A review of the culling of day-old chicks and in-Ovo technologies

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1 Introduction

In 2008, it was estimated that within the European Union, 330 million day-old male chicks are culled annually (European Commission, 2008) and more recent studies predict that globally, approximately 7 billion day-old male chicks are culled annually (Ataei & Kırkpınar, 2021; Ching et al., 2021). This is as a result of a commercial system genetically selecting hybrid chicken that either specialise in high egg production characterised by slim physique and low fattening performance (laying hens (females only)) or that specialise in fast growth rates, large breast muscles and improved feed efficiency (meat chickens(both sexes)) (Ataei & Kırkpınar, 2021; Ching et al., 2021). The male birds from birds genetically selected for egg production have no economic value as they do not lay eggs and do not put on sufficient flesh for meat production and have slow growth rates compared to meat chickens (Cerit & Avanus, 2007; Ataei & Kırkpınar, 2021). Currently, once hatched, day-old chicks are currently sexed manually via cloacal sexing or feather colour sexed (brown strains) and male chicks are culled while females go on to be raised as laying hens. In the UK predominantly colour sexing is used.

The economically motivated culling of day-old male chicks is being debated critically in a number of societies (Woelders et al., 2007; Leenstra et al., 2011; Reithmayer et al., 2021) but also raises questions of sustainability and resource use for these unwanted chicks. Consequently, there are several methods being developed for sex determination as well as sex-allocation of chick embryos during the incubation period (in-Ovo – within the egg) (Reithmayer et al., 2021; Ataei & Kırkpınar, 2021; Ching et al., 2021; Xiang et al., 2022). Although sperm sexing is available in other species, this method is not feasible for poultry (Vishwanath & Moreno, 2018). Manual cloacal sexing has an accuracy of over 98% and is performed at a speed of around 1,000 birds per hour per person (Biederman & Shiffrar, 1987). Thus, any alternative to this method must provide the same or improved accuracy and speed. For an average hatching rate of 80% it requires 2.4 eggs to be set per female chick obtained.

1.1 Interest in alternative methods in other countries

In 2022, Germany banned the culling of one-day-old male layer chicks and the government will decide in 2023 if a proposed ban on in-Ovo sexing methods used after day 7 of incubation will be implemented in 2024 (Poultry World, 2021).

France have announced a ban on chick culling by 2022 (BBC News, 2020. Poultry World 2022a) and will allow identification till day 15. Culling of male sexing error chicks will still be allowed as will male chicks from white feathered genetics. All hatcheries must utilize some gender identification technology from 2023 and government support for equipment and building costs is available.

In Italy, the main trade association of egg producers (Assoavi) has committed to adopt in-Ovo sexing technologies and to promote their application throughout the Italian supply chain once they become commercially viable (The Poultry Site, 2020). Chick culling is to be prohibited form 2026, including for white feathered birds and sexing error chicks.

Switzerland has decided to stop chick culling in 2024 by a combination of gender identification until day 9 and rearing male chicks.

In the US in 2016, a cooperative of around 90% of US egg producers (United Egg Producers) announced they would seek to eliminate the culling of day old male chicks within the next four years, or when "an economically feasible, commercially viable alternative" was found (United Egg Producers, 2020).

1.2 Timing of alternative methods

There are currently four commercially available in-Ovo sex determination techniques (Table 1), however they require several days of incubation - 9 or 13 days. Commercial hatcheries are keen to perform sex determination as early as possible to minimise incubation costs and to have more options to repurpose the unwanted male eggs. Techniques that can be performed before any incubation – e.g. 'Hypereye' – would be the most cost-effective as incubator running costs are effectively halved from the removal of male and infertile eggs (Canadian Poultry Magazine, 2016 and 2018).

Legislators are also keen to find techniques for in-Ovo sex determination as early as possible so eggs can be repurposed before embryo development – especially before any chance of embryo pain perception having developed. Current research suggests that chicks are likely to feel pain after between 7 to 10.5 days of incubation. Whilst sensory nerves are present from day four of incubation, there are no synaptic connections to the spinal cord – and therefore no way for painful stimuli to be centrally processed – until day seven (Krautwald-Junghanns et al., 2018). However, the neural tube does not develop into a functional brain until the second half of gestation (Close et al., 1997) after around 10.5 days of incubation.

2 The welfare impact of all 'potential' methods for killing live chicks

The large-scale killing of unproductive animals includes male day-old layer chicks as well as surplus male day-old breeder chicks (EFSA, 2019). Asphyxiation by carbon dioxide gas or maceration using a high-speed grinder are common methods to cull day-old chicks. However, a study from Turkey (Ataei & Kırkpınar, 2021) suggest the use of cervical dislocation is also used. It may be that cervical dislocation is used for the culling of single chicks or as secondary method if the primary method has failed but, within a hatchery, a group process such as exposure to inert gas or maceration is likely to be more practical.

2.1 Exposure to inert gas

Modified atmosphere killing methods such as exposure to high concentrations of carbon dioxide (at least 75% by volume in air), inert gases such as argon or nitrogen containing less than 2% residual oxygen or a mixture of inert gases and carbon dioxide is routinely used to kill unwanted day-old chicks (<72 hours) in hatcheries (Raj and Whittington, 1995; HSA, 2001; AVMA, 2016). The duration of exposure to gas mixtures required to kill chicks varies according to the species and concentrations of carbon dioxide or residual oxygen levels. The HSA Guidelines recommends a 3 minute exposure time for day-old chicks when exposed to 90% carbon dioxide in air or inert gases with less than 2% residual oxygen (HSA, 2001).

Exposure to gas mixtures involves placing chicks in containers, bins or large skips prefilled with a chosen gas mixture. When using atmosphere killing methods it is important that each batch of chicks is allowed sufficient exposure time to die and that birds are not showing any signs of life before adding the next batch. Similarly, when chicks are contained in trays or crates it is important to ensure that chicks are evenly distributed for full exposure to the gas mixture (EFSA, 2019). Gurung et al. (2018) found exposure of chicks to low atmospheric pressure to be a suitable method and

preliminary trials showed that negative air pressure of 15.3kPa (1 kPa = 7.5 Torr) resulted in 100% mortality. The experimental chicks were subjected to a reduction in chamber pressure from 100.12 kPa to 15.3 kPa over 80 seconds and held in the chamber for 5 minutes, resulting in death.

EFSA (2019), identified various hazards with both the use of gas mixtures in containers and low atmospheric pressure for killing which can have detrimental welfare consequences.

2.1.1 Concerns associated with gas mixtures in containers

During the use of gas mixtures in containers for killing of day-old chicks, if temperatures are too low, caused by the physical property of the gas as well as by a lack of skilled operators, liquid delivery of gas or too fast gas injection rate, this can result in cold stress. Issues such as overloading, too short exposure time and too low gas concentrations are also a welfare concern resulting in chicks remaining alive, conscious, in pain, fearful and experiencing respiratory distress. To prevent these issues arising, staff training is paramount. Additionally, appropriate concentration of gases and temperature should be monitored, containers should be fit for purpose and birds should be checked for life before adding additional batches. Although these risks can be prevented, inhalation of high carbon dioxide concentration alone can result in pain, fear and respiratory distress. This is a serious welfare concern of the method itself.

2.1.2 Concerns associated with low atmospheric pressure killing

For low atmospheric pressure killing, too fast decompression or too short exposure time as a result of lack of skilled operators or the wrong rate of decompression or exposure time, can cause pain and respiratory distress and can result in chicks not being killed, remaining conscious and experiencing respiratory distress. Additionally, expansion of gases in the body cavity due to inefficient equipment can result in pain.

3 The welfare impact of all ‘potential’ methods for killing in-shell embryos

There continues to be debate regarding the stage of incubation in which welfare of the embryo becomes an issue. Unhatched eggs are disposed of, but the methodology used for these eggs should differ if the embryo can experience suffering, an emotional experience. As evidence suggests that the ability to feel pain may occur as early as mid-way through embryonic incubation, requirements for humane-killing techniques are sometimes based on this ability and incubation timescale (American Veterinary Medical Association (AVMA), 2013, Close et al., 1997).

3.1 Maceration

Maceration methods, otherwise known as instantaneous mechanical destruction (IMD), are generally used to kill in-shell embryos but can also be used for chicks up to 72 h post-hatch (Council Regulation (EC) No. 1099/2009). There are two designs of mechanical apparatus: 1) a ‘roller-type’ design which causes the chicks to be crushed between two rollers which are rapidly rotation and 2) a ‘knife-type’ design containing fast moving blades which mince the chicks. Garden shredders should not be used. IMD methods are aesthetically unpleasant but are deemed an acceptable and humane method of chick disposal providing the equipment is well maintained and used responsibly (HSA, 2001). Maceration is used only for large-scale killing and as a killing method only. The method should be sufficient to ensure that all chicks are killed instantaneously, even if they are handled in a large number. Mechanical destruction of chicks should result in slurry, rather than recognisable body parts such as internal organs, legs, wings and heads, to ensure chicks are truly macerated (HSA, 2005).

EFSA (2019), identified various hazards which can have detrimental welfare consequences. For example, slow rotation of blades or rollers or rollers set to wide can cause pain in birds and can result in birds not being killed instantly and remaining conscious. Slow rotation of the blades can

result in chicks accumulating over the blades and not being quickly or sufficiently macerated. If rollers are set too wide there is a risk that the chick's abdomen is crushed without causing damage to the brain, thus a serious welfare concern. Therefore, the gap between rollers must cause instantaneous crushing of the chicks' heads resulting in immediate death (HSA, 2005) and this can be achieved by setting space <10mm between rollers (EFSA, 2019). Additionally, overloading of mechanical apparatus can cause bird pain, distress and fear. To prevent overloading specifically, it is important to avoid adding chicks before the previous batch has gone through and died. The process should be slow enough to avoid jamming, birds rebounding from the blades or suffocating prior to maceration (HSA, 2017). These hazards often stem from a lack of staff training, inappropriate setting of equipment (e.g. rotation per minute, roller settings), the use of equipment not fit for purpose or trying to macerate too many birds or egg embryos at one time (EFSA, 2019).

4 The current status of in-Ovo sexing technologies

To avoid the culling of day-old chicks at a large scale, in-Ovo technologies have been and are continually being developed for gender determination with some focusing on sex-allocation or 'gender reversal'. These technologies are executed at different stages of embryonic development and allow the identification of male chicks during incubation within the egg. Sex determination before incubation is preferred (Leenstra et al., 2011), but is difficult to achieve. Some technologies are commercially available whilst some are still being investigated. For technologies to be commercially feasible the sexing technologies need to be fast, cost-efficient, highly-precise and not have significant impacts on hatching rate, bird health, welfare or performance (Krautwald-Junghanns et al., 2018). Additionally, sex determination needs to occur before pain perception has evolved. The first sensory afferent nerves develop in the chicken embryo on d4 of incubation, but a synaptic connection to the spinal cord is not present before d7 of incubation. Therefore, pain perception is not expected before d7 of incubation (Aleksandrowicz & Herr, 2015).

Within the scientific literature the following methods have been investigated:

4.1 Non-optical methods

4.1.1 Morphological measurements of the egg

Previously, studies have investigated whether the sex of the chick could be determined using the morphological measurements of the egg. Imholt (2010) found no correlation between the maximum length and maximum diameter and sex of the developing chick of 1,223 eggs of 6 commercial layer breeds and 6 fancy breeds. On the other hand, Yilmaz-Dikmen and Dikmen (2013) found that the egg shape index, egg length, egg width and volume of the egg was significantly difference depending on the chick's sex. However, this was achieved using 300 white layer eggs and so studies investigating this method across other breeds and with larger sample sizes would be necessary before this method could be considered commercially viable.

4.1.2 Molecular sexing assays

Clinton et al. (2016) developed a novel sexing procedure based on Hologic Invader® technology. The Invader® sexing assay reagents are proprietary materials owned and produced by Hologic Inc. (10210 Genetic Center Drive, San Diego, Calif., 92121, USA) (http://www.hologic.com/). Hologic Invader® technology is an isothermal 'PCR-free' approach that can determine the sex of an embryo in 5-15 minutes using either, tissue fragments (e.g. 1 ng of DNA), small volumes of whole blood (125 nl of whole blood) or small number of isolated cells (as few as 250 cells). Depending on the stage of

embryo development, blood is collected from either a chorio-allantoic membrane vessel, the vitelline vein, or the heart. This method is therefore invasive. Hologic Invader® technology has also only been developed for use under laboratory conditions (Krautwald-Junghanns et al., 2018).

4.1.3 Genetic engineering

Recent advancements in avian gene technology allow specific marking of the sex-determining chromosome in chickens allowing for the identification of male chicks before hatching. Quansah et al. (2013) and Doran et al. (2017) investigated the production of genetically engineered hens, and described the marking of the Z chromosome of breeding hens with green fluorescent protein. This method was successfully used for sex determination in layers, with the gender being deducted from sex-specific patterns of germinal disc fluorescence in non-incubated eggs (Bruijns et al., 2015). A company called eggXYt received funding from the European Union's Horizon research and innovation programme in 2020 to develop this technology that enables sex detection of chicken embryos immediately after the eggs are laid and before they enter incubation.

4.1.4 Markers and Hormone detection in allantoic fluid

Weissmann et al. (2013) established a method for in-Ovo sex identification on d 9 of incubation by taking a sample of allantoic fluid from each egg and mixing with a reagent that determines the presence of a female sex hormone, estrone sulphate. Compared to females, male embryos were found to have significantly lower hormone levels in the allantoic fluid. Categorising eggs with ≤0.171 ng/mL of estrone sulphate in the egg allotonic fluid on day nine of incubation as male resulted in a sensitivity of 86.0% and specificity of 82.9%. Currently this technology cannot be performed before d 9 of incubation because the hormone in waste products sampled from the egg need time to accumulate. Improvements to specificity and sensitivity are achieved on day 10, however Weissmann et al (2013) results provided a test useable before the onset of embryonic pain perception on Day 10.5 of incubation (Close et al., 1997). Predictive sexing accuracy was above 98% for in-Ovo sexing on d 9. Compared to an untreated control group, the hatching rate of the experimental group was reduced by 1.4 to 3.5 points of percentage (brown layers) and 12.7 points of percentage (white layers) due to sampling of allantoic fluid. For both groups, the hatching weight of the day-old chicks was the same. Further monitoring of the post hatching performance revealed that the use of allantoic fluid has negligible impact on the hens. Although distinctions in weight of control and experimental groups were observed during the rearing period, the adult hens' laying performance, egg and body weight did not differ significantly between the groups.

This technology is considered the most established technology and is used by a German company 'Seleggt' who won a CIWF Best Innovation award in 2018 for developing their endocrine-based method for in-Ovo sex determination (CIWF, 2018). The hole created in the egg is small enough for the inner membrane to reseal but to improve hatching rates, Seleggt now close the hole using beeswax.

A technique developed by Dutch spin-off company from Leiden University, 'In-Ovo' can determine the sex of an egg "within seconds" after nine days of incubation. The latest machine "Ella"is currently working in a single Dutch hatchery (AWC meeting 27/10/22). The 'In-Ovo' website doesn't give much detail on what the technique involves, other than the requirement to create a small hole for sampling in each egg for the procedure (In-Ovo, no date) with the presence of a specific biomarker is determined from the sample by mass spectrometry.

Another German company, 'PLANTegg', appears to be developing a similar technology to Seleggt that requires a small sample of allotoic fluid from the egg after nine days of incubation. The technique is not currently fully automated as it requires the samples to be manually loaded into

separate polymerase chain reaction (PCR) machines, however one hatchery is reportedly already using the process (PLANTegg GmbH, 2020).

There is an increase in the number of eggs required at the start of incubation as in a small percentage, 2-3%, of eggs it is not possible to collect allantoic fluid. However the techniques are continually being developed and this might reduce in the future.

4.2 Optical and imaging methods

4.2.1 Reflectance spectroscopy

Rozenboim & Ben Dor (2011), performed reflectance spectroscopy on 450 White Leghorn eggs from a 24 week old flock. The eggs were measured on d 0, 1, 2, and 10 and using the unscrambler platform and principal component analysis the discrimination of both fertility and gender were determined. A comparison of actual and predicted results indicated that prediction capability is over 95% for fertility tested on d 0 and 90% for gender detection on d 10. Therefore, Rozenboim & Ben Dor (2011), concluded that reflectance spectroscopy was an adequate in-Ovo sexing technology for chicken embryos mid-incubation.

4.2.2 Hyperspectral imaging

Göhler et al. (2017), investigated a non-destructive optical technique that uses hyperspectral images taken through the egg to identify the colour of the chick embryo feathers to determine its sex. Therefore this method can only be used with breeds of chicken where males and females have different feather colours (males are yellow/white, females are brown). In the lab the technique provided a 97% sex determination accuracy of eggs after 13-14 days of incubation (Göhler et al 2017). As the down feathers only start to emerge 11 days after incubation, and reliable results cannot be obtained before 13 days of incubation, this technique cannot be used before pain perception has developed. However, using this technology, a German company, Agri Advanced Technologies (AAT), has developed a fully automated system suitable for commercial use called 'Cheggy' (see Table 1). The technology has been in commercial use by Hy-Line France since early 2020 (Hy-Line France, no date) and in Germany since June 2020, whilst both IBERTEC in Spain and Pluriton in Belgium have also started using the technology (AAT, accessed 2022).

4.2.3 Ramen and fluorescence spectroscopy

Raman spectroscopy, another type of vibrational spectroscopy, uses monochromatic light to illuminate the object under examination. The spectrum of scattered light is analysed following its interaction with the sample. Raman spectra are unique for each molecule and are often referred to as a "molecular fingerprint" (Krautwald-Junghanns et al., 2018).

As the biochemical composition of cells of female and male birds is slightly but significantly different, Raman spectroscopy allows in-Ovo sex identification based on the spectral signature of germinal or blood cells (Galli et al., 2016). Using Ramen spectroscopy, Galli et al. (2016) achieved correct sexing of up to 90% without hindering embryo development. Follow up studies using a combination of Ramen and fluorescence spectroscopy achieved 93% accuracy for the sex determination of 380 eggs at day 3.5 of incubation (Galli et al., 2017a,b) or over 90% accuracy when the inner eggshell membrane is kept intact to become a less invasive approach that doesn't impact egg hatchability (Galli et al., 2018). This final, refined method looks to be what is being further developed by AAT for their Ramen-spectroscopic method. During the Ramen-spectroscopic method, the determination of sex is carried out on d 5 of incubation. The air cell within the egg is first detected, the shell is then perforated with a CO_2 laser and the lid of the shell is lifted. The sex of the embryo is then determined contactless and the shell is re-sealed. This method has been developed under laboratory conditions and continues to be tested. From 2024 onwards, German law will be to determine the sex of the egg

before d 7 due to evidence around the capability for pain perception. AAT are therefore working to develop an early sexing technology that is commercially viable, ideally by 2024.

4.2.4 Magnetic resonance imaging

Davenel et al. (2015) investigated a non-invasive method using magnetic resonance imaging (MRI) to differentiate sex based on measurements of the albumen, vitelline sac and the allantoid and amniotic cavities. However, these measurements did not significantly differ between male and female embryos.

4.3 Trialling of prototypes

A patented scanning technology funded by the Ontario Poultry Industry Council and Egg Farmers of Ontario (EFO), called 'Hypereye', has been developed by McGill University in Canada, with the expected commercial capability to identify the gender and fertility of 50,000 eggs per hour (Canadian Poultry Magazine, 2018). The technology uses hyperspectral imaging which is analysed by a mathematical model to determine if the egg is fertilised or not and whether the embryo is male or female. In 2018, prototypes were being tested in Ontario hatcheries, whilst EFO have partnered with the Livestock Research Innovation Corporation with the expectation to bring a commercially viable product to market later that same year. The US, Holland and Sweden have expressed interest in the technology (Canadian Poultry Magazine, 2018).

A different approach by Israeli company 'SOOS' uses sound vibrations to make genetically male chicks become female. The percentage of female eggs hatched is variable and reported to be between 60-90%, therefore the technique can minimise but not eradicate the need for culling of male chicks (AWC meeting with SOOS 25th Oct 2022). SOOS are currently running pilot tests of their technology and hope to increase the percentage of female chicks as the technology is developed (Poultry World, 2020). Whilst SOOS have found no difference in egg productivity between all female and mixed, female and gonad-reversed male flocks, other research suggests that these genetically male chicks may not lay eggs as adults (Zhao et al. 2022, not yet peer reviewed).

A different technique developed by US-based company 'eggXYt' works by genetically modifying parent hens so that the embryos of their male offspring have a fluorescent biomarker whilst female embryos are unchanged (Canadian Poultry Magazine, 2018). All eggs are then scanned with a 'seXYt' optical scanner developed by the company, which will detect the bio-luminescence from the eggs with male embryos. This method has the drawbacks of being unable to detect unfertilised eggs and potentially resulting in waste eggs that are unfit for human consumption as the male embryos are genetically modified. In 2018, the company expected approval from the Food and Drug Administration in 12-18 months (Canadian Poultry Magazine, 2018).

The Commonwealth Scientific and Industrial Research Organisation (CSIRO), an Australian government agency, is undertaking a proof-of-concept project that differentiates between in-Ovo male and female chicks by placing a biological marker on the chicken's sex-determining chromosome (CSIRO, 2020).

Table 1. Summary table of alternative technologies for the culling of day old male layer chicks.

Product name, company, country	Method description	Key points	Stage of development	
Seleggt HatchTech, Netherlands; REWE and University of Leipzig, Germany	Lasers cut a small hole in each egg and extract a small amount of allotoic fluid which is mixed with a reagent to detect the presence of a female hormone (estrone sulphate)	- semi-invasive - requires 9 days incubation - 97-98% accuracy - 3,600 an hour plus sample result time - approx. 4 million female chicks/year/machine	Used for 'Respeggt' eggs available in France, Germany, Switzerland and Holland; fully-automated SELEGGT Circulus was launched in June 2020	Five SELEGGT Circuit machines currently (2022) in operation in Europe. Nine systems in production 2023
Cheggy Agri Advanced Technologies, Germany	Hyper-spectral imaging of intact egg to detect the colour of down feathers of breeds with sexual dimorphism of feather colour – yellow/white for male and brown for female	- non-invasive - requires 13+ days incubation - over 96% accuracy - over 20,000 eggs an hour Have developed "Stunny" which passes an electrical current across the egg prior to maceration	Commercially available and used by Hy-Line France for brown layers; starting to be used for markets in Germany, Spain and Belgium Cheap to run as no consumables	Currently in seven European countries including Germany, France and Italy Allegedly in every hatchery in France as cheapest technology
Hypereye McGill University and Egg Research Development Foundation, Canada	Hyper-spectral imaging of egg combined with statistical modelling to identify egg fertility and embryo sex	- presumed non-invasive - no incubation required - 99% accuracy - 50,000 eggs an hour	Prototypes being tested on commercial Canadian hatcheries; US, Holland and Sweden have expressed interest	
PLANTegg PLANTegg, Germany	Lasers cut a small hole in each egg and extract a small amount of allotoic fluid which is mixed with a reagent and ran through a polymerase chain reaction (PCR) machine to determine genetic sex of embryo	- semi-invasive - requires 9 days incubation - 98-99% - 3000 eggs/hour - takes an hour to get results prior to sorting	One hatchery using the semi-automated process; ALDI SUD shown an interest in the technology	Operating in Germany and Netherlands
Ramen-spectroscopic method	Ramen-spectroscopic imaging; requires a large hole to be made in	- semi-invasive - requires 5 days incubation	Prototype currently being tested, no market	

Agri Advanced Technologies, Germany	each egg to obtain spectral images, and subsequent resealing of each egg	- 90% accuracy - no speed estimate found	launch date confirmed	
SOOS SOOS Technology, Israel	Incubation system that uses noise vibrations to turn genetically male embryos into phenotypically egg-laying females	- non-invasive - requires 6.5 days incubation - achieved up to 80-90% of eggs hatching 'female' during lab testing -different success rates in different areas of the incubator	Pre-commercial pilots are underway; Won $1 million from Grow NY in November 2020 to continue R&D	
In-Ovo In-Ovo, Netherlands	A small hole is made in each egg and a small sample extracted; the presence of a specific biomarker is determined from the sample by mass spectrometry	- semi-invasive - requires 9 days incubation - accuracy described as >95% - 3,600 an hour plus sample result time	Received a €2.5 million EIC Accelerator grant in June 2020 and €34 million in March 2022.	Currently in single commercial hatchery in Netherlands
eggXYt eggXYt, Israel and USA	Parent hens are genetically modified so male eggs glow under fluorescent light; female eggs are genetically unaltered	- non-invasive - no incubation required - no accuracy or speed information	Company looks to now be more focussed on gene-editing for bird flu resistance in chickens	
Orben Munich, Germany	MRI imaging to differentiate sex based on embryo morphology	-non-invasive -day12-13 incubation -accuracy reported to be 96% -can adjust sensitivity and specificity according to customer requirements -3000 eggs/hour	No currently working in commercial setting Expensive due to costs of MRI scanners	Six systems reported to be being installed in France late 2022.
Dual-purpose breed Co-Op, Switzerland	R&D of dual-purpose chicken breed where male chicks are reared for meat production	- males have comparable ADG to very slow growing meat chicken breeds	Concluded as not economically viable unless male chick culling is banned	

5 Raising male layers for meat production

Use of natural resources, sustainability and economics will not be studied in depth although an article comparing the environmental impact on greenhouse gases, land and water use concluded that meat production from growing the males of layer lines would triple the environmental load of standard broiler production (Bessei 2022).

5.1 Fattening of laying-type males

An alternative to the culling of one-day old male chicks in the context of laying hen production is the rearing of these laying-type cockerels for meat production (sometimes referred to as producing laying hen brothers) (Koenig et al., 2012). Koenig et al. (2010, 2012) reared a commercial meat chicken (Ross 308) and three different genotypes of laying-type cockerels to compare fattening performance and carcass quality. The cockerels were fed standard diets ad libitum and were reared on deep litter. The meat chickens attained the intended carcass weight of approximately 650 g after 19 d, the laying-type cockerels after 47 -49d. Feed conversion was calculated to be 1:1.2 and 1:2.45 for meat chickens and egg-laying types, respectively meaning layer types take 2.5 times as long and twice the feed to reach the same weight as commercial broilers. The weights of valuable parts (e.g. breast, legs) were higher for the meat chickens than for the egg-laying types so meat output was not comparable. Additionally, the preparation and cooking of male laying hens is considered different to that of meat chickens and is not likely to meet consumer expectation of chicken meat (Krautwald-Junghanns et al., 2018).

Another project on suitability of male chicks for capon production studied the effect of castration at 8 weeks old on growth characteristics and meat yield. Caponized birds were more docile and gentler which was thought to contribute to improved FCR and reduce feed intakes. There was no effect on final carcase weight or total meat yields although there was an increase in proportion of breast muscle in capons. This type of production was suggested to generate consumer interest, but future demand was not predicted (Murawska et al 2022).

5.2 Breeding dual purpose chickens

An example of a dual purpose chicken breed is the Lohmann Dual chicken. These birds consume up to 30 g more per day, resulting in feed costs up to 50% higher for the entire laying period than for commercial layers. Additionally, Lohmann Dual hens lay not only fewer but also smaller eggs, thereby lowering egg mass output. Compared to a slow-growing broiler, at 8 weeks of age, the male dual-purpose birds have a live weight of just about 2 kg, whereas the slow-growing broiler has a bodyweight of 3.2 kg. When fed with broiler diets for 70 days, dual cockerels reach a live weight of 3 kg, and a carcass weight of about 2 kg. Unlike meat chicken lines, dual cockerels have a lower portion of breast meat and a higher portion of thigh meat and this may pose a problem in markets where consumers prefer breast meat. Differences in feed utilisation and efficiency and, egg and meat production between dual purpose chickens and specialised layers or broilers results in dual purpose chicken being an economic disadvantage and not commercially viable (Mueller et al 2018, 2020).

Gangnat et al (2018) identified that Swiss consumers are more willing to pay a higher price for eggs from dual purpose hens rather than the meat produced, and knowledge of the poultry industry affected this.

5.3 Potential welfare issues

There is little peer reviewed evidence investigating the welfare of laying-type males reared in environments designed for meat chickens. These environments may not meet the behavioural needs of a laying-type cockerel and research considering the welfare impacts of rearing these birds is required.

Information obtained from industry research suggested that there can be issues with aggressive tendencies in male layer chicks and they are more sensitive to stress and loud noise. Additionally increased activity levels can result in increased dust in the houses (WattPoultry 2022).

Potential welfare implications in raising male layer chicks could include increased aggressive behaviours in part due to single sex grouping, the birds are raised to greater age so reaching maturity (Giersberg and Kemper 2018). Baldinger and Bussemas (2021) identified an issue with high incidence of breast blisters, up to 20% affected, and comb injuries in male dual purpose chicks reared to 15 weeks. These were suggested to be linked to perch use and aggressive fighting behaviour.

Selegett reported (AWC meeting 1/11/22) that there had been issues with rearing male layers due to birds being reared in an unsuitable environment with no specific diet. Also due to the different shape of the birds compared to standard broilers there were problems at the slaughterhouse with stunning and processing. With planning and investment these matters could be resolved.

6 Evidence for sentience and pain

Sentience is the capacity of an animal to experience feelings such as suffering or pleasure. Negative emotions might include pain, fear, boredom, frustration and positive emotions might include contentment and joy. For successful achievement of a sex-determination method in chickens, methods need to be fast, cost-efficient, precise and must not have any negative impacts on hatching rate, animal welfare and performance (Kaleta and Redmann, 2008; Krautwald-Junghanns et al., 2018). This includes ensuring sex determination methods occur before pain perception has evolved in chick embryos (Krautwald-Junghanns et al., 2018). Sentience is difficult to determine, particularly in embryos and therefore, pain perception and/or neural development has been used to assess whether sex-determination methods might negatively impact welfare.

6.1 Capability of perceiving pain at different stages of embryonic development

Numerous reports suggest that sex determination should occur during the first half of an incubation period as avian embryos have evolved a functional brain by day 10.5 of incubation and are therefore capable of perceiving pain in the second half of gestation (Bruijnis et al., 2015; Gohler et al., 2017; Weissman et al., 2013). However, these reports commonly cite Close et al. (1997) who states that from the stage at which a neural tube has developed into a functional brain (>50% gestation) bird embryos may be capable of perceiving pain. However, Close et al. (1997) did not describe the methods on how this conclusion was reached and commented only on bird embryos giving no details on specific species investigated.

Nonetheless, it is known that the central nervous system begins to form as early as day 2, maturing some time prior to hatch on day 21. The embryo's neurological sensory mechanisms develop over stages, including tactile (day 6), proprioceptive-vestibular (day 8–10), taste (day 12), auditory (day 12–14), visual (day 18), and olfactory (day 20) (Deeming, 2011, 6). Similarly, brain waves, measured via electroencephalograph waves, are initiated as early as day 13–14 of incubation, then go through a progressive developmental series in the embryo. Erratic spikes appear by day 15, and by the 18th

day of incubation, EEG waves similar to slow/fast sleep waves appear. By the 19th–20th day, the EEG waves become similar to the waves noted in the hatchling during sleep. Muscular activity appears to begin sometime in the second trimester, although those responses may be an autonomic reflex rather than a reaction to stimuli (Deeming, 2011).

Additionally, although it is known that bird embryos have the ability to experience in-Ovo nociception (Bjørnstad et al., 2015), the first sensory afferent nerves develop in the chicken embryo on d 4 of incubation, but a synaptic connection to the spinal cord is not present before d 7 of incubation, making nociception impossible in the first third of incubation (Eide and Glover, 1995, 1997). Therefore, no sensitivity of the chick embryo is to be expected before d 7 of incubation (Rosenbruch, 1994, 1997; Aleksandrowicz and Herr, 2015).

Based on this evidence, it is believed that the maturity of the neural embryo is lacking the ability to feel pain during at least the first half of the incubation phase (Mellor and Diesch 2006) which coincides with Close et al. (1997). However, it is not necessarily just "pain" that causes suffering of an embryo, but also the ability to experience noxious stimuli (Mellor and Diesch, 2006) and it is important to understand the concept of suffering when determining potential welfare impacts of in-Ovo sexing methods as well as the killing of in-shell embryos.

However, because of the difficulty in understanding when suffering can be felt, science has not come to a clear consensus on a chick embryo's ability to suffer. To feel suffering, an embryo must have a level of consciousness (Campbell et al., 2014). For that to occur, there must be adequate subcortical–cortical neural connections, and there is no evidence to demonstrate when that occurs in the avian brain. Mellor and Diesch (2006) reported that both sentience (meaning that the neural development in the embryo is functional and able to transmit neural impulses to the brain and convert them into some form of sensation) and consciousness (in that the embryonic brain is able to comprehend or perceive the sensations and must sense the negative form of the sensations) must exist in the embryo for suffering to occur**.**

Electroencephalograms indicate the avian embryos are in a sleep-like or unconscious state until after hatching. They also note that the EEG waves found during day 18–20 of incubation are similar to those noted in chick sleeping patterns, further providing evidence of a sleep-like stage prior to hatch. However, the authors also note that this is not a clear area of science. For example, they note that during this latter phase, the embryos actually have an ability to vocalize, which may indicate that some degree of awareness has been achieved, and the embryos must initiate the hatching process, which may require some level of consciousness, and therefore potential suffering (Schwean-Lardner, 2018). Similarly, sensory and neural development of chicks is well advanced several days before hatching and coordinated behaviour and electrophysiological change to tactile, auditory and visual stimuli is present 3-4 days prior to hatching (Broom, 1981).

6.2 Pain and sentience in day-old chicks

Previously, studies have investigated distress calls in chicks when isolated or separated from their mother (Collias, 1952; Andrew, 1964; McBride et al., 1969) and the rate and intensity of distress calls has been found to be positively correlated with corticosterone levels and behavioural measures of fearfulness (at 11 days of age: Jones and Williams, 1992). The emotional experience of the chick's experience is highlighted by the fact that distress calling reduces when chicks are given certain drugs that have anti-anxiety effects on humans (tested on d7 post-hatch) (Feltenstein et al., 2004). Similarly, if chick isolation continues for >1 hour the rate of distress calling is seen to reduce and has been termed a depressive like state (Kim and Sufka, 2011). When chicks are kept in enriched environments (Kim and Sufka, 2011) or are given anti-depressant drugs (Hymel and Sufka, 2012), the severity of this depressive like phase is reduced (recorded on d7 post-hatch). These studies demonstrate the capability for chicks to suffer and feel fear which could be indications of sentience. Nonetheless, these studies have been carried out on chicks older than 1-day-old and therefore the sentience of a 1-day-old chick needs to be investigated.

7 Evidence for societal concerns on the issue of male chick culling

There have been no structured surveys of the public in the UK relating to the current practice of culling of male chicks. A recent white paper was presented to the European Parliament by the European Institute for Animal Law and Policy (Animal-Law-Europe-–-Chick-Killing-Report-2023.pdf (animallaweurope.com) 2023) detailed a YouGov survey carried out in seven European countries. There was a wide range in awareness of chick culling in the different countries ranging from 30% to 87% stating that they were at least partially aware of the practice. When questioned if they thought newly hatched chick culling should be banned across the EU between 64 and 78% agreed.

A Dutch study in 2011 into public opinion of the acceptability of alternatives to chick culling was carried out by focus groups and computer aided interviews. Killing of late embryos was not considered much of an alternative to current practice. At the time of the study there were no commercially available options for sexing of embryos and there was interest in developing sexing techniques of the egg or by genetic modification and not commencing incubation (Leenstra et al 2011). A more recent Dutch study, based on an on line questionnaire identified that the preferred options to culling male chicks were use of in ovo sexing and increased use of dual purpose chickens (de Haas et al 2021).

A German study using images identified reduced preference for sex determination and culling after day 9 of incubation, thought to be due to similarity visually to chicks. Usage of the screened out eggs for pet food was preferred to use by the chemical industry or discarding (Reithmayer et al 2020). A second study from the same research group identified a widespread disapproval for chick culling. A significant percentage (41%) expressed ethical concerns re in ovo technologies if conducted after embryo sentience develops. And there was a marked variation in the additional amount consumers would pay for eggs free from culling (Reithmayer et al 2021). Seleggt is used to produce eggs for the no-kill 'Respeggt' label, which also rears male layer chicks, with eggs available on shelves in France, Germany, Switzerland and Holland.

Multiple online articles are available from animal welfare organisations in Australia, (Farm Transparency Project, Animal Liberation) Israel (Kinder World) and the USA (PETA), detailing day old chick culling and calling for a ban. From a GB perspective the RSPCA details current procedures and states that it will be monitoring developments in technology. The British Hen Welfare Trust, Open Cages and Compassion in World Farming make no mention of culling of male chicks. The Humane League has articles from 2021 referencing chick culling.

What is chick maceration? | RSPCA Assured

https://thehumaneleague.org.uk/article/what-happens-to-male-chicks-in-the-egg-industry

The Truth About The Egg Industry: Uncensored | Watch If You Still Eat Eggs (kinderworld.org)

Eggs exposed - Campaigns - Farm Transparency Project | Australian animal protection charity

Impacts of Egg Farming — Animal Liberation | Compassion without compromise

Articles have been published in broadsheet papers mainly The Guardian and The Independent on chick culling and alternative solutions being trialled in other countries.

https://www.theguardian.com/environment/2022/sep/02/uk-retailers-blocking-moves-to-end-the-killing-of-day-old-male-chicks

https://www.theguardian.com/environment/2018/dec/22/worlds-first-no-kill-eggs-go-on-sale-in-berlin

https://www.theguardian.com/food/2021/jan/31/good-vibrations-sound-waves-eggs-ethical-slaughter-male-chicks

8 Exotic pet and zoo animal food requirements

Nutritionally, are male chicks an adequate/optimum food source for (a) reptiles, (b) raptors and (c) carnivorous mammals? Are there better food sources than male chicks available? This is beyond the scope of a literature review and specialist advice on this matter should be sought.

In Austria an agreement was reached between hatcheries, poultry farmers and zoos where by chick culling was allowed provided the chicks were used as feed. It is reported that all nine million day old cull chicks produced per year are used as food in Austria (Poultry World b 2022).

22-11-30 AWC Alternatives to Male Chick Cull - chicks as exotics food.docx

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