and a late habitual abortion (following the development of the placenta) which begins with the 13th week of pregnancy.

The incidence of recurrent abortions amounts to between about 0.4 and 1.8 % of all pregnancies. Although only about 2 % of women of a reproductive age are affected by habitual abortions, recurrent grounds for such abortions, which may require intensive diagnostic and therapeutic measures, can be expected to be found for these patients.

### a) The pathway of diagnosis

The exclusion of non-immunological causes for a habitual abortion is generally a prerequisite for any therapy making use of immunotherapeutic agents. This diagnosis by exclusion is mandatory for all forms of immunotherapy, including the enzyme therapy which is presented here as a new procedure for the immunotherapy of habitual abortions.

In order to complete the picture, the causes of a habitual abortion which must be excluded, as well as the respective indications for the established therapeutic procedures to be employed before beginning an enzyme therapy, are presented here, once again, in the following.

### b) Diagnosis and therapy for non-immunological causes of habitual abortion

Many early abortions already occur during the first days after conception and, consequently, are

not even noticed consciously by the women [13; 51]. These early losses of an embryo are predominantly due to chromosomal aberrations. In spite of the reduction in chromosomal causes of abortion with an increasing gestational age, these conditions amount to about half of the clinically-identified early abortions [14].

In contrast to the numerous overvaluations of uterus anomalies with regard to the prognosis of a pregnancy, the reproductive rate of these women is evaluated as being more likely normal. A review of the infectious causes of late abortions has been written by Sating [42].

Even today, views concerning the diagnosis and therapy of luteal insufficiency are quite divergent. Immunoregulatory effects of progesterone were described by Nouza [35]. Various authors report on positive experiences made with the use of individual gestagen substitutions for the treatment of luteal insufficiencies, which are more related to the specific findings, rather than employing therapies which may be easier to perform, i.e. with the use of vaginal progesterone suppositories [17]. Nevertheless, the general, prophylactic hormone therapy for renewed pregnancies following habitual abortions could not assert itself [17].

Psychological stabilization along with gentle healthcare is generally considered to be acceptable, and consequently practiced frequently, for the treatment of pregnant women known to suffer from recurrent abortions. Among these modes of treatment is also the so-called tender loving care (TLC) therapy according to Stray-Pederson as well as the use of generous certifications for sick-leave in order for the patient to avoid bodily and psychological stress. Of course, the psychological support is also sensible in the event of immunological causes.

### c) Diagnosis and therapy for the immunological causes of habitual abortions

After eliminating the cases of early abortion without chromosomal aberration, i.e. approximately 50 % of the early abortions, and following the exclusion of endocrinal disturbances (e.g. luteal insufficiency), uterine anomalies, metabolic diseases, infections and clotting disorders, one is left with the habitual abortions which demonstrate immunological causes. These causes are seen to have various degrees of incidence in the different literature. Estimations of the frequency for immunological causes to habitual abortions lie between 30 and 50%. Reviews and brief commentaries concerning the spectrum of immunological causes of abortions are to be found in Carp [6], Coulam [9], von Dithfurt [11], Domke [12], Grosse-Wilde [18], Saling [41], Schblmann [43] and Schwarzenau [44].

In many studies, one is confronted with the highly probable assumption that disturbances in the protective, maternal immune response lead to the expulsion of the semiallogeneic embryos or fetuses, and are consequently responsible for the abortion [3]. Studies considering the concept of the "fetus as a transplant" reveal the multitude of different and partially contradictory theories over the immunology of a pregnancy [3].

More recent publications on immunology during the early weeks of pregnancy show agreement

that disturbances in the immunological interactions between the mother and embryo are to be seen as the cause of habitual abortions. In their position published in "Frauenarzt 4 /1999 [20], Heilmann and Marzusch also demonstrated a similar opinion.

The assumption of a disturbed immune tolerance in habitual abortions has so far led to therapies using quite different active agents like aspirin, heparin, corticoids and immunoglobulins.

#### d) Active immunization

In the meta-analysis by Christiansen performed in 1997 [7], the active immunization with paternal lymphocytes only demonstrated a negligible improvement in the rate of live births for women who had suffered from an abortion following their first pregnancy. However, the risks of a therapy with lymphocytes can only be calculated in part. Consequently, the generation of an immunological disturbance, which may only appear fate during the development of a child, cannot yet be excluded conclusively.

Marzusch, Mayer and Dietl [33] critically evaluated the risk/benefit relationships of an active immune therapy with regard to such different complications as viral infections or the development of autoantibodies.

#### e) Pharmacotherapy used for the treatment of autoimmune causes (antiphospholipid syndrome) to date including ASA, heparin, corticoids and immunoglobulins (passive immunization)

For systemic lupus erythematosus (SLE), which is associated with the development of various autoantibodies, abortion rates of 11- 46 % have been reported. Systemic lupus erythematosus (SLE) and the antiphospholipid syndrome (APLS) are closely-related autoimmune diseases which can nevertheless be distinguished based on the clinical symptoms and the pathogenic, autoimmune antibodies.

In the antiphospholipid syndrome, one primarily finds antiphospholipid antibodies, anticardiolipin antibodies and antibodies against beta-2 glucoprotein-1.

Both clinical conditions are caused by complement activating autoimmune antibodies and the cytotoxicity associated with them (antibody-dependent cell-mediated cytotoxicity = ADCC). The monograph of Asherson, Cervera, Piette and Shoeniteld [1] is recommended for a detailed, pathophysiological description of the antiphospholipid syndrome.

As a result of the alterations in coagulation, the antiphospholipid syndrome leads to thrombotic complications in various organs, for instance to an eventual heart attack or pulmonary embolism, and may lead to habitual abortions in pregnant women [49].

Since the thrombotic symptoms stand in the foreground during the course of an antiphospholipid syndrome, anticolagulatory agents, especially 'low-dose' aspirin and "low-dose" heparin regimens, have been administered thus far for the prophylaxis of abortions.

To date, the low-dose administration of acetylsalicylic acid (40-100 mg/day) still appears to be an effective and, at the same time, well-tolerated therapy. Even the additional administration of prednisolone is considered to be questionable, especially since the administration of a prednisolone therapy alone has not yet reliably demonstrated any effects [17]. Only little experience has been made with the use of a subcutaneous heparin therapy at a daily dose of 10,000—36,000 IU., a treatment which clearly accentuates the considerable side effects attributed to heparin [1].

In the case of an antiphospholipid syndrome, when a recurrent abortion is observed in spite of the administration of acetylsalicylic acid, heparin and prednisolone, an intravenous immunoglobulin therapy (IVIG) is frequently employed during the course of the next pregnancy. This therapy leads to a measurable reduction in the concentration of autoantibodies (see below).

#### f) Passive immunization

In 1999, according to the Study Group on Immunology in Gynecology and Obstetrics of the German Society for Gynecology and Obstetrics (DGGG; Deutsche Gesellschaft für Gynäkologie und Geburtshilfe), the efficacy of an intravenous immunoglobulin therapy (IVIG) for the treatment of recurrent spontaneous abortion could not be verified conclusively [20]. Using placebo-controlled studies. Hinney and Neumeyer [22] also came to the conclusion that a positive effect could not be demonstrated for a therapy with intravenous immunoglobulins. Furthermore, the cost of a passive immunization represents an additional problem [8].

Even without the administration of intravenous immunoglobulin prior to conception, its subsequent use from about the 6th to the 24th week of pregnancy leads to costs of about 22,500 DM (more than \$12,000 U.S.) according to the costs required for a pharmacist [22]. The high costs of a passive immunization are chiefly justified by the advantages seen over an active immunization with leukocytes and, for example, reflected in the avoidance of viral infections and at an undesirable HLA immunization [34].

The considerable difficulties observed with active and passive immunization, and the profile of side effects attributed to anticoagulatory agents, motivated the author to the search for an effective, well-tolerated and reasonably-priced therapeutic alternative. This ultimately led to successful therapeutic experiments using enzyme therapies, a procedure which proved to be an effective immunotherapeutic process for the therapy of women with a history of immunologically-induced habitual abortions.

#### Material and methods

In the course of the diagnosis performed before the administration of an enzyme therapy, the non-immunological causes of habitual abortions noted above were routinely excluded. According to the author, a detailed immunological diagnosis, including an investigation of all

conceivable autoantibodies, must not be considered mandatory before the administration of an enzyme therapy since it is tolerated well at any rate.

After carefully eliminating the possibility of all of the non- immunological causes for a habitual abortion, the classification of the problem as being in the field of immunological causes may possibly lead to an expensive immunological diagnosis.

In 1999, Hinney and Neumeyer also came to the conclusion that a method for the recognition of immunological causes of a habitual abortion (aside from the identification of antiphospholipid antibodies) has not been available to date [22].

The use of HLA-serotyping also remains questionable if one takes into consideration the possible therapeutic consequences.

In regard to the own collective, it must be noted that the women with a history of habitual abortion explicitly demanded the administration of an enzyme therapy even after having previously undergone complex and nevertheless unsuccessful therapies using various methods which have been described above, from corticoids to immunoglobulin therapy. Nevertheless, in an individual declaration of the possible risks to a so-called therapeutic attempt using enzymes during pregnancy, the patients were instructed that clinical studies on the administration of Wobenzym® N and Phlogenzym® for this indication had not yet been performed and that the directions for the use of both of these preparations advise subjects to exercise caution with regard to an administration during pregnancy.

Phlogenzym® is a combination preparation consisting of the active agents bromelain (from pineapple), trypsin (from the porcine pancreas) and rutin for reducing the vascular permeability (in the trophoblast). Aside from the three active agents in Phlogenzym®, Wobenzym® N also contains papain (from papaya) and the animal enzymes chymotrypsin and pancreatin.

## Results

This paper reports on therapeutic attempts made with the use of a systemic enzyme therapy as an immunotherapeutic process for the treatment of patients with a history of immunologically induced habitual abortion.

In the years 1994—1999, 144 women with a history of habitual abortion recorded anamnesticly were treated during a renewed pregnancy with enzyme combination preparations and observed until the birth. Aside from the administration of gestagens, the women had also received enzymes during the renewed pregnancy: initially using only Wobenzym® N and later using Phlogenzym® as well. From the total of 144 women, 71 women had received Wobenzym® N and 73 had received Phlogenzym®

The first administration of the enzyme combination preparations (Wobenzym® N /

Phlogenzym®) occasionally began before a planned conception. The enzyme combination preparations were initially administered at a higher dosage. One portion of the patients received the enzyme preparations until the 15th week of pregnancy, while the majority continued to take the enzyme preparations during the entire course of the pregnancy.

Peculiarities were noted in only two cases of pregnancy: one in which the enzymes had already been administered after a vaginal hemorrhage had begun and another in which a missed abortion had been identified.

From the total of 144 women who had been treated with enzymes, 114 were able to carry out their pregnancies and a total of 114 healthy children were born within the observation period (rate of success 79 %). Because of the various obstetrical problems (conditions subsequent to an enucleation of a myoma, retarded birth, etc.), the deliveries were carried out between the 34th and the 40th week of pregnancy.

Among the 114 children, there were no signs of teratogenic damage and the infants' intrauterine requirements were seen to be optimal until the time of birth, findings which are reflected in the documentation of an inconspicuous asphyxiation index and a normal pH in the umbilical artery.

The preparations were tolerated without reservation. No unpleasant side-effects developed which might have induced the patients to discontinue the therapy.

Comparable experiences with the use of an enzyme therapy during pregnancy, namely problem-free pregnancies with the birth of healthy children following immunologically-induced habitual abortion, have also been reported by Sukhikh in Moscow [48].

Modes of action of the enzyme therapy in the event of autoimmune diseases

Proteolytic enzymes are applied with a great deal of success in the treatment of autoimmune illnesses.

In the table of literature which follows, some of the studies on the use of enzyme therapies for the treatment of autoimmune diseases are included:

The modes of action of enzyme therapy were explored primarily by Lehmann [23, 28], Kunze [251 and Desser [10].

In a mouse model for multiple sclerosis (experimental allergic encephatomyelitis = EAE). the animals received autoantigenic myelin and thereby developed EAE in 100 % of the cases. Lehmann of Case West Reserve University in Cleveland, Ohio treated a group of mice with Phlogenzym® which was administered orally by means of a feeding tube. In this group, the illness could either be suppressed fully or the symptoms could be alleviated at the outbreak of the EAE. Summaries of the results of the Lehmann group published in two issues of NATURE [23, 28] must actually be considered sensational.

In further investigations, Lehmann's group of researchers showed that enzymes reduce the

antigenic presentation of certain adhesion molecules (CD4 and CD44 on autoreactive T lymphocytes and B7 on macrophages) via down-modulation (cf. Fig. 1).

Lehmann draws attention to the fact that this process (an altered response following antigen presentation) only has an effect in autoreactive processes. In comparison to foreign antigens (e.g. in an infection), the quantity of auto-antigens is relatively small. The reduction of the antigen presentation, as well as the subsequent activation and proliferation of the T cells and B cells, is therefore only significant where relatively little antigen is to be found (like that observed during the beginning of an autoimmune disease).

A reduction in the antigen presentation, a process whereby the number of the activated T cells is reduced, occurs through the administration of an enzyme therapy: Autoimmune illnesses do not break out.

According to the investigations of Emancipator in Cleveland, the oral administration of Phlogenzym® also reduces the manifestation of symptoms in cases of arthritis induced in mice via collagen 11[15]. In comparison with Ibuprofen, Phlogenzym® provided a substantially better protection to the cartilage as could be demonstrated by the thickness of the cartilage which was measured.

Bohm investigated the development of type I diabetes mellitus in NOD mice [50]. Here, it could be seen that the enzyme therapy inhibits the outbreak of a type I diabetes. Based on Bohm's results, as well as on the results seen on autoimmune insulinitis in NOD mice [32], Martin and colleagues are presently investigating whether the outbreak of diabetes in children at risk of developing type I diabetes could either be avoided or at least delayed through the oral administration of an enzyme therapy [40]. Autoantigen-specific T cell proliferation is thereby inhibited, while the non-specific activation remains unaffected [40]. The inhibitory effect is mediated particularly through the protease preincubation of antigen-presenting cells. In addition, in response to the autoantigens, the cytokine production-profile of the T cells is influenced to such an extent that a restriction of the TH1 immunity results [40].

A good response could be demonstrated for the administration of an enzyme therapy in cases of glomerulonephritis associated with antigen- antibody complexes. Emancipator explored the effects of an oral administration of Phlogenzym® in rats and established that there was a continuous decrease in the degree of proteinuria and hyperlipidemia [16].

According to Sharma [45], TGF- $\beta$  substantially accelerates the glomerulosclerosis observed to develop during a diabetic nephropathy.

In rats with insulin-dependent diabetes mellitus (IDDM) and diabetic nephropathy, Heidland and Paczek examined the effects of Phlogenzym® on the production of TGF- $\beta$  in the glomeruli and were thereby able to show that Phlogenzym® held the TGF- $\beta$  production at a normal level and that the renal fibrosis was clearly decreased [19, 36].



The immunoregulation observed in a pregnancy which develops subsequent to an immunologically-induced habitual abortion is used as a special case in order to determine the mode of action of an enzyme therapy in the treatment of autoimmune diseases. Here, considering the complexity of the immunological system during early pregnancy and the different possible factors of an immunoregulation brought about by proteolytic enzymes, only a few, selected aspects dealing with the mode of action of enzyme combination preparations in pregnancy are described according to the present status of knowledge.

From the elicitation of an abortion by microorganisms, the cytokine-induced damage to a pregnancy through the influence of the metabolic products of *Yersinia enterocolitica* have been demonstrated in an animal experiment using a mouse model [4, 5].

Since a similar reaction to the acute rejection reaction is to be observed in a habitual abortion, it must also be mentioned that, according to Knulst [24], cytokines like TNF- $\alpha$ , for example, are involved in such processes in the sense of a graft-versus-host or a host-versus-graft reaction.

Mallmann [30, 31] and Malave [29] reported on an increased blood level of the cytokines TNF- $\alpha$  and IL-1 $\beta$ , both before and during an abortion. The increased and systemically demonstrable cytokine synthesis during abortion, as a sign for an activated cellular immunological system, represents an important site of attack of the proteolytic enzymes for the development of the abortion-hindering, immune modulatory effects which arise by way of the  $\alpha_2$ -macroglobulin. Streichhan [46, 47] has described in detail the resorption of orally administered proteolytic enzymes and their influence on the proteolytic serum activity (PROSA).

Proteolytic enzymes alter the concentration and biological half-life of the  $\alpha_2$ -macroglobulin, and consequently reduce the abnormally increased, immunological reactivity in women with a history of habitual abortion [25]. The cytokine binding characteristics of proteinase activated  $\alpha_2$ -macroglobulin have been explained in detail by LaMarre [27]. In addition, Palermo [37] described the effect of  $\alpha_2$ -macroglobulin on controlling the trophoblast. Further details concerning the regulatory effects of  $\alpha_2$ -macroglobulin on the development of a pregnancy have been described by Petersen [38].

Parallel to the increased cytokine synthesis, pregnant women with a history of habitual abortion also demonstrate an activation and an increased expression of their adhesion molecules. According to LaBarre [26], the increased expression of ICAM-1 is instrumental for the faulty immunological reaction of the mother to the embryo or the fetus. Through the down-modulation of excessively activated cells, proteolytic enzymes also reduce the ICAM expression in particular and consequently decrease the risk of a pathological mother-child reaction [25].

The autoantibodies, like antiphospholipid antibodies, develop during disturbed pregnancies as a result of the abnormal T-cell response (especially by way of TH1 cells). In *in vitro* investigations Heumann and Vischer [21] were able to verify the down-modulatory effects of the trypsin-(L2-

macroglobulin complex on different immunoreactive cellular processes like antigen presentation, T-cell proliferation and T cell activation.

Evidently, humoral and cellular immunological reactions work in conjunction in cases of habitual abortion, whereby the activity and the proliferation rate of the cytokine-producing immunocytes increases to a great extent and the complement cascade of tissue-binding antigen antibody complexes is stimulated in such a manner that there is a complex immune response which ultimately results in a habitual abortion.

Proteolytic enzymes have an effect on different sites of this procedure: Enzymes reduce the production of proinflammatory cytokines and the expression of adhesion molecules, as well as the production of injurious immune products (autoantibodies and antigen-antibody complexes). The outstanding effects of proteolytic enzymes on the autoantibody-induced and antigen-antibody-complex-induced illnesses is known from many investigations (cf. table with a review of the literature).

The effects of the rutin on reducing the vascular permeability (high-dosed in Phlogenzym®) may possibly protect the subjects from microhemorrhages which occur as a result of trophoblastic disturbances.

As a summary for the mode of action, the patent specifications [39] can be recommended in conclusion. The patent was granted on Dec. 8, 1999 (Patentblatt 1999 / 49).

Addendum: Recommendations for practice and hospital

The large number of telephone calls received by the Starnberg District Hospital (from women with a history of habitual abortions and their gynecologists) demonstrated the great amount of interest in the treatment described and was ultimately responsible for the production of this publication. The author would be grateful if this method could be employed more frequently by colleagues under their own individual risk-benefit declarations on women suffering from habitual abortions and if he could receive a report on the course of these pregnancies.

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Kraillinger StraBe 12

82131 Stockdorf

Tel. (0 89) 89 97 91 87 — Fax (0 89) 89 97 91 67