

# Differences in Response of *Staphylococcus aureus* protein to serum IgG and secretion of Uterine cervical s-IgA from Fertile and Infertile Couples Women with Non-Specific Vaginitis

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The cause of idiopathic infertility approximately 10-30% and allegedly much related to immunological disorders and previous infections. We explore the response of circular and local humoral adaptive immune system at women of the fertile and infertile couple to infection *Staphylococcus aureus* (*S. aureus*) at a lower genital tract of women with nonspecific vaginitis (NSV). Design: Biocomputational and Invitro study. Result: Five *S. aureus* protein have high homology sequences and structures. Analysis of antigenicity, all of the molecule obtain high antigenicity properties and all of *S. aureus* protein express their epitope, only 80 % molecule of human sperm protein does. So all of the proteins almost confirmed that they allow it to be recognized by the same antibodies. Two isolates of *S. aureus* from a fertile women partner (*S. aureus\_fertile*) and infertile women partner (*S. aureus\_infertile*) is used to collect its outer membrane protein (OMP). The circular and local humoral adaptive immune response occur in the women of a fertile and infertile couple experiencing NSV due to *S. aureus*. The molecular weight (MW) of OMP of *S. aureus\_fertile* that was recognized by the s-IgA\_fertile was 52 kDa while the OMP of *S. aureus\_infertile* was recognized by the s-IgA\_infertile was 49 kDa. The molecular weight (MW) of OMP of *S. aureus\_fertile* that was recognized by the IgG\_fertile was 142, 128, 87, 85, 67, 65, 52, 49, 32, 29, 28, and 19 kDa while the OMP of *S. aureus\_infertile* was recognized by the IgG\_infertile was 143, 138, 123, 117, 61, 57, 52, 49, 45, 36, 33, 28, 26, 18, 17, 15, 14, and 11 kDa. They obtain cross-reaction between OMP of *S. aureus\_fertile* and OMP of *S. aureus\_infertile* via both IgG and s-IgA. s-IgA antibodies more specific than IgG antibodies.

**Keywords:** infertility, cross-reaction, *S. aureus*, Immunoglobulin G, secretory-Immunoglobulin A

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## 1. INTRODUCTION

The worldwide prevalence of infertile couples varies between 10-40%. Data from Indonesian Statistical Bureau (2005 and 2010) pointed out the prevalence of infertile couple in some regions in Indonesia at about 17%, East Java 26%, Surabaya 25%, Malang 18% and Mojokerto 25%.<sup>1,2</sup> According to The Mayo Clinic, USA, about 20% of infertility cases are due to problem in the man, 40% to 50% are due to problem in woman and 30% to 40% are due to problem in both man and woman.<sup>3</sup> The cause of idiopathic infertility approximately 10-30% and allegedly much related to

immunological disorders and previous infections.<sup>3,4</sup> Non-specific vaginitis (NSV) occurring at women infertile couples due to polymicrobial. Preliminary study had been held at the Islamic Hospital Hasanah Muhammadiyah Mojokerto East Java Indonesia for 1,5 years, found that the prevalence of *Staphylococcus spp* infection in women of infertile couples is about 37%. *Staphylococcus aureus* (*S. aureus*) contributes by 21%, *Staphylococcus epidermidis* 16%, *Escherichia coli* 21%, *Streptococcus faecalis* 16,3%, *Streptococcus alpha-haemoliticus* 11,2%, and *Candida albicans* 11,2%.<sup>5</sup> We explore the protein of *S. aureus* and protein of human sperm

whether there is cross-reaction between them. So we retrieve an amino acid sequence of *S. aureus* and human sperm from UNIPROT database (<http://uniprot.org>) for the bio-computational research. We have done alignment by BLASTP NCBI ([http://blast.ncbi.nlm.nih.gov/Blast.cgi?PROGRAM=blast&PAGE\\_TYPE=BlastSearch&LINK\\_LOC=blasthome](http://blast.ncbi.nlm.nih.gov/Blast.cgi?PROGRAM=blast&PAGE_TYPE=BlastSearch&LINK_LOC=blasthome)), molecular weight measurement by ProtScale expasy tools NCBI (<http://web.expasy.org/cgi-bin/protscale/protscale.pl>), molecular modeling by i-Tasser (<http://zhanglab.ccmb.med.umich.edu/I-TASSER/>), structure comparing by Superpose v.1.0 (<http://wishart.biology.ualberta.ca/superpose>), Antigenicity analysis by Kolaskar Tongaongkar method ([http://tools.immuneepitope.org/tools/bcell/iedb\\_input](http://tools.immuneepitope.org/tools/bcell/iedb_input)), Epitope mapping by Discotope (<http://tools.immuneepitope.org/stools/discotope/discotope.do>), and viewing the result by chimera (<http://www.cgl.ucsf.edu/chimera/download.html>). We have found between two molecule can recognize each other. After this finding, we have done another explorative study to exhibit that there is molecular similarity between protein of *S. aureus* and protein of human sperm. We have found that protein of *S. aureus* recognize by antibody secretory IgA from uterine cervix of women of infertile couple and from women of fertile couple. Especially protein 49 kDa of *S. aureus* from women of infertile couple and protein 52 kDa of *S. aureus* from women of fertile couple.<sup>5,7</sup> Because of this finding, we want to explore whether the response of circular adaptive humoral immune system at women of the fertile and infertile couple due to infection of *S. aureus* at their lower genital tract, differ with the response of local adaptive humoral immune system at women of the fertile and infertile couple due to infection of *S. aureus* at their lower genital tract.

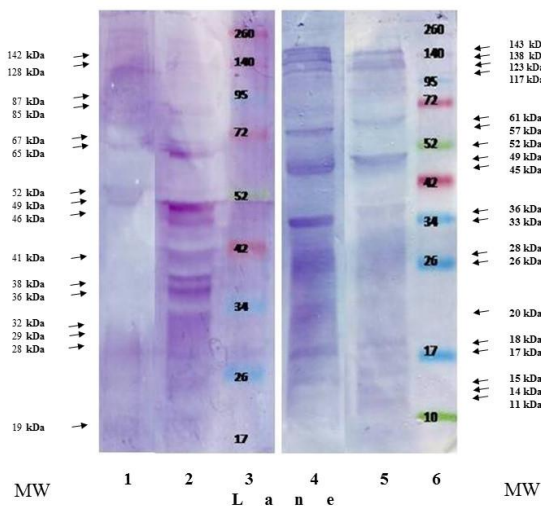


Figure 1 above shows that serum IgG from a woman of fertile couple recognize a band 142, 128, 87, 85, 67, 65, 52, 49, 32, 29, 28, and 19 kDa from Omp of *S. aureus\_fertile* and serum IgG from a woman of infertile couple recognize a band 143, 138, 123, 117, 61, 57, 52, 49, 45, 36, 33, 28, 26, 18, 17, 15, 14, and 11 kDa from Omp of *S. aureus\_infertile* by western blot. Cross-reaction between IgG\_fertile (Omp of *S. aureus\_fertile*) with Omp of *S. aureus\_infertile* and cross-

## 2. EXPERIMENTAL DETAILS

The design of the study is Invitro research. The *S. aureus\_fertile* and the *S. aureus\_infertile* isolates used in this research are thawed and then cultured from preliminary study stock that have been collected and frozen at - 20°C. The outer membrane protein (OMP) of both the *S. aureus\_fertile* and the *S. aureus\_infertile* is isolated by Glucose-EDTA-Tris pH 8 solution. The OMP's of those *S. aureus* are run by electrophoresis. Then we did blotting analysis by dot blotting (the data isn't shown here) and western blotting to evaluate circular adaptive humoral immune response. An immunoglobulin which we used to evaluate the circular adaptive humoral immune response, we have isolated from blood serum from women of the fertile and infertile couple that are collected at preliminary study. All of the participants in this study have asked their permission and they have agreed and have signed agreement form. This research has obtained ethics approval from Ethical Clearance Board of Brawijaya University. To compare the finding of this research, we use the finding of second research ie western blotting of protein of *S. aureus* with secretory IgA from cervical mucus of uterine cervix of both the women of fertile couple and the women of infertile couple as control history.<sup>6</sup>

## 3. RESULTS AND DISCUSSION

The result of this in-vitro research is presented as western blotting picture at figure 1 below.

**Figure 1 Circular Adaptive Humoral Immune Respons of *S. aureus* Protein to serum IgG from Women of Fertile and Infertile Couple.** Lane 1: OMP *S. aureus\_fertile* with serum IgG from a woman of the fertile couple. Lane 2: OMP *S. aureus\_infertile* with serum IgG from a woman of the fertile couple. Lane 4: OMP *S. aureus\_fertile* with serum IgG from a woman of the infertile couple. Lane 5: OMP *S. aureus\_infertile* with serum IgG from a woman of the infertile couple. Lane 3 and 6: protein marker. MW: molecular weight.

reaction between IgG\_infertile (Omp of *S. aureus\_infertile*) with Omp of *S. aureus\_fertile* also detected at that western blot.

As the result of the preliminary study, we mention that there are five kinds of microorganism isolated from non-specific vaginitis woman of the infertile couple. These are *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Escherichia coli*, *Streptococcus faecalis*, and *Streptococcus*

*alpha-haemoliticus*.<sup>5</sup> One of five microorganisms i.e. *Staphylococcus aureus* has high prevalence and the spouse of that categories have rules to be pregnant. So we make research by the bio-computational approach. In this study, bio-computational approach shows that Omp of *S. aureus* can cross-react with Omp of human sperm detected by sequential homology and structural homology. This phenomenon is called molecular mimicry.<sup>5,6</sup>

There is 57% homology sequence from 96 amino acids sequence of human sperm to *S. aureus*, and 5% highest homology as a list at Table 1. Homology range of protein glyceraldehyde-3-phosphate dehydrogenase from human sperm and *S. aureus* is the amino acid sequence 4–327; the amino acid sequence range of homology protein L-lactate dehydrogenase C (human sperm) vs Lactate Dehydrogenase C (*S. aureus*) is in 8–309; protein deglycase DJ-1 (human sperm) vs hypothetical protein1 *S. aureus* the homology range of amino acid sequence is at 2-166; the homology amino acid sequence of sperm acrossome membrane-associated protein 4 vs hypothetical protein2 *S. aureus* is in the range 4-35; and the homology range of amino acid sequence from UDP-N-acetyl hexosamine pyrophosphorylase (human sperm) vs Uridyl transferase (*S. aureus*) is at 7-389. Forty percent of five molecules have highest similarity on its structure (0,312 & 0,332 Å), 40% rather similar, and 20% less similar. The molecular weight of human sperm protein that measured by Protscale Expassy tools range between 13–58 kDa, and molecular weight of *S. aureus* protein range between 18–46 kDa.<sup>6</sup>

Antigenicity properties of all the protein molecule of *S. aureus* are high, and also all the protein molecule of human sperm checked it by kolaskar tongaongkar method. All the protein molecule of *S. aureus* (100%) express their epitope. But not all the protein molecule of human sperm express their epitope, one of them (20%) is molecule *L-lactate dehydrogenase C*.<sup>6</sup>

*Molecular mimicry* is also obtained between tubulin protein in *axoneme principle piece* of human sperm with protein from *E. coli* (strain UTI89), *Morganella morganii*, *Ureaplasma urealyticum*, and *Enterococcus faecalis*.<sup>8</sup> The same researcher also discover a correlation between *Haemophilus pylori* strain CagA+ infection with decrease the quality of human sperm.<sup>9</sup> The smallest molecular weight of human sperm protein and *S. aureus* protein are 13 kDa and 18 kDa, respectively. Protein has immunogenic properties if its molecular weight more than 10 kDa.<sup>10</sup> Both *S. aureus* and human sperm show high antigenicity so that they can stimulate an immune response to produce antibody. Because of they have high similarity both sequence and structure, so the antibody that produced will be similar. Almost all the Omp both *S. aureus* and human sperm show their epitope, only one molecule Omp of human sperm hide its epitope. So almost all the Omp in pairs can produce an antibody that recognizes both proteins, each other.<sup>6</sup>

To prove the result of that bio-computational research we do the in-vitro study. We evaluate the immune response to OMP of *S. aureus*, an especially adaptive humoral immune response both local from uterine cervix secretion. Uterine cervical s-IgA of a woman of the fertile and infertile couple can recognize both OMP of *S. aureus\_fertile* and OMP of *S. aureus\_infertile*.<sup>5</sup> Uterine cervical s-IgA from a woman of fertile couple recognize a band 52 kDa from *S. aureus\_fertile* and uterine cervical s-IgA from a woman of infertile couple recognize a band 49 kDa from Omp of *S. aureus\_infertile* by

western blot. Cross-reaction between s-IgA\_fertile (Omp of *S. aureus\_fertile*) with Omp of *S. aureus\_infertile* and cross-reaction between s-IgA\_infertile (Omp of *S. aureus\_infertile*) with Omp of *S. aureus\_fertile* also detected at that western blot.<sup>5,7</sup> Also, serum IgG of woman of the fertile and infertile couple can recognize both OMP of *S. aureus\_fertile* and OMP of *S. aureus\_infertile*.

But western blot on s-IgA against OMP of *S. aureus\_fertile* and *S. aureus\_infertile* only shows two protein bands. It occurs because at a lower genital tract of a woman of the fertile and infertile couple contains s-IgA produced from basal lamina of a lower genital tract of women\_fertile and women\_infertile. OMP of *S. aureus\_fertile* and *S. aureus\_infertile* stimulate immune cells at that site of infection to produce IgG converted to IgA by somatic hypermutation after conjugation by J chain then secreted as s-IgA in the lumen of women reproductive tract.<sup>10</sup>

If we take a look at the result of adaptive humoral immune response both local<sup>5</sup> and circular on the result of western blot above, there are a different recognition between IgG to OMP of *S. aureus\_fertile* and *S. aureus\_infertile*; also s-IgA to OMP of *S. aureus\_fertile* and *S. aureus\_infertile*. Western blot on IgG against OMP of *S. aureus\_fertile* and *S. aureus\_infertile* shows many protein bands. Because serum IgG produced from immune cells at many lymph nodes sensitized by OMP of *S. aureus* from many sites of the body. Another researcher have obtained cross-reactions between *Staphylococcus enterotoxin A* (SEA) with *Staphylococcus enterotoxin B* (SEB), *Staphylococcus enterotoxin C* (SEC), *Staphylococcus enterotoxin D* (SED) and *Staphylococcus enterotoxin E* (SEE), using either monoclonal antibody IgG from rabbit and polyclonal antibody IgG from goats and rabbits.<sup>11</sup> The conserved domains has been demonstrated in exotoxins of *Streptococcus pyogenes type A*, *Staphylococcus enterotoxin B*, and *Staphylococcus enterotoxin C1*, are likely originated from a common evolutionary ancestor because they can cross-react between others.<sup>12</sup> The toxin of *Staphylococcus enterotoxin* and *Streptococcus pyrogenic* have been found to share a homology sequence that has a similarity of antigenic determinants or in other words can cross-react each other.<sup>13</sup> Other researcher acquired patients with chlamydial double infection both conjunctival and genital tract had a high IgG titer higher compared to a single infection. It due to the anamnestic response that causes the usual cross-reactions between species within a genus.<sup>14</sup>

The carrier status of someone to *S. aureus* provides humoral immunity strains specific, which contribute to protection during septicemia of *S. aureus* because there are specific antibodies to superantigen of *S. aureus*.<sup>15</sup> A cross-reaction between *Streptococcus pneumoniae* and *Staphylococcus aureus* also have been found which resulted in the presence of the same antigen of conserved dehydrogenase. In *Staphylococcus aureus* as *1-pyrroline-5-carboxylate dehydrogenase (P5CDH)* and *Streptococcus pneumoniae* as *SP\_1119*. This leads to a decreased risk of *S. aureus* carrier for colonization of *Streptococcus pneumoniae* in immunocompetent individuals but no in immunocompromised individuals.<sup>16</sup>

#### 4. CONCLUSIONS

Non-specific Vaginitis due to *S. aureus* may trigger adaptive humoral immune response (IgG and s-IgA) which cross-react

to both OMP of *S. aureus* fertile and *S. aureus* infertile. s-IgA can recognize an epitope of protein *S. aureus* more specific than IgG. And some protein of *S. aureus* can cross-react each other.

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