Genome Editing in Poultry:

Opportunities and Impacts

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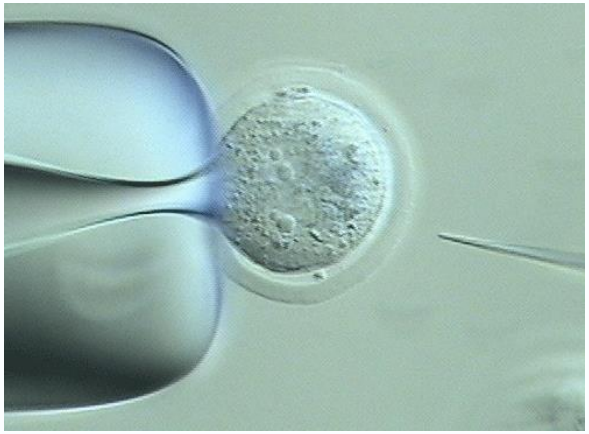
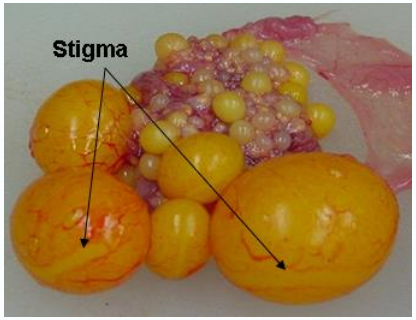
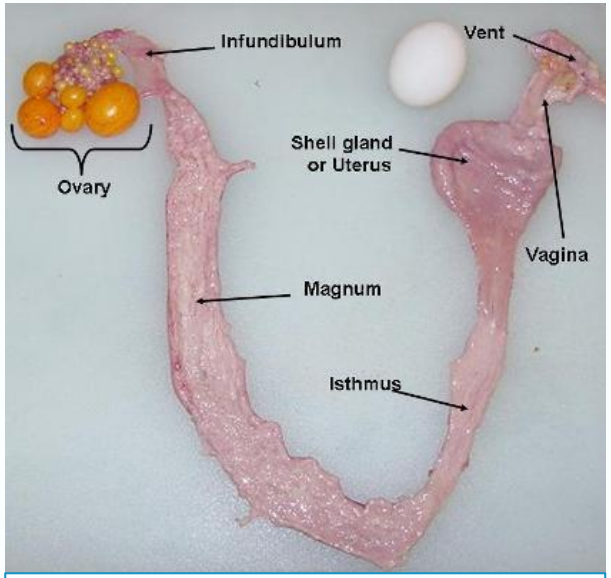
The challenge for precision genome engineering in birds



In mammals (cattle sheep goats):

- Use IVF and edit the single cell zygote (by microinjection)
- Or edit a cell in culture and use Somatic Cell Nuclear Transfer





Images courtesy of Dr Jacquie Jacob
University of Kentucky

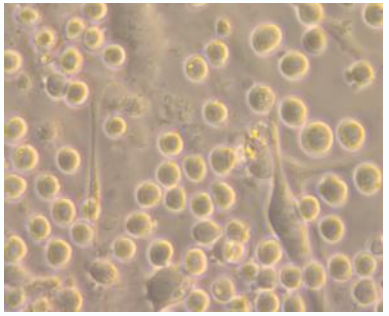
In bird species (poultry):

- The avian ovum on the swelling yolk until its release and fertilization (single cell zygote) at the top of the oviduct
- The single cell zygote is intimately linked with the yolk: almost impossible to manipulate
- ***An alternative approach is required***

Methods to make a transgenic or edited chicken

Primordial Germ Cells (PGCs)

- Progenitors to ovum and sperm forming cells
- In the blood of the 1-2 day old chick embryo

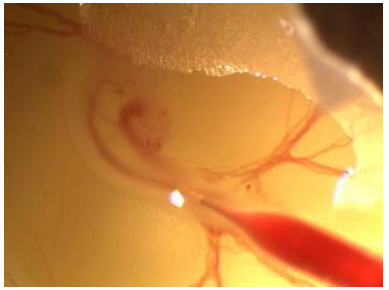


PGCs OUTSIDE – highly skilled culture (not trivial)

- gene targeting (Schusser et al, 2014)
- gene editing (Park *et al*, 2014)

PGCs INSIDE – accessing the germ cells in vivo (in ovo)

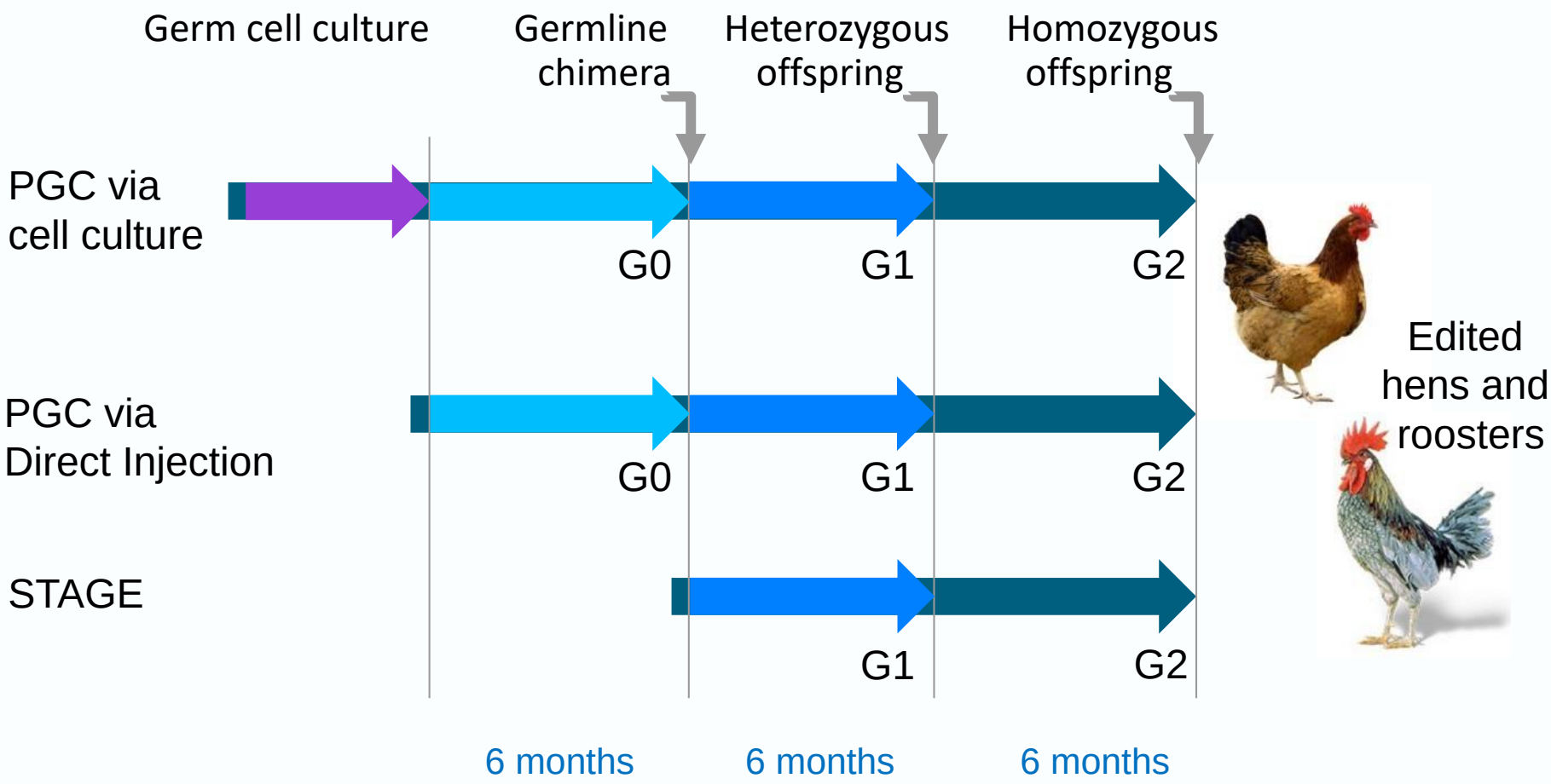
- Integrating lentiviral systems (McGrew et al, 2004)
- Direct Injection in vivo (Tyack *et al*, 2014)



Sperm Transfection Assisted Gene Editing (STAGE)

- Another approach which could speed up gene editing in poultry (Cooper *et al*, 2017)

Timeline approximations for application of the technologies



Gene editing tools and chicken PGCs

TECHNIQUES AND RESOURCES

Efficient TALEN-mediated gene targeting of chicken primordial germ cells

Lorna Taylor[1], Daniel F. Carlson[2], Sunil Nandi[1], Adrian Sherman[1], Scott C. Fahrenkrug[2] and Michael J. McGrew[1,*]

ABSTRACT

In this work we use TALE nucleases (TALENs) to target a reporter construct to the *DDX4* (*vasa*) locus in chicken primordial germ cells

PLOS | ONE

RESEARCH ARTICLE

Germline Gene Editing in Chickens by Efficient CRISPR-Mediated Homologous Recombination in Primordial Germ Cells

Lazar Dimitrov, Darlene Pedersen, Kathryn H. Ching, Henry Yi, Ellen J. Coll
Shelley Izquierdo, Marie-Cecile van de Lavoir, Philip A. Leighton*

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Abstract

The CRISPR/Cas9 system has been applied in a la
for genome editing. In chickens, CRISPR has be
sues, but no CRISPR-mediated germline mor
CRISPR to target the chicken immunoglob
(PGCs) to produce transgenic progeny
tor for homology-directed repair of th
selected. All of the resulting drug
targeted cells gave rise to hea
results show that gene-edi
CRISPR/Cas9 system,
cation in birds.

OPEN ACCESS

Citation: Dimitrov L, Pedersen D, Ching KH, Yi H, Collarini EJ, Izquierdo S, et al. (2016) Germline Gene Editing in Chickens by Efficient CRISPR-Mediated Homologous Recombination in Primordial Germ Cells. PLoS ONE 11(4): e0154303. doi:10.1371/journal.pone.0154303

Editor: Michael S D Kormann, University Tuebingen, GERMANY

Received: March 10, 2016

Intr

Vasa, a DEAD box RNA helicase originally identified in *Drosophila*, is essential for proper germ cell formation in multipl
species (Schupbach and Wieschaus, 1986; Komiya et al., 19

PNAS

Targeted gene knockout in chickens mediated by TALENs

Tae Sub Park[a,b], Hong Jo Lee[a], Ki Hyun Kim[a], Jin-Soo Kim[c], and Jae Yong Han[a,1]

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Edited by James E. Womack, Texas A&M University, College Station, TX, and approved July 30, 2014 (received for review June 7, 2014)

Genetically modified animals are used for industrial applications as well as scientific research, and studies on these animals contribute to a better understanding of biological mechanisms. Gene targeting techniques have been developed to edit specific gene loci in

the mRNA that encodes TALENs or ZFNs was injected to generate target gene-modified animals. However, programmable gene-edited poultry have not yet been reported. Unfortunately, the one-cell stage injection method cannot be used to generate

SCIENTIFIC REPORTS

OPEN

Targeted mutagenesis in chicken using CRISPR/Cas9 system

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Received: 23 October 2015
Accepted: 17 March 2016
Published: 06 April 2016

The CRISPR/Cas9 system is a simple and powerful tool for genome editing in various organisms including livestock animals. However, the system has not been applied to poultry because of the difficulty in accessing their zygotes. Here we report the implementation of CRISPR/Cas9-mediated gene targeting in chickens. Two egg white genes, ovalbumin and ovomucoid, were efficiently (>90%) mutagenized in cultured chicken primordial germ cells (PGCs) by transfection of circular plasmids encoding Cas9, a single guide RNA, and a gene encoding drug resistance, followed by transient antibiotic selection. We transplanted CRISPR-induced mutant-ovomucoid PGCs into recipient chicken embryos and established three germline chimeric roosters (G0). All of the roosters had donor-derived mutant-ovomucoid spermatozoa, and the two with a high transmission rate of donor-derived gametes produced heterozygous mutant ovomucoid chickens as a about half of their donor-derived offspring in the next generation (G1). Furthermore, we generated ovomucoid homozygous mutant offspring (G2) by crossing the G1 mutant chickens. Taken together, these results demonstrate that the CRISPR/Cas9 system is a simple and effective gene-targeting method in chickens.

Chicken is a commercially important animal and its genetic modification is expected to be used for agricultural, industrial, and scientific applications[1–3]. There are various possible and beneficial applications of genetically modified chicken, including improvement of production of meat and eggs, generation of disease-resistant chickens, mass production of therapeutic proteins in egg whites, and establishment of models for studying avian development. Furthermore, gene disruption of egg white allergen genes such as ovalbumin (OVA) and ovomucoid (OVM) has the potential to produce low allergenicity in eggs, thereby reducing immune responses in individuals sensitive to items such as egg white–containing food products and vaccines. To produce these genetically modified chickens, efficient technologies, including suitable transgenic and knockout methods, are required. However, the genetic modification of chickens has lagged far behind that of other organisms because of difficulty in accessing and manipulating the zygote[4]. Therefore, chicken transgenesis has mainly been cultured and genetically modified in vitro. Here, we have been used to generate transgenic chickens using viral vector infection of the early stage embryo[5,6]. Recently, primordial germ cells (PGCs) have been transgenic chickens with these methods, few reports are available on chicken knockout chickens was first reported by Schusser *et al.*[7]. In this study, we transplanted PGCs by homologous recombination disrupted in cultured PGCs by homologous recombination and homozygous gene-targeted chickens were produced by crossing the G1 mutant chickens. Taken together, these results demonstrate that the CRISPR/Cas9 system is a simple and effective gene-targeting method in chickens.

Another gen
(CRISPR)

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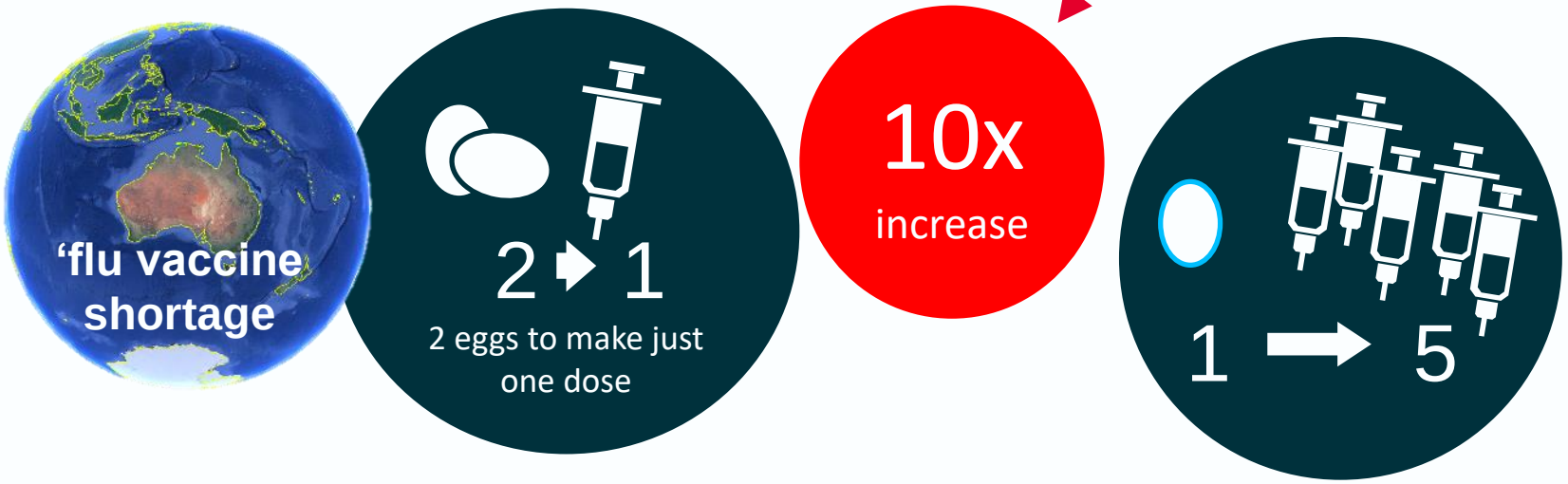
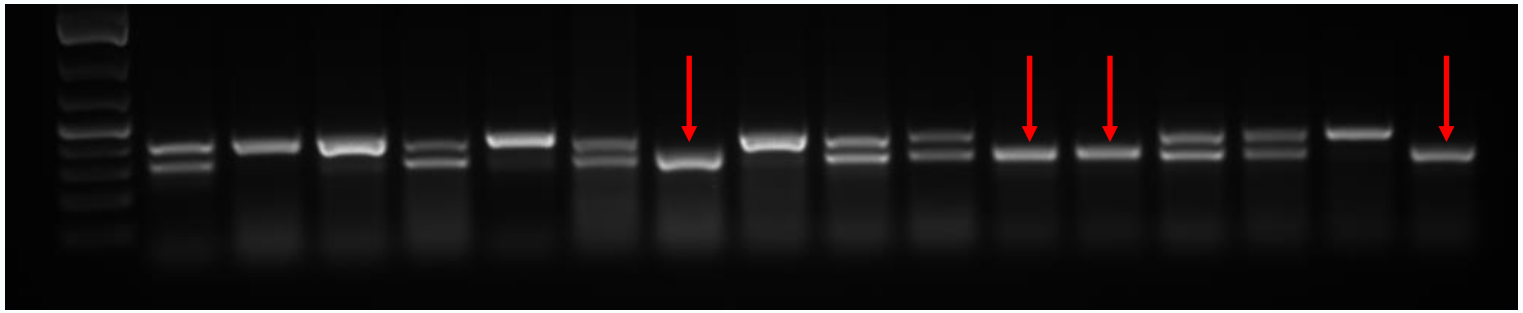
Vaccine manufacture and immune gene deletion



- We have deleted a key **anti-viral** gene in a line of chickens, initially in just one parent then, by crossing, achieving double knock-out and full effect



- Double knockout flock are currently being expanded to provide eggs for trial to assess ***enhanced growth*** of influenza vaccine virus.

Disease Resilience

- Disease impacts are significant to food production and to human health and safety (e.g. “bird ‘flu”)
- Genomics and gene editing are revealing opportunities to enhance resilience to important diseases such as:
  - Avian Influenza

RNA-seq analysis revealed novel genes and signaling pathway associated with disease resistance to avian influenza virus infection in chickens[1]

Developmental and Comparative Immunology 77 (2017) 340–349

Contents lists available at ScienceDirect

Developmental and Comparative Immunology

journal homepage: www.elsevier.com/locate/dci

Precise gene editing of chicken Na+/H+ exchange type 1 (chNHE1) confers resistance to avian leukosis virus subgroup J (ALV-J)

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Nonconserved Tryptophan 38 of the Cell Surface Receptor for Subgroup J Avian Leukosis Virus Discriminates Sensitive from Resistant Avian Species

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Subgroup J avian leukosis virus (ALV-J) is unique among the avian sarcoma and leukosis viruses in using the multimembrane-spanning cell surface protein Na^+/H^+ exchanger type 1 (NHE1) as a receptor. The precise localization of amino acids critical for NHE1 receptor activity is key in understanding the virus-receptor interaction and potential interference with virus entry. Because no resistant chicken lines have been described until now, we compared the NHE1 amino acid sequences from permissive and resistant galliform species. In all resistant species, the deletion or substitution of W38 within the first extracellular loop was observed either alone or in the presence of other incidental amino acid changes. Using the ectopic expression of wild-type or mutated chicken NHE1 in resistant cells and infection with a reporter recombinant retrovirus of subgroup J specificity, we studied the effect of individual mutations on the NHE1 receptor capacity. We suggest that the absence of W38 abrogates binding of the subgroup J envelope glycoprotein to ALV-J-resistant cells. Altogether, we describe the functional importance of W38 for virus entry and conclude that natural polymorphisms in NHE1 can be a source of host resistance to ALV-J.

Improving food safety

- Allergy to chicken egg affects ~ 2.5% of children
- Significant food safety issue
- **OVM** is the most allergenic egg white protein
  - only 10% of total egg white protein (compared Ova at >50%)
  - Ovm allergenicity is ***not lost in cooking/processing***
- No clear role identified for **OVM** in fertility, egg formation or nutritional value
- **OVM** $^{-/-}$ birds have been successfully created

Sex-selection: by detection at point of lay

Marker integrated
(with global genetics company)

$Z^{FP}W$ $Z\,Z$

Detection system identifies
point of lay embryo as male

$Z^{FP}Z$ ♂ → ♂ Egg removed at point of lay

$Z\,W$ ♀ →

No more culling of
day old male chicks

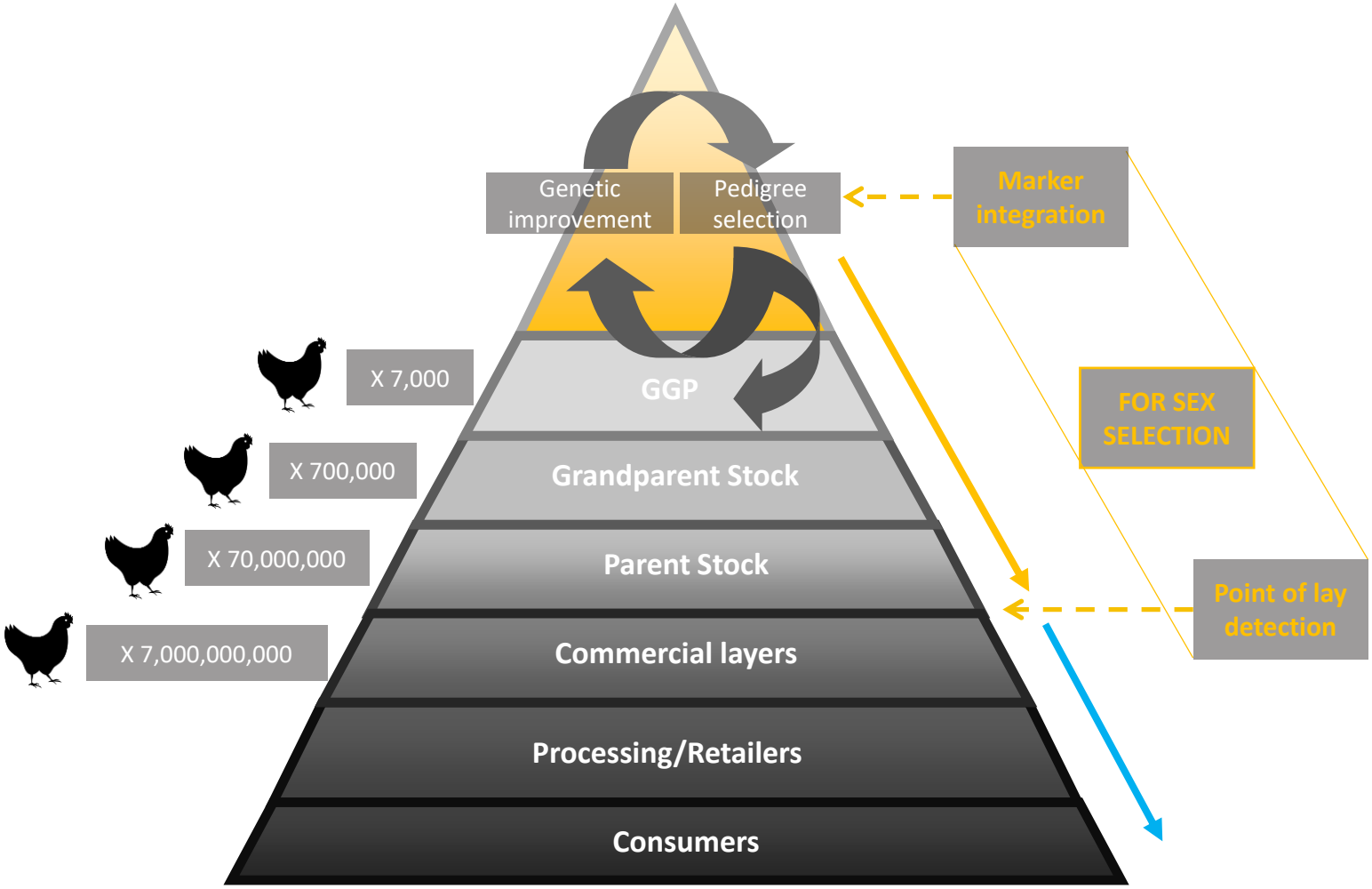
Hens to the farm
exactly the same
as they are today

Currently 7 billion male chicks are culled at day old every year across the world

Marker assisted sex-selection:

- no male egg incubation = $$s
- Egg white and yolk value recovery = $$s
- Female eggs = "null-segregant", no GM present → hens lay eggs for food

Industry integration of GE/GM into poultry must fit the pyramid



Impacts for the poultry industry

- The impacts could lead to….
  - Improved production
    - In sustainability, health & welfare
  - Increased food safety
  - Enhanced vaccine production

….but all of this relies on

- **Safety data combined with effective regulation of the technology**
- Public understanding and attitudes to gene editing (vs GMO)
- How these factors impact on industry's appetite for adoption

Acknowledgements

CSIRO

- Tim Doran
- Kristie Jenkins
- Mark Tizard
- Terry Wise
- Kirsten Morris
- Terri O'Neil
- Caitlin Cooper
- Arjun Challagulla
- Shuning Shi
- Andrew Bean
- Tamara Gough
- Kerri Bruce