Proceeding
International Symposium in Veterinary Science 2018

Strengthening the Regional Veterinary Education and Research for the Future Excellent Veterinary Graduates

February, 28th 2018
Faculty of Veterinary Medicine, Universitas Gadjah Mada
PROCEEDING
INTERNATIONAL SYMPOSIUM IN VETERINARY SCIENCE
Strengthening the Regional Veterinary Education and Research for the Future
Excellent Veterinary Graduates

REVIEWERS:
Prof. Hiroshi Sato (Yamaguchi University, Japan)
Prof. Byeong Chun Lee (Seoul National University, South Korea)
Prof. Koichi Sato (Yamaguchi University, Japan)
Prof. Drh. Srihadi Agungpriyono, Ph.D, PAVet(K) (Institut Pertanian Bogor)
Prof., Dr. drh., Pudji Srianto, M.Kes. (Universitas Airlangga, Surabaya)
Dr. drh. I Nengah Kerta Besung, M.Si. (Universitas Udayana, Bali)
Prof. Dr. drh. Siti Isrina Oktavia Salasia (Universitas Gadjah Mada, Yogyakarta)
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http://fkh.ugm.ac.id
Greetings From The Dean

Assalamu’alaikum warohmatullahi wabarokatuh.
Good Morning.

I take this opportunity to welcome our honorable:
Rector of Universitas Gadjah Mada (Prof. Ir. Panut Mulyono, M.Eng. D.Eng)
Vice President of Foreign Affairs, Yamaguchi University (Prof. Fusanori Miura)

I would love to welcome our distinguished keynote speakers:
Dean of The United Graduate School of Veterinary Medicine, Yamaguchi University
Dean of The United Graduate School of Kagoshima University
Dean of The United Graduate School of Tottori University
Prof. Byeong Chun Lee, College of Veterinary Medicine, Seoul National University
Head of Indonesian Association Faculty of Veterinary Medicine
All Deans of Faculty Veterinary Medicines in Indonesia
as well as dear participants.

Distinguished delegates, Ladies, and Gentlemen,

Faculty of Veterinary Medicine, Gadjah Mada University is proud to become the host of the 2nd International Symposium in Veterinary Sciences. This symposium is an annual meeting that initiated, firstly, in Bogor in 2017 by a group of veterinary faculty representatives from Indonesia and Japan as initiators. The theme of this symposium is "Strengthening the regional veterinary education and research for the future excellent veterinary graduates".

As the Dean of Veterinary School, Universitas Gadjah Mada and on behalf of the Organizing Committee, I am very pleased indeed to welcome all of you to Yogyakarta. For years, Yogyakarta has experienced being a student and cultural-rich city leading to the destination for higher-learning study from all over the country and as the second tourist destination in Indonesia, as well as being endowed with handful of beauty and a wide range of cultures. In addition, the city is generally safe to visit, therefore I do hope you may have some other chance to visit Borobudur and Prambanan temples, a part of the world heritages as awarded by UNESCO. This seminar will allow you the opportunity to experience some of its richness.

In this symposium we invite deans and faculty members from 15 universities as follows: Yamaguchi University, Kagoshima University, Tottori University, Seoul National University, Gadjah Mada University, Bogor Agricultural University, Syah Kuala University, Airlangga University, Udayana University, Hasanuddin University, Nusa Cendana University, Brawijaya University, Wijaya Kusuma University, Nusa Tenggara Barat University and Padjajaran University.

Recent advances in veterinary research and education would be discussed in this symposium. Every faculty representative would present their leading activities in research and its contribution to educational improvements in term of standardization system. During
the discussion, we could do an institutional capacity mapping as a fundamental base for future collaborative works and synergies.

Last but not least, the role of these networks will not be maximized if they are not accompanied by strong mutual commitments from each institution. With this, it is hope every individual will enable to contribute actively and progressively with good outcomes for strengthening the veterinary education and research for the future excellent veterinary graduates.

We sincerely hope that from this symposium will give a positive impact for every institution. We also hope to develop more mutual collaboration in the future. Thank you, have a nice and fruitful discussion and God Bless You.

Wassalamu'alaikum warahmatullahi wabarakatuh

Dean of Faculty of Veterinary Medicine UGM

Prof. Dr. Siti Isrina Oktavia Salasia, DVM
Schedule at Glance

International Symposium in Veterinary Science: Strengthening the regional veterinary education and research for the future excellent veterinary graduates

Faculty of Veterinary Medicine, Gadjah Mada University, February 27th-28th, 2018

Tuesday, February 27, 2018

Yogyakarta City Tour Part I

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<td>07.30</td>
<td>Pick up Participants in Adi Sucipto Yogyakarta Airport</td>
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<tr>
<td>09.40</td>
<td>Go to Hotel (Novotel)</td>
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<tr>
<td>11.30</td>
<td>Pick up Participants in Adi Sucipto Yogyakarta Airport to Hotel</td>
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<tr>
<td>12.30</td>
<td>Pick up in Hotel for Lunch Time and Tour “CUPU WATU RESTO”</td>
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<tr>
<td>14.00</td>
<td>Go to Hindu Temples Prambanan</td>
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<tr>
<td>Time</td>
<td>Activity</td>
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<tr>
<td>16.00</td>
<td>Boko Temples: Sunset view</td>
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<tr>
<td>18.00</td>
<td>Go to Abhayagiri Restaurant: Dinner</td>
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<tr>
<td>20.00</td>
<td>Back to Hotel</td>
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## DAY 2, February 28 2018
International Symposium in Veterinary Science: Strengthening the regional veterinary education and research for the future excellent veterinary graduates

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<td>08.00-09.30</td>
<td>Registration</td>
<td></td>
<td>Auditorium 3&lt;sup&gt;rd&lt;/sup&gt; Floor</td>
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<tr>
<td>09.30-10.00</td>
<td>Opening Ceremony</td>
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<td>10.00-10.30</td>
<td><strong>Group photos &amp; AM Coffee Break</strong></td>
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<td><strong>Oral Session (1):</strong></td>
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<td></td>
<td>Chairperson: Agung Budiyanto, DVM., Ph.D. (Vice Dean of Academic and Students Affairs)</td>
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<tr>
<td>10.30-10.50</td>
<td>Research and Study at FVM of Yamaguchi University</td>
<td>Dean of The United Graduate School of Veterinary Medicine, Yamaguchi University</td>
<td>Auditorium 3&lt;sup&gt;rd&lt;/sup&gt; Floor</td>
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<tr>
<td>10.50-11.10</td>
<td>Research and Study at FVM of Kagoshima University</td>
<td>Representative of FVM of Kagoshima University</td>
<td>Auditorium 3&lt;sup&gt;rd&lt;/sup&gt; Floor</td>
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<tr>
<td>11.10-11.30</td>
<td>Research and Study at FVM of Tottori University</td>
<td>Representative of FVM of Tottori University</td>
<td>Auditorium 3&lt;sup&gt;rd&lt;/sup&gt; Floor</td>
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<td>11.30-12.00</td>
<td>Research and Study at FVM of Indonesia</td>
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<td>Auditorium 3&lt;sup&gt;rd&lt;/sup&gt; Floor</td>
</tr>
<tr>
<td>12.00-12.30</td>
<td>Research and Study at College of Veterinary Medicine, Seoul National University</td>
<td>Prof. Byeong Chun Lee</td>
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<td>12.30-13.00</td>
<td>Discussion</td>
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<td>13.00-14.00</td>
<td><strong>LUNCH</strong></td>
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<td>14.00-15.00</td>
<td>Poster Session</td>
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<tr>
<td>15.00-15.30</td>
<td><strong>Coffee Break</strong></td>
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<tr>
<td>Time</td>
<td>Event</td>
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<td>15.30-16.30</td>
<td>Tour of Gadjah Mada Veterinary Teaching Hospital</td>
<td>Chairperson: Dr. Guntari Titik Mulyani, DVM. Dito Anggoro, DVM., M.Sc.</td>
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<td>Visit to UGM Veterinary Teaching Hospital</td>
<td>Veterinary Teaching Hospital</td>
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<td>16.30-18.00</td>
<td>Back to the Hotel and Break</td>
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<td>18.00-21.00</td>
<td>Farewell Dinner</td>
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*Note: Special events*

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<td>Prof. Fusanori Miura, Sidna Aranto, DVM, M.Biotech., Imron Rosyadi, DVM, M.Sc.</td>
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<td>Merapi Volcanoes</td>
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<td>15.30-17.00</td>
<td>Dean Meeting*</td>
<td>Chairperson: Prof. Koichi Sato</td>
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<td></td>
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EXPRESSION OF DMRT1 IN QUAIL EMBRYOS (Coturnix coturnix japonica)

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Abstract

The sex differentiation in the quail is a complex process of formation and involves many genes. One of the genes suspected of having a major influence on genital formation is DMRT1. This study aimed to see the level of DMRT1 gene expression in the quail embryo. The sample of the study was the gonadal tissue of quail embryos obtained from the fertile quail eggs. Eggs are incubated and taken daily to be isolated from the gonadal genetic material. Genetic material in the form of DNA was used for molecular sexing using CHDW gene. RNA was used to measure the expression of DMRT1 gene in quail embryo. Measurement of gene expression on days 1-8 was confirmed by nanodrop spectrophotometer. The results show that DMRT1 gene expression has differences in male and female embryos. DMRT1 gene expression in male embryos has higher intensity than females. DMRT1 gene is also seen to have maximum expression in male embryo’s aged 3-5 days. This DMRT1 gene may have an important role in the process of genital formation and differentiation of quail embryos, especially in males.

Keywords: Gene expression, DMRT1, embryo, quail

Introduction

Doublesex and mab-3-related transcription factor 1 (DMRT1) are part of the DMRT family genes linked to gonadal formation in vertebrates (Hong et al., 2007). DMRT1 itself is a gene encoding a transcription factor that affects other gene regulation. This gene’s sex expression were detected in some animals such as rats, reptiles, and poultry. In poultry, the gene is located on the Z chromosome so its expression is higher in males (ZZ) than in females (ZW) (Smith and Sinclair, 2004). Excessive DMRT1 gene expression in female gonads is known to cause masculinity and upregulation of genes in the testes (Lambeth et al., 2014; Minkina et al., 2014). Nevertheless, the expression of this gene in the quail has not been widely reported when compared with chickens.

In the field of research, quail is used to observe brain development (Scholz et al., 2010), heart (Bana et al., 2013), resistance to disease (Mohamed and Abdel-Hafez, 2016) and the developmental stage of poultry embryo (Ainsworth et al., 2010; Saraswati and Tana, 2015) including its genetic mapping (Caetano-Anolles et al., 2015). However, studies related to gene expression affecting genital development in the quail has not been done. This study aims to see the expression of DMRT1 gene in quail embryos up to 14 days old. The expression patterns that occur are expected to give a better picture of the expression of these genes in the quail in relation to the process of genital development.
Methods

Japanese quail egg (*Coturnix coturnix japonica*) was obtained from PT Peksi Gunarахardja Hatchery in Yogyakarta. Eggs are placed in incubators at 37-38°C with manual transition. Eggs were opened every day and embryos are taken. RNA extracted from embryonic gonad tissue and DMRT1 gene expression viewed using RT-PCR. RNA extraction using RNeasy Mini Kit (QIAGEN) and RT-PCR was performed using Superscript III One-step RT-PCR System with platinum Taq DNA Polymerase (Invitrogen) in accordance with company procedures. Quail sexing is done by CHDW gene amplification and then separated between males and females. It followed by RT-PCR to see the expression of the gene.

The RT-PCR cycle consisted of 48°C incubation for 15 minutes, initial denaturation 94°C for 2 minutes, 36x PCR cycle consisting of 94°C denaturation for 30 seconds, annealing 55°C for 30 seconds, 72°C elongation for 1 minute, ending with 72°C elongation for 10 minutes. Analysis of RT-PCR results was done with 2% agarose electrophoresis gel. The internal controls in this study were the β-actin gene while molecular sexing was performed with positive CHDW gene amplification in female. The DMRT1 specific primer for quail is designed with the Primer3Plus program (http://www.bioinformatics.nl/cgi-bin/primer3plus/primer3plus.cgi) by taking a printout from GenBank (Table 1).

Results

Sexing on a quail embryo

Sexing on a quail embryo is an early stage before gene expression is observed. CHDW gene were used for molecular sexing. The result obtained electrophoretic band on size 322 bp which is female genitals (Table 1). Each sample of quail embryos giving positive bands on β-actin internal control will then be sexed with the CHDW gene. Positive samples with CHDW will be grouped as female sex, while negative samples will be grouped into male sex.

Table 1. Primer used in this research.

<table>
<thead>
<tr>
<th>Target Gene</th>
<th>Primer Forward 5'→3'</th>
<th>Primer Reverse 5'→3'</th>
<th>Size (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>β-actin</td>
<td>CGTGCTGTGTCCCATCTAT</td>
<td>TTGCTCTGGGCTTCATAC</td>
<td>103 cDNA</td>
</tr>
<tr>
<td>CHDWE</td>
<td>GGGGAGCGGAGTAAAAATGT</td>
<td>AACCCCACAGGCTATGCTA</td>
<td>421 gDNA</td>
</tr>
<tr>
<td>DMRT1</td>
<td>TTCAGGACATCCCTCCATC</td>
<td>TGCCATGTGTTCCCTGACTG</td>
<td>322</td>
</tr>
</tbody>
</table>

DMRT1 gene expression in quail embryo

This study obtained the expression of DMRT1 gene expressed on male sex and females. DMRT1 gene expression in males is expressed with high intensity. The expression of DMRT1 at the age of 3-7 days of embryo was high in males, then gradually decreased and stabilized thereafter (Fig. 1). On the first day, the expression was still low and increased when entering day 3. In the embryo aged 3-5 days, the intensity of expression looks high trough electrophoresis bands were thicker than others. DMRT1 expression then decreases and is stable at 6-14 days. In the female expression the DMRT1 gene persists but does not increase significantly at the age of 3-5 days as in males. This expression in females tends to be stable even at the age of the embryo on the 11th day. In adult quail, DMRT1 gene expression appears to be more dominant in males than in females. This comparison of expression between males and females was clarified using nanodrop spectrophotometers at 2-8 days.
Discussion

Genital development in quail occurs in the gonads, then develops into the genital organs of each. Gonad on the quail is the same as in chicken that has symmetrical shape in males (right and left developed) and asymmetrical in female, because it only develops in the left gonad (Smith and Sinclair, 2004). This asymmetrical development is due to the difference in the meiotic cycle that occurs on both sides of the gonads. Gonadal germ cell stem cells in female left gonads are 80%, more than 60% in males. However, in males both sides of the gonads develop well. The difference in the number of stem cells is very noticeable in the HH35-42 phase of female embryo development, but not different in males (De Melo Bernardo et al., 2015).

Internal control of PCR in this study used sequence of β-actin gene that amplifies cDNA at 113bp and gDNA at 454bp. Determination of sex of males and females was done by molecular method of PCR by amplifying CHDW gene sequence on intron E. CHDW was known to be used to determine sex in poultry (Griffith et al., 1998). Samples that were amplified at 322 bp were identified as females, since the primers used were specific to the W chromosome.

In quail embryo, genital development begins at the age of embryo stepping on 3 days. Genital differentiation is thought to occur in embryos aged 3-5 days when viewed from DMRT1 gene expression. DMRT1 is a Z chromosome-related gene and affects the formation of male genital organs in the embryo. This gene is also expressed in female embryos though not as much in males (Omotehara et al., 2014). DMRT1 expression at the age of 3-7 days embryo looks high in males, then gradually lower and stable. The expression of the gene is seen in females but not high as the male embryo. The expression on females may be due to the presence of Z chromosomes, although the numbers are relatively fewer than males. DMRT1 expression in chickens also experienced a higher difference in males at the gonadal differentiation phase, age 3-7 days (Kamata et al., 2004; Omotehara et al., 2014; Caetano et al., 2014). When compared to chickens that had very high DMRT1 expression on day 14 after gonadal differentiation period (Caetano et al., 2014), quail had the highest DMRT1 expression while in that phase at 3-4.5 days of embryo. Gene expression fell and gradually stabilized after the 7th day. This expression can show that DMRT1 is one of the genes that play an important role in the gonadal differentiation process for male genital development. In females, these gene expression tend to be stable because the gene effect in the formation of Mullerian duct during gonadogenesis in both males and females. The absence of DMRT1 causes a failure in stem cell proliferation and even causes death in stem cells in the testes (Ayers et al., 2015).

Some genes other than DMRT1, such as MHM, SOX9, CYP19A1 AROMATASE, SOX3, DAX1, and FOXL2 are also known to have some influence in the process of gonadal
development and differentiation in chickens (Teranishi et al., 2001; Roeszler et al., 2012; Caetano et al., 2014). However, similar research has not been done on the quail. More detailed research on other genes that play a role in the gonadogenesis process is expected to gather better contribute to more animal-friendly sexing processes.

Conclusions

DMRT1 gene expression has the highest rate at age 3-5 days and higher in male embryo than females. In the females embryo the DMRT1 gene remains expressed with stable and lower levels than males. This proves that DMRT1 may have an important role in the process of genital development and differentiation in quail embryos. Thus can be considered as a major gene of sex differentiation in quail embryos.

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References


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