World Journal of Biology Pharmacy and Health Sciences
eISSN: 2582-5542
Cross Ref DOI: 10.30574/wjbphs
Journal homepage: https://wjbphs.com/

(RESEARCH ARTICLE)

Check for updates

Immunoblot analysis of antibodies obtained from rabbits immunized with a synthetic Binder of Sperm Protein-1 (BSP-1) epitope

Wayan Wariata *, Sulaiman N Depamede *, Made Sriasih, Anwar Rosyidi and Ryan Aryadin Putra

Faculty of Animal Science, University of Mataram, Jl. Majapahit No. 62 Mataram- NTB, 83125, Indonesia.

World Journal of Biology Pharmacy and Health Sciences, 2024, 17(02), 029–032

Publication history: Received on 21 December 2023; revised on 02 February 2024; accepted on 04 February 2024

Article DOI: https://doi.org/10.30574/wjbphs.2024.17.2.0042

Abstract

This study aims to analyze whether antibodies produced through multiple vaccinations of a synthetic Binder of Sperm Protein-1 (BSP-1) epitope to local rabbits, can react against BSP-1 found in the ruminant sperm. BSP-1 is one of the biomarkers suggested to authenticate sire fertility. The method used was immunoblot assay against frozen semen of Bali cattle and local buffalo, also against the extracts of the caput-, and cauda- epididymis, as well as the testis of the buffalo. Analyses showed that the antibodies reacted to antigens found in the frozen semen of cattle and buffalo. The most notable result was that the antibody reacted specifically to buffalo frozen semen. No reactions occurred to the extracts of the caput-, and cauda-epididymis, as well as to the testis of the buffalo. Further research is needed to ascertain whether the antibody can be used to develop a sire fertility detection kit.

Keywords: Antibody BSP-1; Buffalo; Epitope; Immunoblot; Sperm; Vaccine synthetic

1. Introduction

In the livestock industry, especially regarding reproduction and breeding, more attention is generally paid to female fertility management than the sires fertility issues. This is because sire fertility is complex [1]. Assessment of the sire's reproductive quality is more focused on their semen quality. In fact, assessment of semen quality is more appropriate for identifying a sire with reproductive problems, rather than for predicting the relative fertility of the sire and his offspring. For this reason, in the past decade research has been directed towards exploring the potential use of genomic and proteomic markers to predict the fertility performance of a sire [1]. Recently, at least two candidate biomarkers have been proposed, namely the peptides Enolase-1 (ENO-1) and Binder of Sperm Protein-1 (BSP-1)[2].

It has been reported that Binder of Sperm Proteins (BSPs) are the most abundant group of seminal plasma proteins in rams and bulls and have been extensively studied in the last 30 years [3, 4]. BSPs account for more than 50% of bull seminal plasma proteins [5]. In bulls, there are three BSP proteins involved in fertilization, namely BSP1 (originally known as PDC-109), BSP3 (BSP-A3), and BSP5 (BSP-30 kDa) [6].

Since BSP-1 has the capacity to stabilize the sperm membrane and mediate the sperm binding to the oviduct's epithelium [7,8], we recently focused on BSP-1 and tried to develop antibodies against it. For this purpose, we developed immunogenic epitopes of BSP-1 in silico [9]. In this paper, we report the analyzing results of the antibodies by means of immunoblot assay.

* Corresponding author: Wayan Wariata and Sulaiman N Depamede

2. Material and methods

In this study, the antibodies tested were developed in our laboratory (called BSP1-Ab), derived from local rabbit serum that had previously been multiple vaccinated using peptide synthetic from BSP-1 with the sequence of LPEDSVPDEERVFPFTYRNRKHF [9].

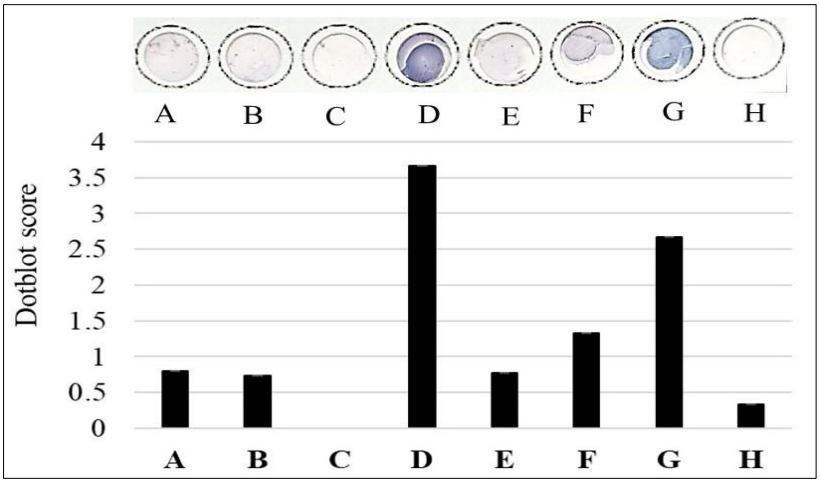
Before being used as a vaccine, the peptide was modified and conjugated with keyhole limpet hemocyanin (KLH) for the vaccine, or with bovine serum albumin (BSA) for positive control, which modified from [10].

Assays for the antibodies were performed using the immunoblot method, modified based on [10] and [11]. Antigens used were derived from local buffalo testis extracts. The extracts included the whole testis extracts, extracts of parts of the caput-, and the cauda epididymis. Frozen semen from Bali cattle and local buffalo were also used. Each antigen, 10 µg each, was dripped onto a nitrocellulose membrane. After incubation at 4°C overnight, the membrane was blocked with 4% skimmed milk at 37°C, 60 min. After incubation and washing, the membrane was dripped with BSP1-Ab (10 ug/ml) and allowed to react for 60 minutes at 37°C. After washing and incubation, the secondary antibody, alkaline phosphatase (AP) conjugated goat-anti rabbit IgG (diluted 1/5000; Sigma Aldrich, USA) was added to the nitrocellulose membrane. The membrane was then incubated for 60 min at 37°C, then subjected to a color reaction by a substrate that reacts specifically with the enzyme to form a dot-like color that can be observed by the naked eye. The test was repeated three times (n=3), then scored for each dot-like color observed. Scoring consisted of 0, 1, 2, 3, and 4, for negative, weakly positive, -moderate, -strong, and -strongly positive, respectively. The observation results were analyzed descriptively.

All activities related to the use of experimental animals were handled in accordance with the procedures set out in the Faculty of Animal Science, University of Mataram

3. Results and discussion

The main aim of this study was to analyze whether antibodies against BSP-1 could be obtained through repeated vaccination of BSP-1 peptide in local rabbits. These antibodies are needed to develop a fertility detection kit for male ruminants (cattle and buffalo), especially if the bulls are intended to be used as sires. Some of these detection kits include those based on antigen-antibody reactions, such as ELISA as well as lateral flow immunoassay, all of which require antibodies. One of the target analytes or biomarkers that has recently been suggested is BSP-1 [2].



(A) Testicular extract, (B) Extract from caput epididymis, (C) Extract from cauda epididymis, (D) BSP-1 peptide vaccine, (E) Skim milk, (F) Frozen semen of Bali cattle, (G) Frozen semen of local buffalo, and (H) BSA.

Figure 1 Top panel: Representative immunoblot of BSP1-Ab reaction against antigen samples; bottom panel: average scoring of immunoblot results (n=3)

In our laboratory, we injected an in silico developed BSP-1 synthetic epitope vaccine into local rabbits. Two weeks after the third vaccination, blood was harvested, and immunoglobulins (IgG) were partially purified from the serum using 33% ammonium sulfate as modified from [12]. The specificity of the IgG (called BSP1-Ab) was then analyzed by means of immunoblot, and a representation of the results is presented in Figure 1.

Figure 1 shows that based on the immunoblot results, the serum IgG obtained after repeated vaccination using synthetic epitope vaccines gave a positive response against some sampel antigens. It is quite interesting that of the antigen samples used, positive results that equivalent to the color intensity of the positive control (i.e. the peptide vaccine) were exhibited by antigen derived from frozen semen of buffalo (dot G). A similar reaction was not shown by the frozen semen derived from Bali cattle, i.e. its color intensity was relatively weaker (dot F). These results illustrate that the antibody (BSP1-Ab) obtained in this study is specific to buffalo semen. This specificity is likely because the vaccine used was derived from the peptide sequence of BSP-1 *Bubalus bubalis* [9].

From these immunoblot results there are some interesting points to note. Firstly, although the BSP1-Ab antibody reacted specifically to frozen buffalo semen, a similar reaction was not shown against antigens derived from the extracts of the caput-, and cauda-epididymis, as well as the testis of the buffalo used in this study. There are several possibilities to explain this phenomenon. Technically, there may have been a problem in the process of extracting testicular tissue and parts thereof. For example, the use of buffer containing phosphate to make the extracts, whereas phosphate is known to be an inhibitor of the alkaline phosphatase-labelled secondary reaction used in the immunoblot method in this study [13]. Whether this is the case still needs to be investigated further.

Further consideration that needs to be taken into account is the results of previous research. It was revealed that the amount of BSP-1 was found to be highest in seminal plasma protein i.e. 31 mg/ml compared to BSP-3 and BSP-4 which were 3 mg/ml and 4 mg/ml respectively [14]. While we understand that seminal plasma proteins are mostly derived from seminal vesicles and the prostate, which produce ~65-75% and ~20-30% of the volume of semen, respectively. Only a small proportion is sourced from the testes, epididymis, and bulbourethral and periurethral glands [15]. It can be argued therefore that the presence of BSP-1, which is found only in small amounts in the testis and epididymis, could have been washed away during the tissue extraction process in this study.

It should be noticed here that the main point of this result is that the synthetic epitope vaccine available in our laboratory [9] has successfully triggered the local rabbit immune system to produce antibodies against BSP-1. Another study, such as the one that conducted by Ardon and Suarez [9], although using a residue sequence of the BSP-1 peptide different from us, i.e. "dqdeg vsteptqdg" as the vaccine, produced antibodies against BSP-1 with an immunoblot pattern that similar to ours. They revealed that the relative band intensity of BSP-1 in seminal plasma and frozen semen was significantly greater ($p<0.05$) than that in fresh sperm. However, they also mentioned that the results were strongly influenced by the semen diluent used [16].

Our study is not yet at the level of application of the antibodies against BSP-1 we obtained. Results with positive prospects that have been expressed by other researchers suggest that further research should be conducted, especially whether BSP-1 can be used as a biomarker to authenticate sire fertility, using BSP-1 antibody.

4. Conclusion

This study has successfully produced polyclonal antibodies against BSP-1 through repeated vaccination of BSP-1 peptide vaccine in local rabbits. The antibody reacted specifically against frozen semen of local high breed buffalo stud. Further research is needed, particularly whether BSP-1 can be used as a biomarker to authenticate sire fertility, utilizing antibodies against BSP-1.

Compliance with ethical standards

Acknowledgments

This research was funded by the Institute for Research and Community Engagement, University of Mataram, PPK Scheme - DIPA BLU No. 1819/UN18.L1/PP/2022. The frozen semen of Bali cattle and local buffalo were generously provided by UPTD-BIB Banyumulek, Livestock and Animal Health Services of NTB Province, Indonesia.

Disclosure of conflict of interest

No conflict of interest to be disclosed.

References

[1] N. Diether, M.K. Dyck. Male fertility evaluation using biomarkers in livestock. JSM Biomarkers 2017; 3(1):1011.

[2] M.K. M. Aslam, V.K. Sharma, S. Pandey, A. Kumaresan, et al. Identification of biomarker candidates for fertility in spermatozoa of crossbred bulls through comparative proteomics. Theriogenology 2018; 119:43–51; https://doi.org/10.1016/j.theriogenology.2018.06.021

[3] T. Pini, V. Labas, S. P. de Graaf, et al. Binder of Sperm Proteins 1 and 5 have contrasting effects on the capacitation of ram spermatozoa. Biology of Reproduction, 2018, 98(6), 765–775. doi:10.1093/biolre/ioy032

[4] G. Plante and P. Manjunath. Epididymal Binder of SPerm genes and proteins: what do we know a decade later? Andrology, 2015, 3, 817–824. doi: 10.1111/andr.12089

[5] V. Nauc, P. Manjunath. Radioimmunoassays for Bull Seminal Plasma Proteins (BSP-A1/-A2, BSP-A3, and BSP-30-Kilodaltons), and their quantification in seminal plasma and sperm. Biol Reprod 2000; 63:1058–1066.

[6] P. Manjunath, J. Lefebvre, P.S. Jois, J. Fan, M.W. Wright. New nomenclature for mammalian BSP genes. Biol Reprod 2009; 80:394–397.

[7] P. Rodríguez-Villamil, V. Hoyos-Marulanda, J.A.M. Martins et al. Purification of binder of sperm protein 1 (BSP1) and its effects on bovine in vitro embryo development after fertilization with ejaculated and epididymal sperm. Theriogenology 2016; 85:540–54; https://doi.org/10.1016/j.theriogenology.2015.09.044

[8] P. Rodríguez-Villamil, D. Mentz, F.L. Ongaratto, L.H. Aguiar et al. Assessment of binder of sperm protein 1(BSP1) and heparin effects on in vitro capacitation and fertilization of bovine ejaculated and epididymal sperm. Zygote 2020;28(6):489–94; https://doi.org/10.1017/S0967199420000374

[9] W. Wariata, M. Sriasih, A. Rosyidi, M. Ali, S.N. Depamede. Preliminary study on designing the binder of sperm-1 synthetic vaccine using sequence-based methods and molecular docking. J Adv Vet Anim Res. 2022; 9(1):122–127. http://doi.org/10.5455/javar.2022.i576

[10] D. Kisworo, S.N. Depamede. Antibody against synthetic peptide of prolactin-inducible protein homologue precursor (PIP-HP) of Bali Cattle (Bos Javanicus) saliva as a potential biomarker for immunoassay development. Journal of Advanced Veterinary and Animal Research. 2018; 5(2):182-187. http://doi.org/10.5455/javar.2018.e264

[11] W. Zhu, K. Li, T. Cui, Y. Yan. Detection of anti-ganglioside antibodies in Guillain-Barré syndrome. Ann Transl Med 2023;11(7):289. http://dx.doi.org/10.21037/atm-20-2285.

[12] Nurhaerani, W. Wariata, D. Kisworo, S.N. Depamede. Purification of polyclonal antibody against pork extracts antigens using protein A column as material for developing halal food detection kit. AIP Conference Proceedings 2199, 070014 (2019); https://doi.org/10.1063/1.5141328

[13] L. Zhang, R. Buchet, and G. Azzar. Phosphate Binding in the Active Site of Alkaline Phosphatase and the Interactions of 2-Nitrosoacetophenone with Alkaline Phosphatase-Induced Small Structural Changes. Biophysical Journal Volume 86 June 2004 3873–3881. doi: 10.1529/biophysj.103.034116

[14] V. Nauc and P. Manjunath. Radioimmunoassays for bull seminal plasma proteins (BSP-A1/-A2, BSP-A3, and BSP-30-kilodaltons), and their quantification in seminal plasma and sperm. Biology of Reproduction (2000); 63 1058–1066. (doi:10.1095/biolreprod63.4.1058)

[15] I. Batruch, I. Lecker, D. Kagedan, C. R. Smith, et al. Proteomic Analysis of Seminal Plasma from Normal Volunteers and Post-Vasectomy Patients Identifies over 2000 Proteins and Candidate Biomarkers of the Urogenital System. J. Proteome Res. 2011, 10, 941–953. dx.doi.org/10.1021/pr100745u

[16] F. Ardon and S.S. Suarez. Cryopreservation increases coating of bull sperm by seminal plasma binder of sperm proteins BSP1, BSP3, and BSP5. Reproduction (2013) 146 111–117. DOI: 10.1530/REP-12-0468.