Towards a Humane and Sustainable Solution:
A Genetic Model to Eliminate Male Chicks Culling in the Egg Industry

Yuval Cinnamon
The GOLDA project
The hens that lay eggs from which only WT females hatch

Why do we need GOLDA?
The poultry industry is the major contributor of dietary protein, worldwide
THE PROBLEM

Unwanted by the egg and meat industries, ~7 billion male chicks are culled around the world annually by inhumane and costly methods.

INHUMANE

7 Billion Day-Old Male Chicks

COSTLY

~7 Billion US$

A global 50% increase in demand for eggs from 2020 to 2035 will intensify the issue of male chick culling at a faster pace.

- Major sustainability problem
- The EU are committed to ban the culling of day-old male chicks
- The US is also committed, but still seeking for a practical and viable solution

Number-wise, this is most devastating animal welfare issue worldwide

What are the requirements for a viable solution?
The solution should be highly accurate, very fast, safe to the layers and cost-effective.

1. Methods must be applicable to eggs prior to setting for incubation.

2. Methods must not affect the integrity of egg-shells and the interior of eggs.

3. Eggs containing male blastoderm cells need to be used for reasonable purposes.

4. Applied methods should have no negative effects on embryonic development.

5. Rate of hatchability of chicks must not be affected.

6. Necessary technical equipment must be available and its use economically feasible.

7. Methods must be applicable to large numbers of eggs.

8. Sorting by gender must be possible within short periods of time.

9. Methods must be free of any negative effect on female reproduction.

10. Post-embryonic physical and behavioral development of chicks remains unchanged.

11. Methods must be acceptable from a humanitarian and ethical point of view.

12. The level of errors must be minimal and acceptable to the industry.

13. There must be an accepted solution to unwanted hatched males.
Addressing the Culling of Day-Old Male Chicks

Previous and ongoing attempts:

- Attempts to bias the sex ratio
- Development of dual purpose breeds
- Sorting strategies
Sorting = Errors
Addressing the Culling of Day-Old Male Chicks

*Previous and ongoing attempts:*

- Attempts to bias the sex ratio
- Development of dual purpose breeds
- **Sorting strategies**

*Despite all attempts, to date there are no viable solution.*
Henner Schönecke, chairman of Bundesverband Ei, which is the branch organisation for the German layer sector.

Photo: Koos Groenewold

**Germany: ‘Only a few hatcheries will survive’**
Egg-Tech Prize for tech solutions to end male chick culling remains unclaimed as ‘no applicants met the criteria’

April 20, 2023    Elaine Watson

Organizers of the $6 million Egg-Tech Prize—a high-profile global competition to find scalable technologies to sex chicks before they hatch—have not yet issued a final award because “none of the applicants met the criteria,” AFN has learned.
Addressing the Culling of Day-Old Male Chicks

Previous and ongoing attempts:

- Attempts to bias the sex ratio
- Development of dual purpose breeds
- Sorting strategies

Despite all attempts, to date there are no viable solution.

The rationale behind our solution is based on two key insights:
1. Practically, there are no differences between male and female embryos
2. Chickens' sex is determined by sex chromosomes, so they are key to the solution.
Genetic Solution to End the Culling of Day-Old Male Chicks

- Normal female chick layer
- Male development stops at the earliest embryonic stage

Diagram showing genetic difference between male (ZZ) and female (ZW) chickens.
The European Commission has confirmed that the layer hens and the table eggs produced by NRS Poultry technology are wild-type (i.e., non-GMO).

As a result, the table eggs produced by NRS Poultry Technology can be marketed immediately in all EU member states without any restrictions or special labeling.

The CVM has indicated in a written response that they “…may consider exercising enforcement discretion for some of the activities/phases described...”.

Implying that the wild-type layer hens and table eggs could be exempt from regulatory approval once sufficient evidence is provided.
Genetic Solution to End the Culling of Day-Old Male Chicks

How does the solution integrate with the breeding program of the genetic lines?
The production pyramid

- **Pure Elite lines**
- **Grandparents**
- **Parents**
- **commercial layer hens**
- **Table eggs**
Integration into Breeding Program

Pure lines
A  B

Grandparents
X

Parents
X

Layers
X

Blue light illumination

Integration into Breeding Program
Integrating into Breeding Program

What is the workflow of generating genetically transformed chickens?
Generating Genetically Transformed Chickens

Primordial Germ Cells (PGCs) are unique embryonic cells that transfer genetic information across generations.

In males, PGCs will colonize the testes. Upon sexual maturity, PGCs will differentiate into sperm.

In females, PGCs will colonize the ovary. Upon sexual maturity, PGCs will differentiate into eggs.

Embryonic PGCs retrieval → Culturing PGCs → Genomic transformation → Single cell FACS → Pure colonies validation → Microinjection to surrogate embryo → Breeding surrogate chimera → Screening for founders → Breeding founders F0
What are the requirements for the targeting vector to be introduced on the Z chromosome?
Requirements from the HolyGrail

1. Integration to the Z chromosome
2. Single ubiquitous promoter
3. Light inducible molecular “switch” – Optogenic system
4. Gene to stop embryogenesis
5. Security “Safe-lock” element to prevent unintended activation
Integration site on the Z chromosome
Integration site on the Z chromosome
Integration to the Z chromosome

The integration site on the Z chromosome

Co-transfection

CRISPR complex
HolyGrail

PGCs

Sperm and Eggs

AtACCAGATAACGTGCTTTATTTG
CRISPR Cas9 sgRNA
The integration site on the Z chromosome

Integration to the Z chromosome

5'HA

HDR

Chromosome Z

3'HA

ATACCAGATAACGTGCCCTATTG

CRISPR Cas9 sgRNA

[Image of gel electrophoresis with red arrows indicating bands]
Injection of genetically transformed PGCs

Colonization of PGCs in the gonads
Design of the HolyGrail construct

The “Safe-lock” mechanism
FlipChick – GFP positive FLPo

WT GFP negative
A 

Possible genotypes:

♂ Z HG1-SL/FlpO □ Z FlpO
♂ Z HG1/WT □ Z WT

B

C

D

5' LHA pCAGG FlpO IRES GFP RHA-3'

Female S18

♂ Z HG1-SL/FlpO □ Z FlpO □ Z WT □ Z HG1/WT

FlpO WT
GOLDA
The GOLDA genome – The HolyGrail without the GFP “Safe-Lock” mechanism

So what is the Opotogenic molecular switch?
The light-inducible molecular - The optogenic system

What are optogenes?
Design of the HolyGrail construct

The molecular light-inducible switch - Optogenic system

The Cre loxP system
Design of the HolyGrail construct

The molecular light-inducible switch - Optogenic system

Optogenic system-Cre

Combining Optogenes with Cre
The GOLDA genome – The HolyGrail without the GFP “Safe-Lock” mechanism

HolyGrail-Safe-lock
Light-activation of the lethality inducing gene
Bone morphogenetic protein-4 is required for mesoderm formation and patterning in the mouse

Glenn Winnier,1 Manfred Blessing,1,2 Patricia A. Labosky, and Brigid L. M. Hogan
Howard Hughes Medical Institute and Department of Cell Biology, Vanderbilt University Medical School, Nashville, Tennessee 37232-1475 USA

GENES & DEVELOPMENT 9:2105-2116, 1995
PCR validation to confirm the activation of the Lethality-inducing mechanism
GOLDA’s embryos, following illumination and 72h of incubation

Normal Females

Malformed and dead males
Hatchability Analysis of Blue-Light Illuminated GOLDA eggs

<table>
<thead>
<tr>
<th></th>
<th>♀</th>
<th></th>
<th>Hatched %</th>
<th>♂</th>
<th></th>
<th>Hatched %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dark Control</td>
<td>102</td>
<td>90</td>
<td>88%</td>
<td>82</td>
<td>71</td>
<td>87%</td>
</tr>
<tr>
<td>Blue-light</td>
<td>267</td>
<td>173</td>
<td>65%</td>
<td>208</td>
<td>0</td>
<td>0%</td>
</tr>
</tbody>
</table>

Distribution of the hatched chicks

Dark Control
- 49% ♀
- 51% ♂

Blue-light induction
- 0% ♂
- 100% ♀
Current status and future prospective of the research

• Fine tuning the illumination process to ensure that no males hatch

Light intensity $\times$ Duration = Illumination dosage

• Performances

• Excluding male eggs, infertile and early embryonic mortality eggs
Advantages:

- Reliability and Accuracy: Genetic Solution with 100% Accuracy
- Compatibility with Current Breeding Program
- Maintenance of Genetic Diversity of the Pure Lines
- Easy Integration with Hatchery Workflow
- The Illumination can take place (maybe) during storage, or pre-incubation.
- Male Embryos Cease Development at an Early Stage
- High Nutritional Value of Male Eggs
- No Need for Egg Sampling
- No Expensive Sorting Technologies Required (Hyperspectral, MRI, Mass Spectrometry, etc.)
- Applicable to a Variety of Chicken Strains and Egg Types/Colors
- Elimination of Timely and Costly Sorting of Male Chicks
- Environmental and Sustainability Benefits
- Saves 50% of Energy and Space Required for Incubation
- No Need to Dispose Dead Males
- The end product – The layers and the table eggs are Non-GMO and identical to the currently used

The Practice of Sorting and Culling Billions of Male Chicks Will Come to An End
Always seeking for highly motivated excellent scientists to join our team: yuvalcinnaman@gmail.com
Collaborations:
Dr. Shelly Druyan
Head of the Poultry and Aquaculture Dept.
The Agricultural Research Organization – The Volcani Institute.
Dr. Dimitri Dima Shinder
Poultry Farm
Mark Ruzal
Alex Kantor
Sergey Oblezin

Thank you!