Allergy 1995: 50: 179–183
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ALLERGY
ISSN 0105-4538

Case report

Severe anaphylactic reaction to bovine serum albumin at the first attempt of artificial insemination

Wüthrich B, Stern A, Johansson SGO. Severe anaphylactic reaction to bovine serum albumin at the first attempt of artificial insemination. Allergy 1995: 50: 179–183. © Munksgaard 1995.

A 33-year-old woman without history of previous atopic diseases or drug allergies developed a severe anaphylactic reaction with asthma, vomiting, itching, generalized urticaria, and angioedema during artificial insemination with her husband's sperm. The sperm-processing medium contained bovine serum albumin (BSA). Skin prick test and RAST demonstrated an IgE-mediated hypersensitivity to BSA as well as a polyvalent atopic sensitization to pollens, animal danders, cow's milk, beef, pork, and mutton. SDS–PAGE studies indicated serum albumin to be the appropriate allergen with a high degree of cross-reactivity between serum albumin from different animal species. Artificial insemination with fluid containing potential allergens can, therefore, represent an unnecessary risk for atopic females, even in the absence of prior clinical symptoms of allergic diseases. Preoperative testing with the medium is recommended.

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Key words: anaphylaxis; bovine serum albumin (BSA); insemination; insemination fluid; prick tests; RAST.

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Accepted for publication 5 August 1994

Bovine serum albumin (BSA) is considered a minor allergen in cow's milk allergy (3, 16, 25). Nevertheless, the clinical relevance of BSA among other purified milk proteins has been proved in various studies (9, 10, 19). Several cases of allergic reactions caused by BSA have been reported in recent years. A male patient with eosinophilic gastroenteritis and high IgE levels reactive to mammalian albumin (24), and an anaphylactic reaction following infusion of autologous bone marrow (14) have been linked to BSA. A young female laboratory technician who developed recurrent rhinoconjunctivitis and shortness of breath immediately after repeated exposure to purified crystalline BSA (11) has also been described. Serum-sickness-like syndromes of patients after having undergone procedures of *in vitro* fertilization (IVF) have been postulated to be due to IgG1-mediated sensitization to BSA (17). Similarly, Gamboa et al. observed an immune-complex-related reaction due to BSA days after the aspiration of graafian follicles (8). They attracted clinicians' attention to this adverse effect and warned against the use of a heterologous protein during IVF.

To our knowledge, there are only two (previously published) cases linking an anaphylactic reaction during artificial insemination directly to BSA (7, 20).

This report of a woman who experienced a severe IgE-mediated reaction to BSA immediately after an intrauterine insemination underlines the need to use media free from heterologous proteins for human application.

Case report

A 33-year-old woman was referred for allergologic investigation because of a severe anaphylactoid reaction connected with infertility therapy. The patient underwent her first intrauterine insemination (IUI) with her husband's sperm 18 months earlier. Five minutes after the insemination, she developed dyspnea, bronchospasm, vomiting, itching on hands and palms, generalized urticaria, and angioedema of the eyelids and lips. The patient's past medical history was negative for atopic diseases, drug allergies, or other allergic disorders. She denied that there was atopy or allergies in her family. Her surgical history

was insignificant; she never had received heterologous sera and had had no operations.

The semen for the IUI was processed in a standard fluid medium (INRA-Menezo, B2-medium). In addition to inorganic salts, it contains organic compounds such as different amino acids, lipids, vitamins, and BSA, as well as the antibiotics, penicillin and streptomycin. A polyethylene catheter was used during the intervention.

Material and methods

Skin tests

Skin prick tests (SPT) were performed with routine inhalant allergens including animal epithelia and latex (Alyostal extracts, Stallergènes), using a standardized needle (Stallerpoint, France). The major and minor determinants of penicillin were tested (SPT and intracutaneous) with commercially available extracts (Allergopen, Allergopharma, Germany). SPT to streptomycin and to the fluid medium for the sperm processing (INRA-Menezo, B2), as well as to BSA (10 mg/ml, distilled water), of a 1% solution were also carried out. Histamine 1 mg/ml for SPT and 0.1 mg/ml for intradermal testing (ID) was used as positive control with glycerol for SPT and normal saline for ID as negative controls, respectively. The results of the skin-testing were evaluated at 20 min, and the positive results were graded in the following manner: SPT (+) diameter of the wheal 3–5 mm, (++) wheal 5–10 mm, (+++) wheal 10–15 mm.

IgE analyses

Total and specific serum IgE was determined by the ImmunoCAP technology (IgE-FEIA and RAST-FEIA, respectively; Pharmacia CAP-system, Uppsala, Sweden), according to the manufacturer's recommendation). The RAST results, expressed in kU/l, were subdivided into six classes, where concentration less than 0.35 kU/l represented a negative result (class 0).

SDS–PAGE and immunoblotting

To evaluate the allergen specificity of IgE binding, a polyacrylamide gel electrophoresis separation in the presence of sodium dodecyl sulfate (SDS–PAGE), followed by immunoblotting, was performed, essentially as described by Alenius et al. (1) on 10–15% polyacrylamide gel, using the Pharmacia Phast system (Pharmacia Biosystem, Uppsala, Sweden). The wells were loaded with 4 µl purified BSA and sera from different animal species, respectively, all diluted 1/5 in buffer containing 15% sodium dodecyl sulfate. The Pharmacia molecular weight protein marker (Pharmacia Biosystem) was included in all gels. After migration, protein bands were transferred to nitrocellulose membrane (Trans-Blot, Bio-Rad, CA, USA) by diffusion and after blocking with 1% Tween 20 (Fluka Chemie AG, Switzerland) for 1 h at 37°C, stained with Coomassie brilliant blue.

The membrane was incubated in a box with six slots, each containing serum from the patient and a nonatopic control (diluted 1/5 in phosphate buffer), overnight at room temperature with continuous shaking. IgE binding was detected by rabbit anti-IgE-β-galactosidase conjugate (Pharmacia Diagnostics). After washing, the molecular marker was cut off and stained with Auro Dye Forte® (Janssen). Another part of the membrane was stained for protein with Fast garnet GBC Salt and 6-bromo-2-naphtyl β-galactopyranoside in phosphate buffer.

Results

Skin tests

Skin prick testing demonstrated an immediate hypersensitivity to various pollens and to mammalian epithelia (Table 1). In addition, SPT was clearly positive to the undiluted INRA-Menezo, B2-medium (+++) and elicited a strong reaction to the 1% solution of BSA (+++). SPT to latex, different house-dust mites, and molds were negative. Prick and intracutaneous testing to minor and major determinants of penicillin and to streptomycin were also negative.

Table 1. Results of skin prick test (SPT)

Allergen	SPT	Allergen	SPT
Inra-Menezo, B2	+++	*Pollen*	
Bovine serum albumin	+++	Grass mixture	++
1% distilled water		Birch	++
Cow's milk	–	Alder	++
		Ash	+
Epithelia		Hazel	+
Cow	+	Mugwort	–
Cat	++		
Dog	++	Latex	–
Horse	+	Penicillin	–
		Major/minor determinants	
		Streptomycin	–

IgE analyses

The total IgE concentration was 1640 kU/l. The IgE antibody level of the patient's serum to BSA represented RAST class 3 (13.5 kU/l). RAST was negative to seminal fluid, latex, ethylenoxide, chloramine T, and formaldehyde. Other RAST results indicated a polyvalent sensitization to epidermal and animal proteins (Table 2).

Table 2. Results of specific IgE determination given as RAST class (kU/l within parentheses)

Allergen	RAST classes (kU/l)	
	18 months after anaphylaxis	24 months after anaphylaxis
Bovine serum albumin	3 (13.5)	3 (10.5)
Beef (f27)	3 (5.3)	3 (5.0)
Pork (f26)	4 (29.1)	4 (26.6)
Mutton (f88)	n.d.	4 (23.9)
Cat dander (e1)	6 (> 100)	5 (95.4)
Dog epithelium (e2)	5 (82.0)	5 (67.1)
Horse dander (e3)	3 (15.4)	3 (17.1)
Guinea pig epithelium (e6)	n.d.	3 (8.6)
Rabbit epithelium (e82)	n.d.	2 (2.5)
Hamster epithelium (e84)	n.d.	4 (39.2)
Rat (e87)	n.d.	4 (26.2)
Mouse (e88)	n.d.	4 (41.2)
Milk (f2)	3 (8.5)	3 (6.8)
α-Lactalbumin (f76)	0	0
β-Lactoglobulin (f77)	0	0
Casein (e1)	n.d.	0
Fish (cod) (f3)	n.d.	0
Egg white (f1)	n.d.	0

n.d.: not done.

SDS–PAGE and immunoblotting

Figs. 1–4 demonstrate the SDS–PAGE and immunoblotting of purified BSA and the sera of the eight different animals. The most prominent allergen in all tested sera was identified as serum albumin.

A significant IgE immunoreactivity was also observed against several unidentified protein fractions with higher molecular weights than serum albumin, present in the sera from all the different animal species except pig. The reactivity to the latter serum was limited to serum albumin. The immunoblot of cat and dog sera demonstrated additional strong IgE binding to a protein band of 44 kDa. The immunoblotting analyses of nonatopic control serum showed an IgE reaction neither with BSA nor with any animal serum.

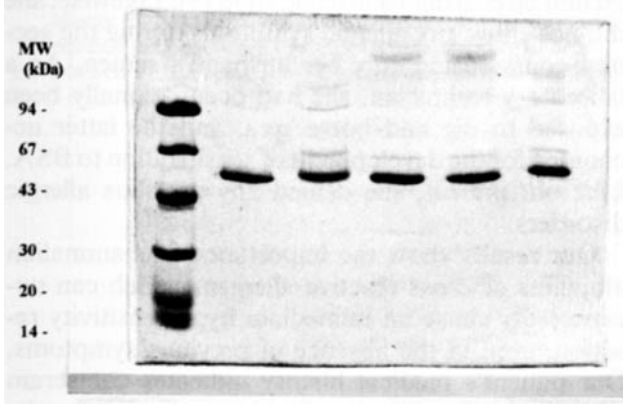

Fig. 1. SDS–PAGE Coomassie brilliant blue staining shows separation of BSA and sera of guinea pig, sheep, goat, and rabbit; 56–60 kDa serum albumin from above-mentioned animal species (MW = molecular weight marker).

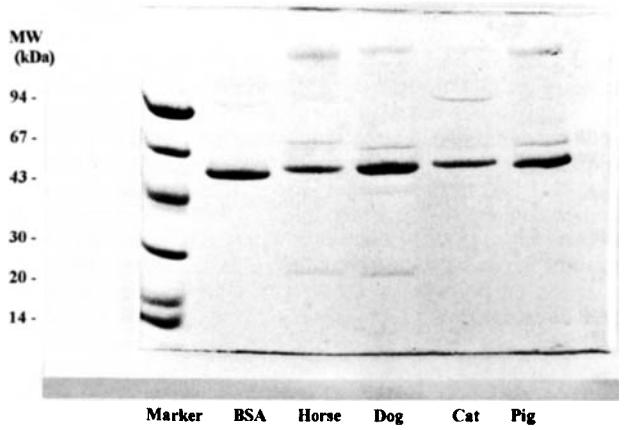

Fig. 2. SDS–PAGE Coomassie brilliant blue staining shows separation of BSA and sera of BSA, horse, dog, cat, and pig; 56–60 kDa serum albumin from above-mentioned animal species (MW = molecular weight marker).

Immunoblot analysis 1

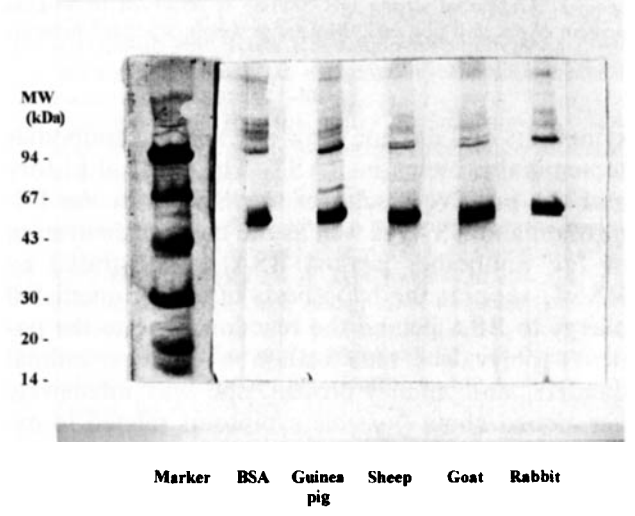

Fig. 3. IgE immunoblots with patient's serum on strips coated with BSA and different animal sera. Patient's serum reacted mainly with serum albumin fraction from all tested animal species. Molecular weight standard proteins are indicated.

Discussion

In the presented patient, BSA in the medium added to the semen for homologous insemination could be identified as the cause of severe anaphylactic reaction. Several cases of adverse reactions to semen have been reported before (4), but this is unlikely in this case, as the patient never had had any postcoital

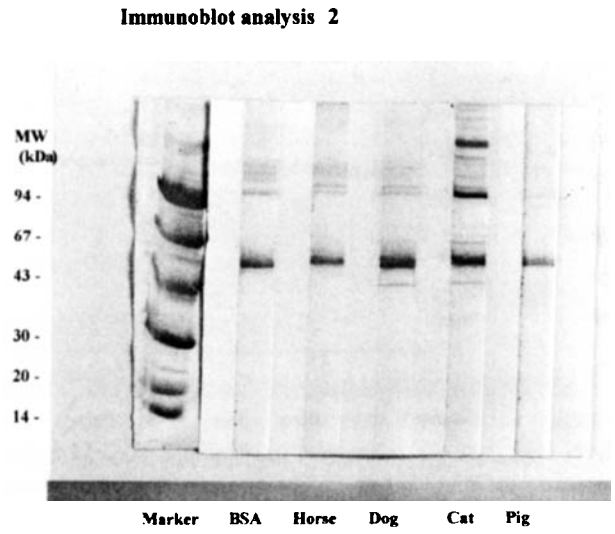

Fig. 4. IgE immunoblot analyses with patient's serum on strips coated with BSA and different animal sera. Patient's serum reacted mainly with serum albumin fraction from all tested animal species. Additional strong IgE binding is observed to 44-kDa protein of cat and dog sera. Molecular weight standard proteins are indicated.

symptoms and did not show any specific antibodies to seminal proteins in RAST. The medical history and the positive results of the SPT with the B2-medium and BSA, as well as the high concentration of IgE antibodies against BSA demonstrated by RAST, support the hypothesis of an IgE-mediated allergy to BSA behind the reaction. Due to the patient's polyvalent sensitization to pollens, animal danders, and animal protein, she was intensively questioned about possible symptoms related to exposure to such allergens. She kept two cats at home and only when cat's saliva came in contact with her scratched skin did pruritus and a moderate wheal appear at this site. She had neither gastrointestinal symptoms related to eating meat or drinking milk nor skin or respiratory complaints. Her past surgical history was also negative.

Since RAST with cow's milk was positive class 3, but negative with the whey proteins α-lactalbumin, β-lactoglobulin, and casein, it can be assumed that the positive RAST to milk was due to BSA (22).

Evidently, as the patient had already reacted after the first course of insemination, she must have developed the sensitization to BSA after natural contact; e.g., by eating meat or drinking cow's milk (16). Another possibility is through inhalative exposure to animal epithelia, dander, or saliva containing serum albumin cross-reactive with BSA. Allergenic cross-reactions between serum and epithelium proteins from various animals are well documented (18, 23, 26). Recently, investigations have confirmed the presence of cross-reactive determinants on cat and dog albumin (6, 21).

Our immunoblot analyses demonstrate an allergen cross-reactivity between serum albumins from different animal species.

Interestingly, only the unusual way of introduction of the unheated, native allergen (BSA), i.e., intrauterine, led here to an allergic reaction. Bovine serum albumin (BSA) is a heat-labile protein. Previous investigations concerning milk allergies (5, 10, 12, 15) have shown a modification of the allergenicity of milk proteins through heating.

On the other hand, local, protective factors in the mucosa of the gastrointestinal tract and the amount of allergen (lower dose by inhalation) could be responsible for the absence of any reaction after oral intake or inhalation of the allergen, despite strong IgE sensitization. Provocation tests with milk and egg have demonstrated a decreasing tissue sensitivity for allergens in the order: skin > nasal > buccal > gastric (2).

During the last few years, a number of patients with serum-sickness-like syndromes due to BSA have been reported (8, 13, 17). Common to all was that the avoidance of contact with bovine proteins made the different symptoms disappear and the concentration of antibodies slowly decline.

Two cases of anaphylactic reactions to BSA after initial artificial insemination have been previously published. The report of a woman with a history of asthma, minor surgery, and anaphylactic reactions secondary to penicillin, among other drugs, demonstrates the importance of reviewing the personal history before any intervention (20). A month later, the second course of IUI accomplished with her own heat-inactivated serum as sperm-processing medium, instead of BSA, was well tolerated. The etiology of the sensitization remained unclear.

The resemblance is close to the case of a woman who reacted with urticaria and angioedema to BSA, 30 min after artificial insemination (7). Likewise, she did not show any allergic symptoms during the second course using only her husband's semen. As a laboratory technician, she had occupationally been exposed to pig and horse sera, and the latter accounted for the development of sensitization to BSA. Like our patient, she denied any previous allergic disorders.

Our results show the importance of mammalian albumins as cross-reactive allergens which can unexpectedly cause an immediate hypersensitivity reaction, even in the absence of previous symptoms. Our patient's medical history indicates cat serum albumin as the primary sensitizing allergen for the IgE-mediated reaction to BSA and the following anaphylaxis. In conclusion, these three cases demonstrate the risk of allergic reactions for atopic patients during artificial insemination when heterologous proteins are supplemented. Since a negative

history cannot exclude sensitization through food or inhalation of mammalian epithelia, a preoperative SPT or RAST with the medium to be inseminated is recommended.

Acknowledgments

The authors thank Maria Lundberg, Uppsala, for preparing the different animal sera and performing the SDS-PAGE and immunoblotting, and Dr A. Fontana, Zurich, for referring the patient.

References

1. ALENIUS H, TURJANAMAA H, PALOSUO K, et al. Surgical latex glove allergy. Characterization of rubber protein allergens by immunoblotting. Int Arch Allergy Appl Immunol 1991: 96: 376–80.
2. AMLOT PL, URBANEK R, YOULTEN LJF, et al. Type I allergy to egg and milk proteins: comparison of skin prick tests with nasal, buccal and gastric provocation tests. Int Arch Allergy Appl Immunol 1985: 77: 171–3.
3. BAHNA SL. Allergies to milk. New York: Grune and Stratton, 1980.
4. BERNSTEIN IL, ENGLANDER BE, GALLAGHER JS, et al. Localized and systemic hypersensitivity reactions to human seminal fluid. Ann Intern Med 1981: 94: 459–65.
5. BLEUMINK E, YOUNG E. Identification of the atopic allergen in cow's milk. Int Arch Allergy Appl Immunol 1968: 34: 521–43.
6. BOUTIN Y, HÉBERT J, VRANCKEN ER, MOURAD W. Mapping of cat albumin using monoclonal antibodies: identification of determinants common to cat and dog. Clin Exp Immunol 1989: 77: 440–4.
7. DE BLAY F, TOMB C, VOUILLOT R, et al. Urticaria and angioedema during insemination with fluid containing bovine serum albumin. Contact Dermatitis 1993: 28: 119.
8. GAMBOA P, JAUREGUI I, ANTEPARA I, BARBAZAN MJ. Serum sickness related to current procedures of *in vitro* fertilization – a case report. J Inv Allergol Clin Immunol 1992: 2: 333–4.
9. GOLDMAN AS, ANDERSON DW Jr, SELLERS WA, et al. Milk allergy. I. Oral challenge with milk and isolated milk proteins in allergic children. Pediatrics 1963: 32: 425–43.
10. GOLDMAN AS, SELLERS WA, HALPERN SR, et al. Milk allergy. II. Skin testing of allergenic and normal children with purified milk proteins. Pediatrics 1963: 32: 572–9.
11. JOLIAT TL, WEBER RW. Occupational asthma and rhinoconjunctivitis from inhalation of crystalline bovine serum albumin powder. Ann Allergy 1991: 66: 301–3.
12. JOST R. Physicochemical treatment of food allergens: application to cow's milk proteins. In: SCHMIDT E, ed. Food allergy. New York: Raven Press: 1988: 17, 199–207.
13. LEFVERT AK. Recurrent angioedema caused by circulating immune complexes containing antibodies against bovine proteins. Int Arch Allergy Appl Immunol 1993: 102: 112–16.
14. MACY E, BULPITT K, CHAMPIN R, SAXON A. Anaphylaxis to infusion of autologous bone marrow: an apparent reaction to self, mediated by IgE antibody to bovine serum albumin. J Allergy Clin Immunol 1989: 83: 871–5.
15. MCLAUGHLAN P, ANDERSON KJ, WIDDOWSON EM, COOMBS RRA. Effect of heat on the anaphylactic-sensitising capacity of cow's milk, goat's milk and various infant formulae fed to guinea pigs. Arch Dis Child 1991: 56: 165–71.
16. MONERET-VAUTRIN DA. Hypersensitivity to milk in adults: present aspects. In: DELMONT J, ed. Milk intolerances and rejection. Nice: Karger, 1983: 138–41.
17. MONERET-VAUTRIN DA, WAL JM, GUILLET-ROSSOF F, et al. Bovine serum albumin immunization: a new risk of allergy protocols for *in vitro* fertilization. Allergy 1991: 46: 228–34.
18. OHMAN JL Jr, BLOCH KJ, KENDALL S, LOWELL FC. Allergens of mammalian origin. IV. Evidence for common allergens in cat and dog serum. Clin Allergy 1976: 57: 560–8.
19. ROTHBERG RM, FARR RS. Antibovine serum albumin and anti-alpha-lactalbumin in the serum of children and adults. Pediatrics 1965: 35: 571–88.
20. SOHNENTHAL KR, MCKNIGHT T, SHAUGHNESSY MA, et al. Anaphylaxis during intrauterine insemination secondary to bovine serum albumin. Fertil Steril 1991: 56: 1188–91.
21. SPITZAUER S, SCHWEIGER C, SPERR WR, et al. Molecular characterization of dog albumin as important cross-reactive indoor allergen [Abstract]. Allergy 1993: 48 (Suppl. 16): 137.
22. STÖGER P, WÜTHRICH B. Type I allergy to cow milk proteins in adults. Int Arch Allergy Appl Immunol 1993: 102: 399–407.
23. SZEPFALUSI Z, EBNER CH, URBANEK R, et al. Detection of IgE antibodies specific for allergens in cow milk and cow dander. Int Arch Allergy Appl Immunol 1993: 102: 288–94.
24. VERDAGUER J, COROMINAS M, BAS J, et al. IgE antibodies against bovine serum albumin in a case of eosinophilic gastroenteritis. Allergy 1993: 48: 542–6.
25. WAHN U, PETERS T, SIRAGANIAN RP. Allergic and antigenic properties of bovine serum albumin. Mol Immunol 1981: 18: 19–28.
26. YMAN L, BRANDT R, PONTERIUS G. Serum albumin – an important allergen in dog epithelia extracts. Int Arch Allergy Appl Immunol 1973: 44: 358–68.