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PROCEEDINGS

The 9th Annual BaSIC Science International Conference

"Recent Advance in Basic Sciences Toward 4.0 Industrial Revolution"

March 20-21, 2019
MIPA CENTER, Brawijaya University
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PREFACE

Conference in a brief

The 9th Basic Science International Conference (BaSIC 2019) was a scientific meeting aimed to promote mutual exchange between scientists and experts, to exchange and share their experiences and research results on all aspects of basic science. The BaSIC 2019 also has provided a premier interdisciplinary platform for researchers, practitioners and educators to present and discuss the most recent innovations, trends, and concerns as well as practical challenges encountered and solutions adopted in the fields of basic sciences.

The conference was carried out with regards of the Rector of Brawijaya University's program to increase the number of publications of scientific paper in international journals or proceedings indexed by Scopus. Therefore, the selected full papers will be published in conference proceedings indexed by Scopus, IOP Conference Series: Materials Science and Engineering.

The conference has recorded **344 registered delegates** (presenters and non-presenters), among which **350 participants** attended the conference. The participants consist of both international and national researchers, university lecturers, and college students in the field of basic sciences. In terms of country of origin, the participants of the BaSIC 2019 are coming from 7 countries, including Indonesia, Japan, Malaysia, Gambia, Libya, Saudi Arabia, and Thailand.

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6. Dr rer nat Rino M Mukti (ITB, Indonesia)
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8. Prof Moh Sasmito Djati (Universitas Brawijaya, Indonesia)

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10. Dr Siti Mariyah Ulfa (Universitas Brawijaya, Indonesia)
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Identification on Osteopontin Promoter Gene Polymorphism and Post-thawing Quality in Dairy Bull Peranakan Friesian Holstein

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Abstract. This study aims to determine the existence of osteopontin promoter gene polymorphism in dairy bull Peranakan Holstein Friesian (PFH) and its relationship with the quality of PFH bull frozen semen. A total of 10 Holstein Friesian dairy cow blood samples were taken and then DNA extracted and amplified using SPP1F and SPP1R primers. The target band 306bp was detected in all samples and continued by sequencing to analyze the nucleotide bases. The results showed that sample with low quality frozen semen were deleted in the 10098 base and a transition (T-C) in bases 10054. The results indicated that this mutation site could be related to the trait susceptibility to frozen semen quality. Comprehensive studies are highly needed to address other parameters related to any abnormality in sperm during cryopreservation.

Keywords : polymorphism, osteopontin, post-thawing quality, spermatozoa, diary bull Friesian Holstein

1. Introduction

Examination of semen in diary bull of Friesian Holstein as a benchmark for fertility has only been carried out through macroscopic and microscopic examination. In addition to these examinations, it can also be seen from pedigree, namely selection based on the reputation shown by the ancestors of the cow concerned, but this test is less accurate, because a bloodline or descendant from a good individual does not necessarily mean that the good characteristics will inherited through selection and marriage [1,2].

The basis for determining osteopontin as the main bio-marker in determining the fertility of male Holstein dairy cows is based on several previous studies [3-5] which show that seminal plasma osteopontin Holstein dairy cows with good fertility have 2.5 times osteopontin concentrations when compared to dairy cows with low fertility. Erikson et al., (2007) said that male dairy cows that were examined for fresh semen and containing osteopontin had great potential in the success of fertilization in vitro and in vivo compared to those without osteopontin [6]. This is reinforced by previous studies that prove the link between osteopontin and the quality of fresh semen of FH dairy cows in Indonesia, and the addition of osteopontin to frozen semen diluter increases the quality of post-thawing FH dairy

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cows through various observations of cellular and molecular parameters [7,8] and increasing the success of fertilization in vitro and in vivo.

The polymorphism of the osteopontin promoter gene is related to male fertility in Holstein dairy cows. Some evidence suggests that there is a relationship between osteopontin promoter gene polymorphism, one of which is the osteopontin promoter gene polymorphism with motility and viability of fresh semen semen spermatozoa [9,10]. Determination of osteopontin promoter gene regions based on previous research conducted by Rori et al., (2016) identified seven SNP regions acting as osteopontin promoters, including: 3379 bp, 3490 bp, 3492 bp, 5075 bp, 5205 bp, 5209 bp, and 5263 bp from the osteopontin promoter gene [10]. Then, substitution of thymine into guanine at 3379 bp correlated with an increase in the percentage of spermazoa motility, but for the parameters of viability it was not identified. Study also reported that regular male testing in the artificial insemination program was very important as a fertility indicator.

The development of dairy cattle populations in the future should be selected based on breeding value, the use of genetic identifiers, especially those that control reproduction because the male will spread superior trait to the population. The development of male testing through identification of genetic markers for semen quality has been carried out in several developed countries, this is expected to support the acceleration of the quality of the superior PFH dairy cattle population. Genetic selection systems require identification of genetic markers as candidate genes that control reproductive properties, especially in male cattle.

2. Materials and Methods

2.1. Tools and materials

The tools used in the study include a glove, mask, ice box, paper labels, microsentrifuge tube (1.5 mL), micro PCR tube (200 mL), micropipette, white tip, yellow tip, vortex engine, sentrifugator, incubator CO2, freezer, thermocycler, EDTA, Horizaontal SDS-PAGE (Biorad), Gel Documentation (Biorad), thermocycler (Biorad), Nano-200 Micro-spectrophotometer nucleic acid. Materials used in research PFH Bull blood samples, Genomic DNA mini kit tissue, ddH2O, forward primer (SPP1_F) 5'-GCAAATCAGAAGTGTGATAGA-3' and reverse primer (SPP1_R) 5'-CCAAGCCAAACGTA TGAGTT-3', the PCR mix, DNA ladder 100 bp and 1 kb, TBE, agarose 1% and 2%, loading dye, alcohol 70%, aluminum foil, red gel.

2.2. Blood Sample Selection of PFH Bull

Ten blood samples of PFH diary bull were collected and placed into EDTA- vacutainer tube. PFH diary bull were estimated 3-5 years old obtained from local diary bull, Malang, East Java, Indonesia. Location blood sampling performed on coccygea vein. The volume of blood samples were 3cc of each individual bull. The blood sample later be labeled according to the name of the individual samples of cattle. Samples were then stored at a temperature of 40^0 C.

2.3. Isolation of DNA

Isolation of DNA from blood samples of PFH diary bull was performed by Geneaid® namely Genomic DNA mini kit tissue and blood. Following the protocol for the isolation of specific DNA blood. The main principle in the isolation of DNA as followed: destruction (lysis), DNA extraction or separation of solid materials such as cellulose and proteins, and DNA purification (Nita, 2013). DNA quantity test were done using Micro-Nano-200 spectrophotometer nucleic acids. The wavelengths between 260 nm and 280 nm were used to analyzed the purification.

2.4. Primer design

Primers used for DNA amplification by polymerase chain reaction technique (PCR) was designed using NCBI Genebank: AY878328.1. Forward primer and reverse primer obtained through primer3plus using data AY878328.1 with 12,300bp linear DNA. A pair of forward primer (SPP1_F)

5'-GCAAATCAGAAGTGTGATAGA-3 '(Length: 21 bp, Tm: 53.7, GC: 38.1% and the reverse primer (SPP1_R) 5'-CCAAGCCAAACGTATGAGTT-3' (Length: 20 bp, tm: 56.3, GC: 45%).

2.5. DNA Amplification using Polymerase Chain Reaction (PCR)
DNA samples were amplified using the PCR thermalcycler method. A pair of primers used are forward primer (SPP1_F) and reverse (SPP1_R). PCR amplification using Thermal-cycler (Biorad®) by mixing the DNA template 3μL, 1μL 10 pmol forward primer, 1μL 10 pmol reverse primer, 10μL PCR mix and 5 mL ddH20 into PCR tube 200 mL. According Zuhriana (2010), amplification stages starting from predenaturation 940C for two minutes, denaturation 940C for 30 seconds, and then annealed at a temperature of 55-600C for 30 seconds. Extension at a temperature of 720C for 30 seconds and post extension at 720C for 7 minutes. The process will be repeated for 30-35 cycles.

2.6. Purification of PCR Products
Purification of the PCR product aimed to purify DNA and eliminate the remnants of PCR mix covering dNTPs, Taq polymerase, Mg ions, as well as ddH20 and PCR primers located within the tube. DNA sequencing later be done after the amplicons were purified.

2.7. Data analysis
Here we performed sequencing result using NCBI Blast and Bioedit to identify the polymorphism from the whole samples. Using NCBI Blast program we can detect the percentage of homology and molecular variation in isolates a sample of SNPs (Single Nucleotide Polymorphism) such as insertions, deletions, and substitutions (transition or transversion) by aligning the results of the fourth sample sequence with the NCBI database Genebank: AY878328.1 alignment using algorithm ClustalW multiple allignment. Further analysis of the molecular variation performed by Bioedit program to see what kind of mutation that occurs and the type nucleotide mutations.

3. Result and Discussion

3.1. Post-thawing Frozen Cement Quality of PFH Bull
Post-thawing quality of 10 dairy bull of crossbred PFH were examined twice. Post-thawing quality on its percentage motility and percentage viability are shown in Table 1.

Table 1. Post-thawing quality from each samples

Code	Average post-thawing motility %	Average post-thawing Viability %	General Quality
A	35	58	Poor
B	55	80	Moderate
C	50	75	Moderate
D	60	80	Moderate
E	55	80	Moderate
F	60	80	Moderate
G	40	60	Poor
H	65	85	Moderate
I	30	52	Poor
J	65	85	Moderate

3.2. DNA Isolation

DNA isolation was carried out using blood samples using Genomic DNA mini kit (Geneaid®) according to the procedure. The results obtained in the form of total DNA extraction which is then tested for quantity and purity test as shown in Table 2.

Table 2. Total DNA concentration and purity of genomic PFH Bull

Code	Concentration ng/ µL	purity
A	14.22	1.90
B	18.56	1.90
C	13.98	1.80
D	10.04	1.85
E	9.71	1.80
F	11.02	1.92
G	14.21	1.90
H	8.35	1.81
I	13.47	1.98
J	19.62	1.78

Based on the results of the quantity test, it is known that almost all samples have good purity levels which are still in the range of 1.8-2.0. If the purity of DNA below 1.8 indicates that the DNA from the extraction results, there are contaminants in the form of protein compounds. Contamination in the form of protein compounds in DNA can be caused by the absence of protease enzymes in the DNA isolation protocol. The purity value of DNA above 2.0 indicates that there are still contaminants in the form of RNA. This might be due to the lack of ribonuclease addition in this study. According to [11] the nano drop test results are in the form of DNA purity values on Å260 / Å280 and DNA concentration values. Good quality DNA based on the nano drop test has a purity of 1.8-2.0 and concentrations above 100 ng / µL.

3.3. Osteopontin Gene amplification by PCR Method

Primers used to amplify the gene osteopontin taken from Genebank with number sequences AY878328.1 as listed in Table 3.

Table 3. Primary Nucleotide Sequence Cow Osteopontin gene PFH Bull

Primary	Oligo Nucleotide Sequence
forward (SPP1_F)	GCAAATCAGAAGTGTGATAGA 5'-3 '
Reverse (SPP1_R)	CCAAGCCAAACGTATGAGTT 5'-3 '

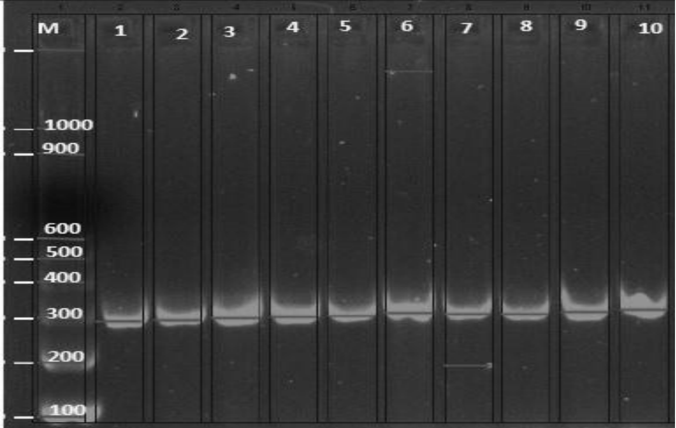

Figure 1. 2% agarose gels of PCR amplification products with a target of 306 bp band was detected in all samples (n = 14) (Gel Doc, Biorad)

3.4. Results of Osteopontin gene sequences analysis

A total of seven samples from a total of ten samples were successfully sequenced. Sequencing results in the form of graphs that shows the content of adenine, thymine, guanine and cytosine contained in DNA fragments and data formats in the form of fasta. The ten sequencing results were included in the NCBI BLAST program to align with the bank's NCBI AY878328.1. The results of the alignment to see the magnitude of ident and alignment on sample bases with the NCBI database Table 4.

Table 4. Identity with NCBI database

NO	Sampel	Identity
1	A	98%
2	B	99%
3	C	98%
4	D	99%
5	E	99%
6	G	98 %
7	I	99%

Alignment results with the NCBI database shows almost all samples above 95%, this shows that all samples have good similarities with NCBI AY878328.1. The osteopontin gene of all male PFH cows is aligned with the reference, namely the NCBI osteopontin database database AY878328.1. The sequenced sequence of sample sequences starts from base to 9900-10100 Figure 2.

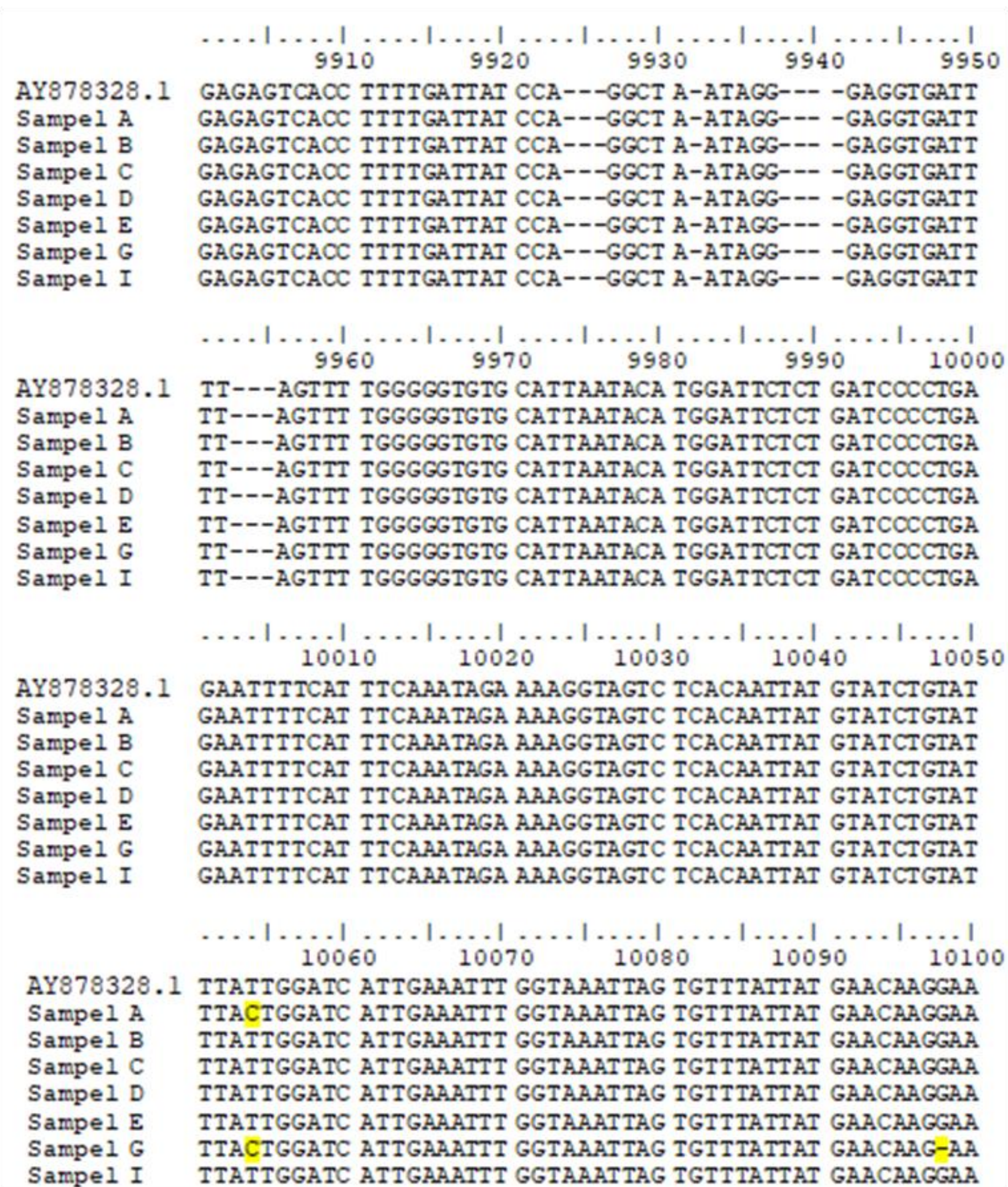

Figure 2. The results of nucleotide base alignment using Bioedit

In samples A and G the base of 10054 experiences a transition (T-C). Transition mutations means mutation that occur when the pyrimidine base in the DNA nucleotide chain is replaced by another pyrimidine base, or the purine base is replaced with another purine base. The sample G deleted in the 10098th base, the presence of deletions affected the amino acid formation, because of the presence of one missing nucleotide base.

The comparison of samples A and G with other samples has a low quality of post thawed spermatozoa compared to the group. But in sample I the low quality of post thawing did not affect changes in the composition of the formed nitrogen base. The results of the study indicate that these mutations can be attributed to the susceptibility of properties to post-thawing quality. It is possible that other genes associated with these molecular genetic markers affect sperm quality.

Samples A and I have similarities to the formation of amino acids formed, not the formation of amino acids glutamic acid. Glutamic acid functions to maintain the quality of spermatozoa, especially to protect the plasma membrane from any damage due to lipid peroxide, especially caused by crypreservation method. This mechanism is an antioxidant mechanism to protect cells from free radicals. The mechanism of action of glutamic acid by blocking and preventing agents from inhibiting the process of sugar fraction in spermatozoa. This factor supports metabolic activity and increases the energy availability of spermatozoa during spermatogenesis. The result is that the quality of the sperm quality is good.

Protein: Sampel A
Length = 88 amino acids
Molecular Weight = 10100,22 Daltons

Amino	Acid	Number	Mol%
Ala	A	1	1,14
Cys	C	2	2,27
Asp	D	3	3,41
Glu	E	0	0,00
Phe	F	7	7,95
Gly	G	6	6,82
His	H	4	4,55
Ile	I	8	9,09
Lys	K	2	2,27
Leu	L	12	13,64
Met	M	1	1,14
Asn	N	5	5,68
Pro	P	2	2,27
Gln	Q	0	0,00
Arg	R	7	7,95
Ser	S	6	6,82
Thr	T	7	7,95
Val	V	3	3,41
Trp	W	1	1,14
Tyr	Y	2	2,27

Protein: Sampel I
Length = 94 amino acids
Molecular Weight = 10850,16 Daltons

Amino	Acid	Number	Mol%
Ala	A	1	1,06
Cys	C	2	2,13
Asp	D	4	4,26
Glu	E	0	0,00
Phe	F	8	8,51
Gly	G	6	6,38
His	H	5	5,32
Ile	I	8	8,51
Lys	K	2	2,13
Leu	L	14	14,89
Met	M	1	1,06
Asn	N	5	5,32
Pro	P	2	2,13
Gln	Q	1	1,06
Arg	R	6	6,38
Ser	S	6	6,38
Thr	T	7	7,45
Val	V	4	4,26
Trp	W	1	1,06
Tyr	Y	3	3,19

Figure 3. Results of amino acid sample A and sample I

The results of the comparison between gene sequences with the results of post-thawing quality for each sample were obtained in samples experiencing deletions in their gene sequences having a low post-thawing quality compared to the group, for example in sample G. While samples A and I have similarities in the arrangement of amino acids formed. Both do not have glutamic acid amino acids.

4. Conclusion

The results showed that sample with low quality frozen semen were deleted in the 10098 base and a transition (T-C) in bases 10054. The results indicated that this mutation site could be related to the trait susceptibility to frozen semen quality. In conclusion, the osteopontin genes can be used as a reference for the selection of broodstock quality bull, but further research is needed between the results of sequencing with several amino acids formed. Suggestions for further research are using cows from the same place and more thoroughly in each stage the process.

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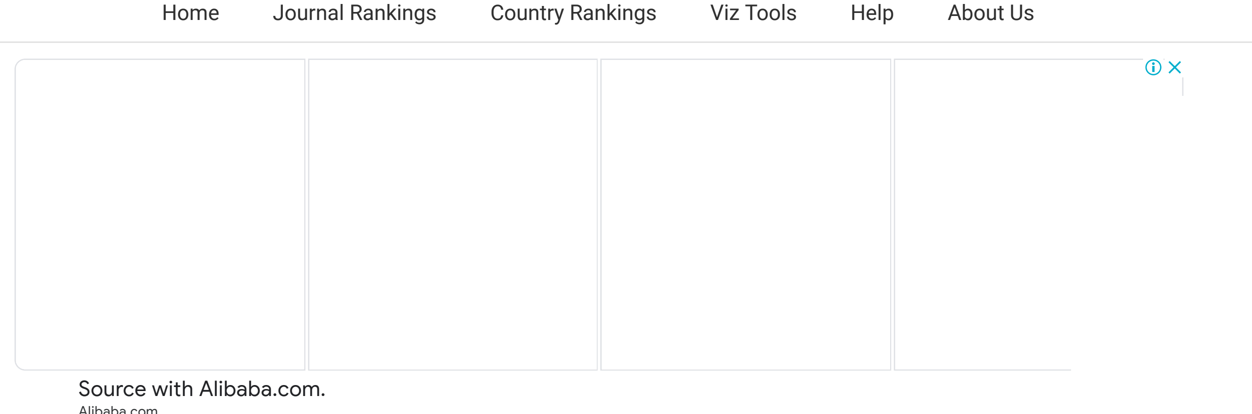
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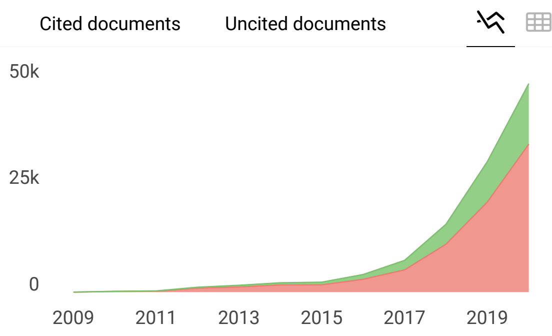

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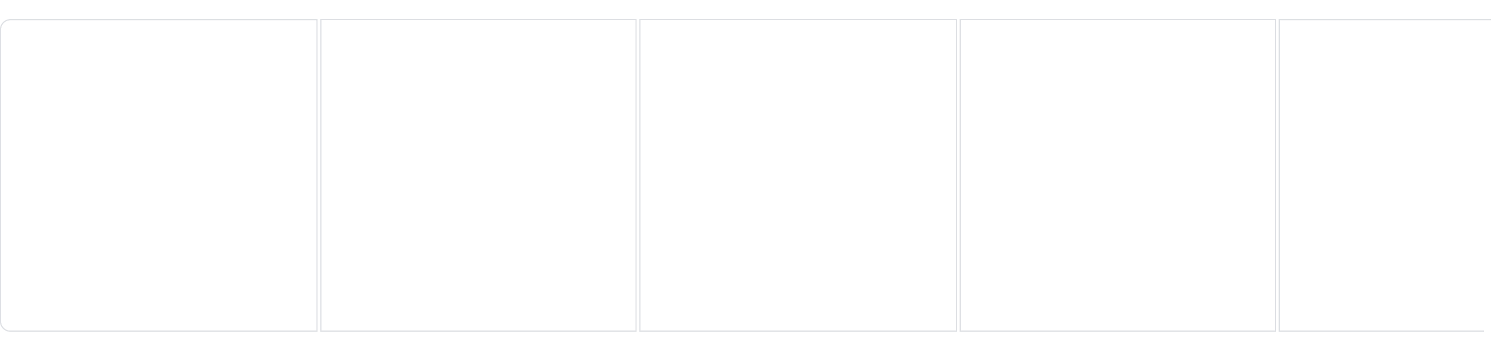

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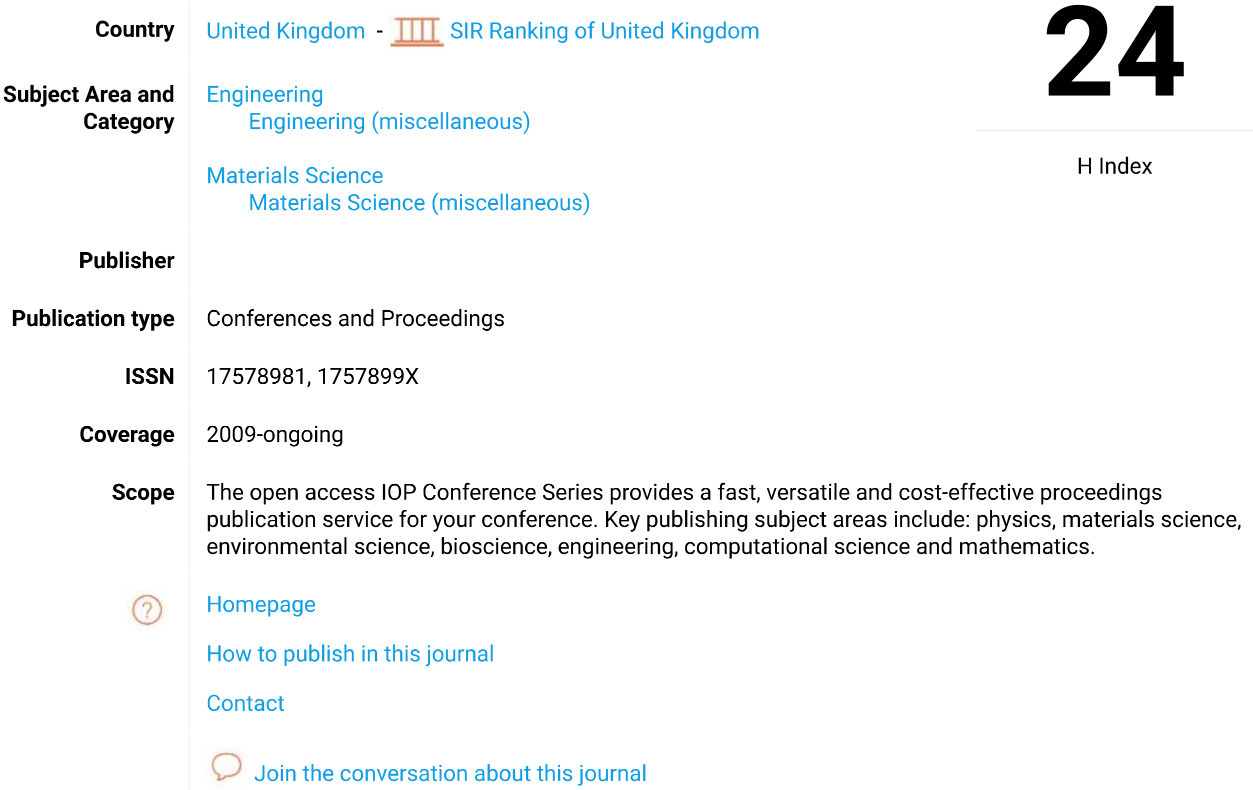

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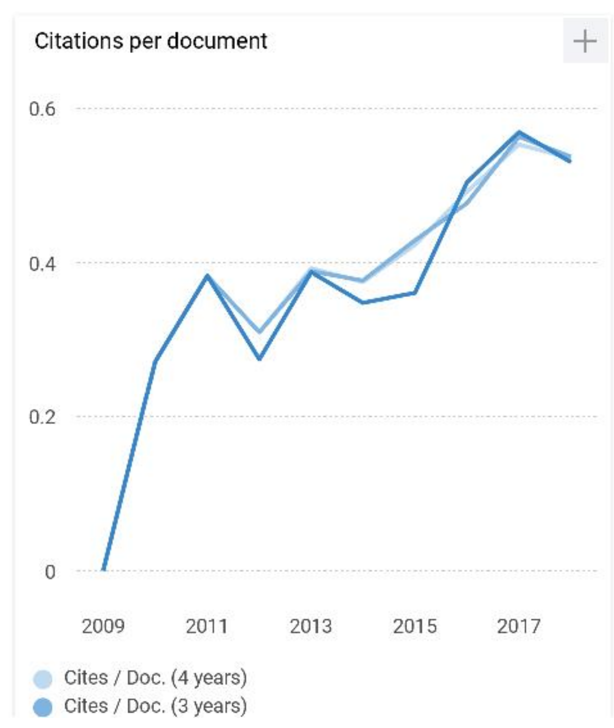

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