

University of Mosul
College of Veterinary Medicine



**Using Genetic Monitoring to Eggshell Proteins in
Chicken Oviduct Transcriptome During Formation of
The Eggshell**

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MSc / Thesis

Veterinary Medicine / Veterinary Physiology

Supervised by

Assistant Professor

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Abstract

Eggshell formation is a complex process that is tightly controlled by a variety of genes and hormones in the laying hen's oviduct. These genes play an important role in the eggshell's integrity and strength during its formation. This study aimed to monitor eggshell proteins in the uterine transcriptome during eggshell development. Thirty-eight mature laying hens of the Mezo white breed, aged 14-24 weeks were used in the study. Group A consisted of twenty-eight of young laying hens, while group B consisted of ten non-laying chickens with latent ovaries. Most of the tissues were collected the oviduct of young laying and non-laying hens', while the egg was still inside the uterus during the egg formation cycle. After sample collection, the tissue samples were stored at -80° C until the RNA extract, and the homogenization was performed to extract the RNA. The homogenization process was carried out using a tissue homogenizer in the Physiology Laboratory for the RNA extract. Total RNA was extracted from oviduct tissues using the total RNA extraction kit. according to the manufactur instructions for RNA extraction, the RNA integrity was then assessed using the Thermo Scientific Nano Drop ONE system as the initial step in the (PCR) process. A reverse transcriptase kit was used to convert the extracted RNA into cDNA. Selected genes on three distinct oviduct segments (magnum, isthmus and uterus) in both laying and non-laying chickens were subjected to qPCR analysis. Using the 2004 initial draft of the chicken genome assembly, which matched the information displayed on the slides, a corrected annotation of the genes observed on the microarray was created. The reference genome (Gallus_gallus-2.1, May 2006 assembly) and (NCBI nr, Ensembl transcripts) the non-redundant transcript databases were used and compared to NCBI megablast for gene ontology (GO) analysis of the *Gallus gallus* species (Taxid: 9031). The results showed a significant difference in gene expression between laying and non-laying hens. ineddition,

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HIGHLIGHTS	GRAPHICAL ABSTRACT																																																																																			
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جامعة الموصل
كلية الطب البيطري

استخدام المراقبة الجينية لبروتينات قشرة البيض في الجسم النسخي
لقناة البيض أثناء تكوين قشرة البيض

رسالة مقدمة من قبل
فاضل عامر فاضل سليمان

رسالة ماجستير الطب البيطري / الفلسفة البيطرية

بإشراف
الأستاذ المساعد الدكتور
علي سعيد حمودي الجلبي

الخلاصة

تشكّل قشرة البيض هو عملية معقّدة تخضع لتنظيم دقيق من قبل مجموعة متنوعة من الجينات و الهرمونات في رحم الدجاجة البياضة. تؤدي هذه الجينات دورًا مهمًا في سلامة القشرة وقوتها أثناء تكوّنها. تهدف هذه الدراسة إلى تتبع البروتينات المرتبطة بقشرة البيض ضمن النص المورثي (الترانسكربتوم) الرحمي خلال مراحل تطوّر القشرة.

تم استخدام 38 دجاجة بياضة ناضجة في الدراسة، من سلالة Mezo البيضاء، بعمر 24 اسبوعاً وبعمر 14 اسبوعاً. تكوّنت المجموعة (أ) من 28 دجاجة بياضة، بينما ضمت المجموعة (ب) عشر دجاجات غير بياضة ذات مبايض خاملة. و قد تم جمع معظم الأنسجة من قناة البيض لدى الدجاجات البياضة الصغيرة، بينما كان البيض لا يزال داخل الرحم خلال دورة تكوين البياضة. و من ثم تم حفظ عينات الأنسجة عند درجة حرارة -80 درجة مئوية حتى استخلاص الحمض النووي الريبوسومي (RNA) تم استخلاص الحمض النووي الريبسي الكلي من أنسجة الرحم باستخدام عدة استخراج مخصصة لذلك، ووفقاً لتعليمات الشركة المصنّعة. ثم تم تقييم سلامة الحمض النووي الريبسي باستخدام نظام NanoDrop ONE** من شركة Thermo Scientific كخطوة أولى في عملية تفاعل البلمرة المتسلسل (PCR). بعد ذلك تم تحويل الحمض النووي الريبسي المستخلص إلى DNA مكمل (cDNA) باستخدام عدة إنزيم النسخ العكسي. و تم إخضاع الجينات المختارة في ثلاثة أجزاء مختلفة من قناة البيض، في كل من الدجاجات البياضة وغير البياضة، لتحليل qPCR. تم إنشاء تعليق توضيحي مصحح للجينات الظاهرة في المصفوفة الجينية استناداً إلى المسودة الأولية لتجميع الجينوم للدجاج الصادرة عام 2004، والتي تطابق المعلومات المعروضة على الشرائح. كما تم استخدام الجينوم المرجعي Gallus gallus-2.1 (تجميع مايو 2006) وقواعد بيانات النسخ غير المتكررة (NCBI nr) ، ونسخ (Ensembl) ومقارنتها باستخدام أداة NCBI Megablast لإجراء تحليل دلالات الجينات (GO) (لنوع) Gallus gallus** (المُعرف التصنيفي: 9031). أظهرت النتائج اختلافاً معنوياً كبيراً في تعبير الجينات بين الدجاجات البياضة وغير البياضة. بالإضافة إلى ذلك، تم رصد زيادة في التعبير لأربعة جينات في جزء "الماغنوم"، وزيادة في عشرة جينات في

منطقتي "برزخ الرحم" و"الرحم". تم تصنيف الجينات المعبر عنها وظيفيًا بين المجموعتين ضمن فئات تشمل: الجينات المرتبطة بالكالموديولين، وعوامل النمو، والسييتوكينات، والارتباط بمستقبلات الإشارة، والبروتينات المعقدة الرئيسية للتوافق النسيجي، وناقلات ثانوية، وبروتينات مصفوفة خارجية، والكادهيرين، ومثبطات البروتياز، والفسفوليبيز.

تم تصنيف الجينات المعبر عنها وفق ثلاث فئات وظيفية رئيسية:

* العملية الحيوية (Biological Process)

* المكون الخلوي (Cellular Component)

* الوظيفة الجزيئية (Molecular Function)

وقد تبين أن سبعة من هذه الجينات ترمز لبروتينات المصفوفة وبروتينات نقل الكالسيوم، فيما يرمز جينان إلى نشاط السييتوكين ومثبطات الإندوبيبتيداز، بالإضافة إلى جينات أخرى مرتبطة بربط أيونات الكالسيوم والمناعة الفطرية، ونشاط الفوسفوليبيز C ، وربط بيتا-كاتينين وجزيئات الالتصاق الخلوي، ونشاط مستقبلات الإشارة الغشائية، وناقلات الكاتيونات المعدنية. وقد اعتُبر أن جميع هذه الجينات تشارك في تكوين قشرة البيضة.

خلصت الدراسة إلى أن التعبير الجيني خلال تطور قشرة البيض أظهر وجود عدد من الجينات ذات التعبير المتغير في قناة البيض لدى الدجاج. وقد استخدمت هذه الدراسة تحليل دلالات الجينات (GO) لإجراء تحليل وظيفي للجينات المعبر عنها والبروتينات الناتجة عنها. وقد ساعد هذا النهج الترانسكريبتومي في الكشف عن الجينات العالية التعبير في أجزاء قناة البيض، والتي تسهم في تكوين بروتينات أو مكونات أولية لقشرة البيض، أو تُقرز في السائل الرحمي للمساهمة في تكوين القشرة، والحماية من الميكروبات، والحماية الميكانيكية، ويُعتبر جهاز RT-PCR أفضل وسيلة لمتابعة النسخوم في دراسات النصوص الجينية.