
Case report

Anaphylactic reaction after artificial insemination

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Background: Bovine seroalbumin is known as an allergen for human beings, but reactions to it in an artificial insemination procedure are much rarer. We report a case of anaphylaxis after intrauterine insemination (IUI) in which sensitization to bovine serum albumin (BSA) is demonstrated.

Objective: Report the allergy evaluation performed in a patient who suffered a severe reaction immediately after an IUI procedure.

Methods: A 33-year-old woman was referred because of an anaphylactic reaction after a second trial of IUI. She developed pruritus, abdominal pain, nausea and vomiting, bronchospasm, and generalized urticaria. She had an atopic medical history of pollen allergy and sensitization to cat epithelium. She had never had trouble with minor surgery and she usually uses latex material. She had never received heterologous sera before. Her husband's semen for the IUI was processed in a standard fluid medium called upgraded INRA B2 (Laboratoires CCD, Paris, France), which contains amino acids, lipids, vitamins, BSA, penicillin, and streptomycin in addition to inorganic salts.

Results: Skin prick tests with the medium and BSA 10 mg/mL were positive. In vitro studies demonstrated an immunoglobulin E binding protein of 60 to 65 kDa and mast cells and basophil activation (CD63 expression) against BSA contained in the medium. Cutaneous and challenge tests with penicillin and streptomycin were negative.

Conclusions: We consider the BSA in the semen culture medium to be the factor which triggered the anaphylactic reaction. This case supports the authors who state that media free from heterologous proteins should be used for human application, especially on atopic patients, to avoid sensitization.

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INTRODUCTION

Anaphylaxis is an immediate and life-threatening reaction resulting from the release of bioactive mediators from mast cells and basophils. Such reactions have already been described in cases of intrauterine insemination (IUI) or in vitro fertilization because of sensitization to penicillin,¹ streptomycin,² or bovine serum albumin (BSA).^{3–8} In this kind of practice serum sickness-like syndrome has also been described as a cause of failure.^{9,10}

We report a case of sensitization to BSA which is considered to be the origin of the anaphylactic reaction suffered by the patient after a second trial of IUI performed for infertility. In addition to in vivo tests, we performed a complete in vitro study: specific immunoglobulin (Ig)E determination of BSA-related antigens, sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) immunoblotting, enzyme aller-

gosorbent test (EAST) inhibition tests, histamine release determination, antigen-specific sulfidoleukotriene (sLT) production, and basophil activation test.

CASE REPORT

A 33-year-old woman was referred for allergologic evaluation because of an anaphylactic reaction during an artificial insemination procedure. The patient had undergone her first IUI with her husband's sperm 1 year earlier, without any significant clinical symptoms, but the procedure was not successful. On the second occasion, several minutes after insemination she developed generalized pruritus, abdominal pain, nausea, and vomiting followed by bronchospasm and urticaria. She is an atopic patient suffering from pollen allergy and bronchial asthma attributable to cat epithelium, but without a previous history of drug or food allergy, although she reported hand edema when touching fresh rabbit meat. She usually uses latex material, and she had not been operated on or received heterologous sera before. The semen for the IUI was processed in a standard fluid medium called upgraded INRA B2 (Laboratoires CCD, Paris, France), which contains inorganic salts, organic compounds (different amino

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acids, lipids, vitamins, and BSA), and antibiotics (penicillin and streptomycin).

MATERIAL AND METHODS

Skin Tests

Skin prick tests (SPTs) were performed with routine inhalant allergens including cat, dog, cow, pig, and horse epithelia and latex (ALK-Abelló, Madrid, Spain), different food extracts including cow's milk, beef, pork, rabbit, and lamb (Leti, Barcelona, Spain), using a standardized needle (ALK-Abelló). The major and minor determinants of penicillin were tested (SPT and intracutaneous) with commercially available extracts (Allergopen, Allergopharma, Reinbek, Germany). SPT, prick, and intracutaneous tests for reactions to streptomycin (10 mg/mL), SPT to the fluid medium for sperm processing (upgraded INRA B2) as well to BSA (10 mg/mL) were carried out. They were also performed on 5 atopic and 5 nonatopic control subjects. Histamine was used as the positive control (1 mg/mL for SPT and 0.1 mg/mL for intradermal test) and saline solution as a negative control.

Results of the skin testing were evaluated 15 minutes after the prick and they were considered positive when the diameter of the wheal was at least 3 mm larger than that of the negative control.

Specific IgE Measurement and Inhibition Assay

Total serum IgE was determined by radioimmunoassay. Specific serum IgE to cat dander, pig, and cat seroalbumin, rabbit serum protein, human and bovine seroalbumin 10 mg/mL, and formaldehyde and ethylene dioxide was measured by CAP-System Pharmacia (Pharmacia, Uppsala, Sweden) following the manufacturer's instruction and EAST. Results were expressed in kU/L and values less than 0.35 kU/L were taken to be negative results. The possible cross-reaction between cat epithelium and culture medium extracts was evaluated by means of EAST inhibition technique using Bial-Aristegui disks (Bial-Aristegui, Bilbao, Spain) with the extracts coupled, following the method reported by Yman et al.¹¹ The development was carried out with the HY-TEC EIA kit for specific IgE (Hycor Biomedical, Garden Grove, CA) following the manufacturer's instructions.

SDS-PAGE and Immunoblotting

SDS-PAGE was carried out according to Laemmli's method,¹² and 12.5% and 4% were used for separating and stacking gel, respectively. The samples were prepared in 0.125 M HCl-Tris pH 6.8 and were dissociated with 0.1% SDS and 5% β -mercaptoethanol at 100°C for 5 minutes. Twenty μ g of protein, as described by Bradford,¹³ was applied per lane, and after electrophoresis, gels were stained with the Coomassie brilliant blue R-250 staining method. When immunoblotting was performed, proteins were electrophoretically transferred to Immobilon-P transfer polyvinylidene fluoride membranes (PVDF membranes) essentially as described by Towbin et al.¹⁴ After blocking the membrane with Tween-20 0.05%, it was incubated with 0.5 mL patient's serum and a chemolu-

minescent detection reagent (Lumigen PS-3; Pharmacia) was used for developing (Western blotting detection system ECL+Plus Amersham Biosciences; Piscataway, NJ).

Histamine Release Test (HRT)

In vitro antigen-specific histamine release using complete blood was determined by fluoroenzyme immunoassay¹⁵ with a Bran Lubbe autoanalyzer (Evanston, IL). The antigens were upgraded INRA B2 medium (1/10, 1/100), pork and beef extracts, α -lactoalbumin, β -lactoglobulin, cat epithelium, and latex extracts (Bial Aristegui) at 20 μ g/mL as well as BSA (250, 25, 2.5, 0.25 μ g/mL). Total histamine release with buffer was used as the negative control and anti-IgE anti- ϵ (4.5 ng/mL) (Caltag, Burlingame, CA) as the positive control.

Interpretation of the results was carried out using the formula:

$$\% \text{ histamine release} = \frac{\text{Ag-basal}}{\text{total-basal}} \times 100$$

where Ag = histamine release by the allergen; basal = spontaneous histamine release; total = total histamine content in basophils after treatment with perchloric acid 2.5%. Histamine release greater than 10% was considered positive.

sLT Production

Antigen-specific sLT production was measured by means of cellular assay stimulation test (Bühlmann, Allchwill, Switzerland) following the manufacturer's instructions with the same allergens as in HRT. Basal production of sLTs and production with Bühlmann anti-IgE anti-receptor at 0.2 μ g/mL were measured as negative and positive controls, respectively.

Basophil Activation Test

The basophil activation test originally developed by Sainte Laudy et al¹⁶ determines the percentage of basophils which express CD63 as an activation marker, by means of flow cytometry, after in vitro stimulation with allergen and using double-labeling with monoclonal antibody anti-CD63 phycoerythrin and anti-IgE fluorescein isothiocyanate. We performed a modified test.¹⁷ Briefly, isolated leukocytes were incubated 40 minutes at 37°C with BSA at 25, 2.5, and 0.25 μ g/mL concentrations and upgraded INRA B2 medium (1/1, 1/10, 1/100), pork meat 1.25 mg/mL, beef meat 1.25 mg/mL, α -lactoalbumin 1.25 mg/mL, β -lactoglobulin 1.25 mg/mL, and cat dander 5 μ g/mL. We measured in parallel the basal response (negative control) and anti-IgE response (positive control).

Drug Challenge Tests

Controlled single-blind challenge tests were performed on separate days with:

- 1) penicillin (phenoxymethylpenicillin potassic salt; Peni-level, ERN Laboratories, Barcelona, Spain) using oral doses: 150,000 IU = 100 mg, 300,000 IU = 200 mg, and 600,000 IU = 400 mg, separated 15 minutes each;

- 2) streptomycin (Estreptomycin Ceba, Ceba Laboratories, Madrid, Spain) using intramuscular unique dose 1 g; and

3) vitamin complex (Redoxon Complex, Roche Laboratories, Madrid, Spain) containing A, B₁, B₂, B₆, B₁₂, C, D, E, and H vitamins, using one complete unique oral dose.

RESULTS

Skin Tests

SPTs demonstrated an immediate hypersensitivity to various pollens (*Olea europaea*, *Secale cereale*, *Lolium perenne*, and *Cynodon dactylon*) and mammalian epithelia (cat and dog dander; Table 1). The SPT was also clearly positive to the undiluted upgraded INRA B2 medium and to BSA solution (10 mg/mL), both of which were negative for the atopic and nonatopic control subjects. However, SPT was negative to latex, house-dust mites (*Dermatophagoides pteronyssinus* and *farinae*, *Tyrophagus putrescentiae*, and *Lepidoglyphus destructor*) and mold extracts (*Alternaria alternata* and *Cladosporium*), as well as to another semen culture medium containing human serum albumin (HSA) instead of BSA (SIL Select, Ferti Pro N.V., Beernem, Belgium). Prick and intracutaneous tests for major and minor determinants of penicillin (benzylpenicilloyl polylysine, minor determinant mixture) and streptomycin were also negative. We obtained positive SPT to commercial extracts of beef, rabbit, and lamb (tested because of their extensive consumption in the Mediterranean diet), and also to commercial cow's milk extract, although the test was negative for each of its main proteins (casein, lactalbumin, and lactoglobulin) other than BSA.

Table 1. Prick-Test Diameter (mm Wheal) for Different Antigens Tested and Specific IgE Determined by CAP System Pharmacia

Ag	Prick-test diameter (mm)	Specific IgE-CAP System (kU/L)
<i>Olea europaea</i>	5	*
<i>Secale cereale</i>	11	*
<i>Lolium perenne</i>	10	*
<i>Cynodon dactylon</i>	12	*
Cat dander	9	110
Rabbit meat	13	*
Cow milk	4	*
Pork meat	20	*
Lamb meat	11	*
Beef meat	12	*
Pig seroalbumin	*	14.1
Cat seroalbumin	*	26.3
Rabbit serum proteins	*	7.24
HSA	*	<0.35
BSA (10 mg/mL)	7	3.24
Upgraded INRA B2	12	*
SIL Select	0	*
Formaldehyde	*	<0.35
Ethylene dioxide	*	<0.35

*, Non-tested.

Ag, histamine release by the allergen; Ig, immunoglobulin; HSA, human serum albumin; BSA, bovine serum albumin.

IgE Analysis

Total IgE concentration was 2,810 kU/L. The IgE antibody level of the patient's serum to BSA represented 3.24 kU/L, 26.3 kU/L to cat seroalbumin, 14.1 kU/L to pig seroalbumin, 7.24 kU/L to rabbit serum proteins, 4.7 kU/L to raw lamb meat extract, <0.35 kU/L to cooked lamb meat extract, 4.9 kU/L to raw rabbit meat extract, 1.63 kU/L to cooked rabbit meat extract, 1 kU/L to raw calf meat extract, <0.35 kU/L to cooked calf meat extract, 6 kU/L to culture medium extract, 110 kU/L to cat epithelium, and <0.35 kU/L to HSA (Table 1). CAP was negative to seminal fluid, latex, ethylene dioxide, formaldehyde, and penicillins G and V (data not shown).

SDS-PAGE Immunoblotting and EAST Inhibition Tests

An IgE-binding band of 60 to 65 kDa was revealed by SDS-PAGE immunoblotting in every sample studied except the HSA (Fig 1). EAST inhibition assays were performed using culture medium extract and cat epithelium extract as solid phase and both extracts and BSA as free phase (0.0001 to 1 mg/mL). Neither BSA nor culture medium produced any kind of inhibition when they were used as free phase and cat epithelium extract as solid phase. However, cat epithelium produced 75% inhibition when culture medium extract was used as solid phase.

HRT

HRT was positive for α -lactoalbumin (44.32%), β -lactoglobulin (32.95%), cat epithelium (71.59%), pork (79.5%), and beef (12.5%), undiluted upgraded INRA B2 (18.18%), and diluted 1/10 (57.95%) and 1/100 (64.77%), and for BSA 25 μ g/mL (56.82%), BSA 2.5 μ g/mL (63.07%), BSA 0.25 μ g/mL (59.09%; Table 2).

Antigen-Specific sLT Production

Positive production of sLT stimulated by BSA, INRA medium, pork and beef extract, α -lactoalbumin, β -lactoglobulin, and cat epithelium extract was found (Table 2).

Basophil Activation Test.

Positive basophil activation stimulated by INRA medium at three different concentrations (33.6, 67.1, 85.6%), BSA (77.87, 89.4, 84.1%), pork meat extract (90.63%), cow's milk extract (87.35%), and cat epithelium extract (92.97%) was found, with a basal activation of 4.44% and the one obtained by IgE of 90.4% (Table 2; Fig 2).

Drug Challenge Tests

Challenge tests performed with penicillin, streptomycin, and vitamins were negative.

DISCUSSION

Immunologic reactions during IUI have been reported as a cause of unexpected problems in assisted reproduction. Both serum sickness^{9,10} and allergic reactions to bovine seroalbumin³⁻⁸ have been described. In our study, high serum levels of IgE antibody against BSA and semen culture medium extract have been found; SDS-PAGE immunoblotting of BSA, culture medium extract, and various animal meat extracts re-

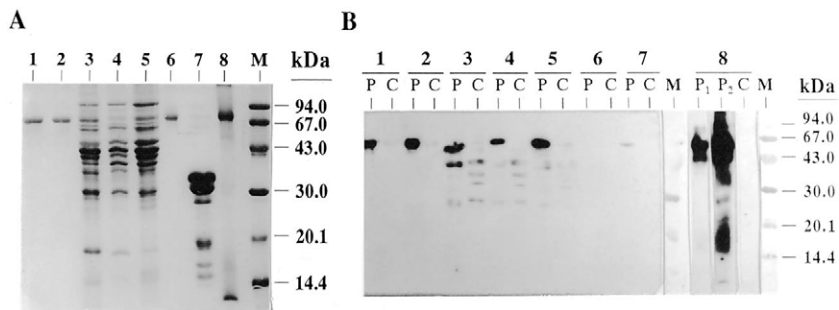


Figure 1. A) SDS-PAGE after Coomassie brilliant blue R-250 staining. Lane 1, Culture medium extract; lane 2, BSA; lane 3, raw beef extract; lane 4, raw lamb extract; lane 5, raw rabbit extract; lane 6, HSA; lane 7, cow's milk extract; and lane 8, cat epithelium extract. M, molecular mass pattern. B) SDS-PAGE immunoblotting. Lane numbers as in Coomassie gel. P, patient's serum; C, control serum (pool from nonatopic subjects' sera); P1, patient's serum diluted 1/60; P2, patient's serum diluted 1/40.

Table 2. Histamine Release Test (HR), Sulfidoleukotriene Production (sLT, CAST Technique), and Basophil Activation Test (BAT; Percentages)

	sLT (pg/mL)	HR (%)	BAT (%)
Basal	188	—	4.44
Anti-IgE	5,848	25	90.4
BSA 25 μ g/mL%	2,581	56.82	77.87
BSA 2.5 μ g/mL%	2,738	63.07	89.13
BSA 0.25 μ g/mL%	3,203	59.09	84.19
INRA	414	18.18	33.6
INRA 1/10	1,214	57.95	67.1
INRA 1/100	2,328	64.77	85.6
Pork meat 1.25 mg/mL	2,608	79.55	90.63
Beef 1.25 mg/mL	865	12.5	—
α -lactoalb 1.25 mg/mL	3,914	44.32	87.35
β -lactoglob 1.25 mg/mL	3,544	32.95	—
Cat dander 5 μ g/mL	1,931	71.59	92.97

BSA, bovine serum albumin; Ig, immunoglobulin.

vealed an IgE-binding band of 60 to 65 kDa, which could mean that probably the albumin-specific IgE found in the patient's serum recognizes some common albumin epitopes present in cow, rabbit, and lamb albumins. This "recognition" could be the cause of cross-reactivity between them, but it is not present in HSA. Further, HRT, activation of basophils expressing CD63 postantigen stimulation, and antigen-specific production of sLTs showed a high activation of mediator-releasing cells when exposed to BSA. As allergic reactions to penicillin¹ and streptomycin² during in vitro fertilization and IUI are known, both possibilities were ruled out, as were vitamin reactions, by negative results. All in vitro findings as well as the medical history and the in vivo tests (positive SPT with upgraded INRA B2 medium and BSA, and positive results with cow's milk extract but negative with each protein component apart from albumin) strongly suggest that BSA in the medium added to the semen for insemination is the allergen responsible for the anaphylactic reaction.

The literature reports four other reactions during IUI procedures mediated by IgE antibodies against bovine seroalbumin using different laboratory techniques. The first one was described in 1991 by Sonenthal et al,⁴ who detected specific IgE against BSA from the semen culture by enzyme-linked immunoadsorbent assay and enzyme-linked immunoadsor-

bent assay-inhibition procedures. de Blay et al⁵ in 1993 had a BSA sensitization in a patient who was a laboratory technician handling pig and horse sera, which were considered the sensitizing agents. Prick-test and specific IgE to BSA from the semen culture were positive, as were those to albumin extracts or sera of pig, horse, cat, dog, and bovine origin. The whole study made with penicillin and streptomycin contained in the medium was negative, as in our own case. Wüthrich et al⁶ in 1995 identified by SDS-PAGE a common allergen among different mammalian sera (beef, pork, and mutton) corresponding to BSA 56 to 60 kDa. The patient reacted with the albumin fraction from the animal sera. This was a first attempt at insemination but, as in our case, the patient had had a cat at home for a long time. Matheu et al⁷ identified another case of sensitization to BSA in a patient experiencing anaphylaxis after the first IUI attempt with her husband's seminal fluid. Specific IgE determination to BSA present in the semen culture medium INRA Menezzo B2 (Laboratoires CCD) done by immunoblotting and inhibition studies identified it as the allergen which was presumably responsible.

Our patient is polysensitized, and at the time of the reaction she had a cat at home and suffered asthma attacks after pet contact. She had never had gastrointestinal symptoms when eating any kind of meat or drinking milk. As the first course of insemination was performed with a semen culture without BSA, the anaphylactic reaction after the second course may well have been produced by natural previous contact with some animal albumin: eating meat (often undercooked as is common in the north of Spain) or drinking milk, but in our opinion, mainly, by permanent contact with cat epithelium or cat saliva containing serum albumin that is cross-reactive with BSA. This idea is reinforced by the EAST inhibition results performed: 75% inhibition was obtained when culture medium extract was used as solid phase and cat epithelium extract as free phase, whereas no inhibition was obtained when cat epithelium was used as solid phase and BSA or medium extract was used as free phase. These results and levels of specific IgE to different animal albumins found (the highest value to cat albumin and the lowest to bovine albumin), point to cat albumin as the most likely sensitizing agent to have cross-reactivity with BSA. Thus, we should say cat-allergic patients may be particularly at risk of such reactions to BSA, but we have also detected some specific IgE

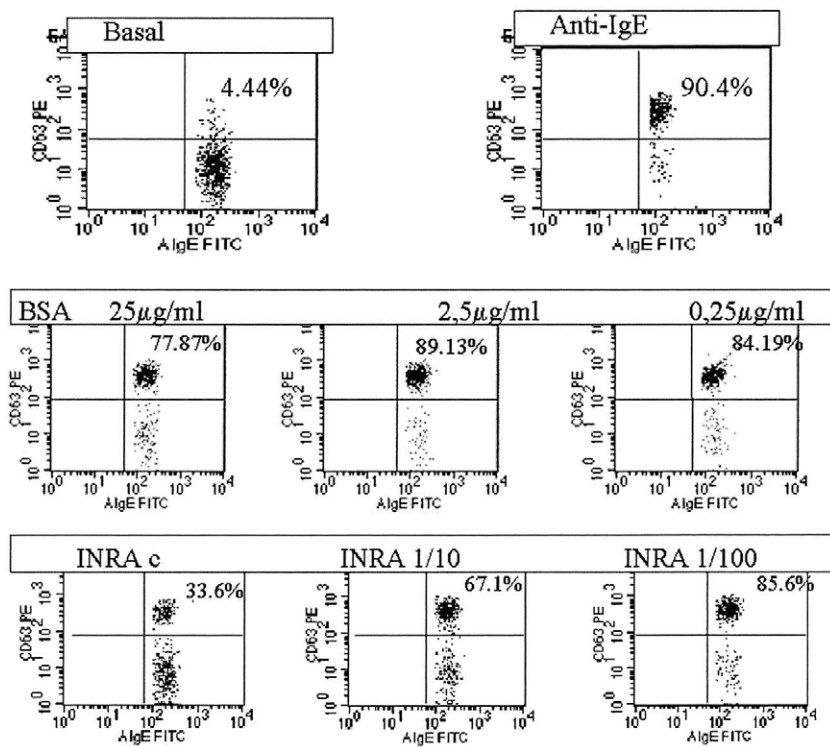


Figure 2. Basophil activation test results determined by means of flow cytometry, after in vitro stimulation with allergen and using double-labeling with monoclonal antibody anti-CD63 phycoerythrin and anti-IgE fluorescein isothiocyanate.

antibodies from patient serum likely to recognize common structures present in serum albumin from different mammalian species (cow, rabbit, sheep), not present in HSA as tests in vitro (EAST, immunoblotting) and in vivo (SPT) show.

Basophil activation tests also showed an important cellular activation when patient blood was exposed to BSA at different concentrations, and also when using pork, beef, and lamb as allergens because of the similarity of their albumins. The sensitization the patient presents to BSA is so high the activation of basophils is maximal even when testing the lowest BSA concentration in vitro, with no observation of a dose-response effect. We can conclude that atopic patients with allergy to mammalian epithelia could be at risk of developing such reactions to BSA allergen. The non-meat-allergic state of the patient and the decrease in levels found between specific IgE values for raw and cooked meat extract reflect the thermosensitive character of these epitopes.^{3,18}

CONCLUSION

Regarding the results of the in vitro study performed by this group, to our knowledge the most complete way of identifying BSA as a relevant allergen, the techniques used are useful for the diagnosis of some allergic reactions where there is some difficulty in identifying the responsible allergen. We support the hypothesis expressed by other authors affirming that artificial insemination procedures with semen culture media containing potential allergens such as BSA represent an unnecessary risk especially to atopic females.⁴⁻⁸ Preoper-

ative SPTs with the medium are recommended as they have been demonstrated to be useful. Thus, our patient had negative SPTs with another medium not containing BSA, and she did not have any trouble in her subsequent IUI attempt after culturing semen with this medium. She is now the mother of a healthy, although atopic, child.

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REFERENCES

1. Smith YR, Hurd WW, Menge AC, et al. Allergic reactions to penicillin during in vitro fertilization and intrauterine insemination. *Fertil Steril* 1992;58:847-849.
2. Abeck D, Kuwert C, Segnini-Torres M, et al. Streptomycin-induced anaphylactic reaction during in vitro fertilization (IVF). *Allergy* 1994;49:388-389.
3. Martínez-Alonso JC, Moneo I, Gomez M, Alday E. Hipersensibilidad a carne de cordero y reacción cruzada a otros mamíferos. *Rev Esp Alergol Inmunol Clin* 1998;13:359-362.
4. Sonenthal K, McKnight T, Shaughnessy M, et al. Anaphylaxis during intrauterine insemination secondary to bovine serum albumin. *Fertil Steril* 1991;56:1188-1191.
5. de Blay F, Tomb R, Vouillot C, et al. Urticaria and angioedema during insemination with fluid containing bovine serum albumin. *Contact Dermatitis* 1993;28:119.
6. Wüthrich B, Stern A, Johansson SG. Severe anaphylactic reac-

-
- tion to bovine serum albumin at the first attempt of artificial insemination. *Allergy* 1995;50:179–183.
7. Matheu V, Caloto M, De Barrio M, et al. Life-threatening anaphylaxis after artificial insemination. *Lancet* 2002;359:1779.
 8. Moneret-Vautrin A, Wal JM, Guillet-Rossof F, et al. Bovine serum albumin immunization: a new risk of allergy during protocols for in vitro fertilization. *Allergy* 1991;46:228–234.
 9. Moneret-Vautrin A, Guillet-Rossof F, Gérard H, et al. Maladie sérique liée aux protocoles de fécondation in vitro responsabilité de la serumalbumine bovine. *Presse Med* 1989;18:1932–1933.
 10. Gamboa P, Jáuregui I, Antépara I, Barbazan MJ. Serum sickness related to current procedures on in vitro fertilization—a case report. *J Investig Allergol Clin Immunol* 1992;2:333–334.
 11. Yman L, Ponterius G, Brandt R. RAST-based allergen assay methods. *Dev Biol Stand* 1975;29:151–165.
 12. Laemmli UK. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* 1970;227:680–685.
 13. Bradford MM. A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principles of protein-dye-binding. *Anal Biochem* 1976;72:248–254.
 14. Towbin H, Staehelin I, Gordon J. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedures and applications. *Proc Natl Acad Sci USA* 1979;76:4350–4354.
 15. Siraganian RP, Brodsky MJ. Automated histamine analysis for in vitro allergy testing. I. A method utilizing allergen-induced histamine release from whole blood. *J Allergy Clin Immunol* 1976;57:525–540.
 16. Sainte Laudy J, Sabbah A, Vallon C, Guerin JC. Analysis of anti-IgE and allergen induced human basophil activation by flow cytometry. Comparison with histamine release. *Inflamm Res* 1998;47:401–408.
 17. Sanz ML, Sánchez G, Vila L, et al. Allergen-induced basophil activation: CD63 cell expression detected by flow cytometry in patients allergic to *Dermatophagoides pteronyssinus* and *Lo-lium perenne*. *Clin Exp Allergy* 2001;31:1007–1013.
 18. Fiocchi A, Restani P, Riva E, et al. Heat treatment modifies the allergenicity of beef and bovine serum albumin. *Allergy* 1998;48:798–802.

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