

Gender Identification of Chicks Prior to Hatch

Introduction

Sex separation of chicks at hatch is an important aspect of the poultry industry. Varied segments of the poultry industry must sex segregate offspring for different reasons. All turkeys are sex segregated due to the large differences in the growth rate, market age, management practices and nutritional requirements between male and female birds. In the layer or table egg industry, chicks must be sexed at hatch as only the females, or birds which lay eggs, are kept while all male chicks are discarded. The broiler breeder and turkey breeder industries produce both male and female lines that are eventually crossed to produce a hybrid commercial bird. The males, or off-sex chicks produced from the female lines, are discarded while the female chicks, or off-sex chicks from the male lines, are discarded. Disposal of unwanted chicks creates animal welfare and waste disposal issues for the industry. The meat type chicken or broiler industry prefer to sex segregate males from females to gain feed efficiency, improve uniformity at the processing plant and reduce production costs. The broiler industry often refrains from sex separate rearing due to the high costs of sexing and the need for trained sexers. Currently, only 27% of U.S. broiler hatcheries sex segregate chicks. The segment of chicks sex segregated are those targeted for increased size and further processing, the fastest growing segment in the broiler industry. These chicks are grown for an extended period of time and sexing costs can be recovered. A higher percentage of the broiler industry would sex segregate if a rapid, inexpensive, automated method were available.

Current sexing methods used in the poultry industry involve manual procedures that require highly trained professionals with specialized skills. Sexing methods involve either the Japanese method of vent sexing or feather sexing of chicks that carry a slow feathering gene (Schroeder, 1933; Taylor, 1933; Warren, 1976). Both manual sexing methods have significant drawbacks, such as 1) require hiring and management of a dwindling supply of trained manual labor 2) increase the processing time before chicks can be placed in the field with access to food and water 3) result in handling stress and damage to the chicks or poults 4) represent a rate limiting step during processing and 5) remains one of the few manual processing steps practiced in the highly automated poultry industry. Embrex is developing an automated gender sorting device to alleviate these issues for the poultry industry.

Manual Sexing Methods

The Japanese or vent sexing method originated in the 1920's when the Japanese discovered that within the first or second fold of a chick's vent there are distinctive characteristics such as spots, lines and folds that reveal its sex (Jull, 1934; Masui and Hasimoto, 1933). Vent sexing is considered an art and principally practiced by Asian individuals with excellent manual dexterity and Zen-like concentration (Hoh, 1997). Vent sexers attend school for six months and then undergo a trainee program for two to five years until they are considered a qualified sexer based on speed and accuracy. Qualified sexers can vent sex 2,000 chicks per hour at 98 % accuracy but only 5 to 10 % of individuals attending vent sexing school attain qualified sexer status (Hoh, 1997). The rapid rate at which a vent sexer must pick up, sex and sort baby chicks can result in a 1.4

to 1.8 % increase in cull rate and 0.5 to 1.0 % increase in early chick mortality due to chick damage during handling. Vent sexers are contract workers who travel from hatchery to hatchery working long tedious hours. Travel of these contract workers from hatchery to hatchery poses a bio-security threat for the poultry industry.

Feather sexing of chickens inbred for the *K* gene for slow feathering is another commonly used method for sex segregation at hatch (Warren, 1976). Day-old chicks carrying the *K* gene can be sex segregated by examining the relative length of the primary and covert feathers of the wing, with the females carrying genes for fast feathering and the males carrying genes for slow feathering. Feather sexing is less expensive than vent sexing and does not require as skilled a labor force. Feather sexing is not applicable to turkeys, the majority of broiler breeds; some layer breeds and also carries disadvantages. The *K* gene for slow feathering is closely linked to an endogenous virus (Bacon et al., 1988), which can cause immunological tolerance to lymphoid leukosis (Crittendon et al., 1987) and is therefore a disadvantage in breeding stock. It is noteworthy that slow feathering males usually do not feather well in the brooder house especially during hot weather often resulting in slower growth and increased cannibalism and that females carrying the *K* gene exhibit reduced egg production. Some poultry breeds exhibit sex specific color differences in feathers, however these breeds are not as commercially viable as other breeds. Color sexing can result in a higher rate of sexing mistakes than feather sexing.

Methods to sex segregate eggs before hatching have been previously attempted without success. A sex-linked gene, S^{al} for imperfect albinism was reported by Hutt and Cole (1973) to have the potential to sex chicks at one day of age on the basis of eye color. The albino female chicks have pink eyes while the normal males have black eyes. The difference in eye color can also be used to accurately sex segregate embryos by candling the eggs on Day 10 of incubation. Candling or light illumination of the intact egg reveals the dark eye of the male chicken embryo but not the pink eye of the albino female. Use of the S^{al} gene to sex segregate poultry was not pursued as the gene carries deleterious effects in turkeys and negative production effects in chickens.

Development of a quick, accurate, inexpensive automated method to gender sort eggs before hatching would significantly increase profitability of the global poultry industry. Benefits of automated gender sorting would include 1) a reliable way to sex broilers so that increased production efficiency could be realized 2) Sex specific vaccination 3) reduced incubation space requirements in the layer industry as males will not be hatched 4) genetic selection of breeds without constraints of selection for slow feathering gene 5) reduced liability and reliability concerns associated with eliminating manual labor 6) speedier hatchery processing and quicker access to feed and water 7) Quicker processing of chicks at hatch and 8) reduced animal welfare concerns associated with discarding male chicks in the layer egg industry.

Basis for Automated Gender Sorting

Automated gender sorting of avian embryos will require a sex specific marker that can be detected with 98.5% accuracy, at a rate of 20,000 to 30,000 eggs per hour, and at a cost equivalent to manual sexing. Potential sex specific targets include 1) Differences in physiological parameters such as heart rate (Laughlin et al., 1976; Glahn et al., 1987) 2) PCR identification of the W specific chromosome in females (Clinton, 1994; Uryu et al., 1989; Petite, 1992) 3) Presence of anatomical differences such as gonads (Vandenabeele, et al., 1993) or sex specific compounds present in the blood or extra-embryonic fluid (Gill et al., 1983; Tanabe et al., 1979).

The basis of Embrex's automated gender sorting device is the detection of estrogenic compounds present in the allantoic fluid of female embryos but not male embryos. The estradiol levels in the allantoic fluid of Day 17 broiler chicken embryos were measured using a commercially available RIA kit (Diagnostic Products, Los Angeles, California). The estradiol level in the allantoic fluid of male embryos was either non-detectable or less than 42 pg/ml. The estradiol level in the allantoic fluid of female embryos was between 113 and 830 pg/ml, at least three times higher than the levels detected in male embryos. The results of the analysis of 490 allantoic fluid samples suggested that estrogens would be an excellent chemical target by which to gender sort avian embryos. Avian embryos can be gender sorted on the basis of the presence of estrogenic compounds in the allantoic fluid of female embryos between days 13 and 18 of incubation, in broiler, broiler breeder, turkey and layer embryos and regardless of flock age or strain.

Analytical Detection of Estrogenic Compounds Present in Allantoic Fluid

The automated gender sorting device will utilize a LiveSensor™ (LifeSensors Inc. Malvern PA) to detect the estrogenic compounds in the allantoic fluid. The LiveSensor™ is a genetically modified yeast transformed with yeast expression vector for the human estrogen receptor, the reporter gene that contains promoter with estrogen response elements coupled with *E. coli* β -galactosidase (Butt and Chen, 1999). In the presence of estrogens the estrogen receptor binds to the estrogen response elements and initiates transcription of the reporter gene. The concentration of estrogens in the allantoic fluid is correlative with the level of induction of the reporter gene. The activity of the reporter gene product β -galactosidase is measured using ONPG substrates which yields a yellow colorimetric signal. The LiveSensor™ can detect femtomolar levels of estrogens. The strain of yeast the LiveSensor™ is comprised is the same strain commonly used in the baking industry, *Saccharomyces Cervisiae*.

The LiveSensor™ can distinguish between male and female embryos using only 4 ul of allantoic fluid. The automated device under development will collect 20 ul of fluid to insure sexing accuracy. The intensity of the yellow color produced is measured in pixels by a CCD camera. The number of pixels scanned represents a 500 to 1 signal to noise ratio.

Sampling the Allantoic Fluid

The allantoic fluid is recognized as an excretory medium for the nitrogenous metabolites of the avian embryo (Romanoff, 1967). The allantoic fluid begins to form around Day 5 of incubation. It attains a maximum volume of 6.1 mls on Day 13 of incubation, wanes in volume as incubation continues due to moisture loss and fluid resorption but is still present in significant volumes, 1 to 2 mls, on Day 18 of incubation (Romanoff, 1967).

The allantoic fluid is separated from the eggshell only by the inner and outer shell membranes and the chorioallantoic membranes. Although the allantoic fluid encompasses the entire periphery of the embryonated egg, the allantoic fluid accumulates at the top of the egg directly underneath the membranes overlying the air cell. The accumulation of the allantoic fluid at the top of the egg is due to gravity and displacement by the dense embryo and yolk sac. Attempting to accurately sample the allantoic fluid through the top of the egg while the egg is upright would be difficult due to the variability of the air space from egg to egg.

Gravity can be used to pool the allantoic fluid in a localized site. When the egg is turned on its longitudinal axis the allantoic fluid will pool at the top side of the egg, directly underneath the shell. Laying the egg on its longitudinal axis renders the allantoic fluid an easy target to access. Laboratory experiments with an automated one egg prototype have demonstrated the allantoic fluid can be sampled from Day 15, 16 and 17 embryonated broiler eggs with an accuracy of 97 to 100%. The prototype lays the egg on its side at a 15 to 20 degree angle, centers the egg and inserts a needle at a depth of 5 to 6 mm slightly above the midpoint of the egg. Experiments to increase the sampling accuracy are currently underway. The type of needle, punch speed, injection depth, site of injection and egg angle all contribute to sampling accuracy.

Automated Gender Sort Commercial Prototype

Embrex is currently developing a commercial prototype to conduct field trials. The first prototype being developed is targeted for the Broiler Breeder Industry. The automated gender sorting device will be comprised of three independent modules linked by a network. The first module is an allantoic fluid sampling module. Flats of eggs are manually removed from a setting incubator on either Day 15, 16 or 17 of a 21-Day incubation cycle and fed onto a conveyor belt. An optical based sensor then automatically identifies live eggs and eggs are transferred into a series of egg cradles. The egg cradles reposition the eggs on their side and center the eggs. Needles are then inserted at a depth of 5 to 6 mm into the midpoint of the egg and 20 ul of allantoic fluid is withdrawn from each egg. The fluid samples are then deposited into a well depressed in a thermoformed bar-coded plastic template. Each template contains approximately 3000 wells and the wells are in the same array as the egg flat. The needles are cleaned between each egg. The eggs are repositioned upright and returned to a bar-coded egg flat and manually placed back into the setting incubator. The templates are manually stacked into a shelved cart. The network matches the barcodes of the egg flat and template containing the allantoic fluid samples.

The second module is the automated assay module . An operator will load the cart containing the templates into the automated assay module. The templates will be moved underneath a dispensing head by a conveyor system. The dispensing head will dispense 30 ul of the LiveSensor reagent into each well. Addition of this reagent to every well within one template will take 5 to 6 minutes. Each template will then be progressed through an environmentally controlled chamber for an incubation period of approximately 3 hours. ONPG substrate will then be automatically dispensed into each well and the templates will be incubated for an additional 30 minutes to allow color development. A CCD camera will then scan each well and determine if the egg was a male or female. This data will be tracked backed to the egg flat. The templates containing the assay reagents will then be automatically collected back into the cart and heated to kill the LiveSensor™ prior to disposal.

The third module is an egg vaccination, sort and transfer module . Eggs are manually removed from the setting incubator on either Day 18 or 19 of a 21 day incubation cycle and flats are manually fed onto a conveyor belt. Eggs are moved through two Inovoject vaccination heads. One vaccination head contains male specific vaccine and is injected only into male eggs. The other vaccination head contains female vaccine and is injected into the female eggs. The network link identifies which eggs are male and female based on the barcode present on the egg flat. The eggs are then sorted by gender and transferred into gender specific hatching baskets. The hatching baskets are transferred into the hatching incubators until Day 21 of incubation. Alternatively, eggs of one gender can be discarded and not vaccinated or transferred into hatching baskets.

Impact of sampling the Allantoic Fluid on Hatchability

Trials to evaluate the impact of the automated gender sorting device on hatchability and chick performance are underway. Two trials have been completed that evaluated the impact of the one egg sampling prototype on hatchability of eggs sampled on Day 17 of incubation. No sanitation was used but only viable eggs were sampled. The percent hatchability of viable is presented in the table below. Treatment means are based on 5 replicates of 54 eggs for each trial (n=540 for both trails). These results suggest there is no impact on hatchability if the prototype samples allantoic fluid from Day 17 eggs even if no sanitation is used. Hatchability and chick performance studies are underway to evaluate sampling fluid from eggs Day 15 and 16 of incubation and to combine the process of sampling fluid for automated gender sorting with the egg vaccination, sort and transfer module.

	Non Treated Controls	Sampling Needle Inserted	Allantoic fluid Withdrawn
Trial 1	94.01	93.68	94.42
Trial 2	94.30	95.50	93.7
Mean	94.15	94.59	94.06

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Speaker: Dr. Patricia Phelps

Question 1

From: Dominic Elfick

Have you considered the effect on only placing viable embryos into the hatcher?

Response:

The gender sorting device is being designed to allow variable numbers of eggs to be transferred into the hatching baskets. Hatchery managers will be able to select backfill rates between 70% and 100% based upon egg mass and hatcher type. Embrex envisions the removal of nonviable eggs will improve air-flow and the quality of the hatching chicks.

Question 2

From: Dr. Douglas Rhoads

Your assay is based on a steroid hormone. Could you not find a protein target in the allantoic fluid, such as aromatase, that you could target for immune diagnostic assays?

Response:

Aromatase would be a viable sex specific marker but investigation into immune diagnostic assays or antibody- based assays suggested this type of technology would not be cost effective. The yeast-based biosensor containing the estrogen receptor is more cost effective.

Question 3

From: Dr. Joseph Darden

With all the diseases floating around in the industry, there are may be transmission of disease from egg to egg and then you discover it after the fact, what measures are taken to avoid cross contamination between eggs?

Response:

A chlorine based sanitation system similar to the sanitation system used in the Inovoject system will be incorporated into the gender sort device. The needles used to sample the allantoic fluid will be sanitized between each egg. The Inovoject system sanitation process has proven effective in the global commercial arena. Methods to clean device components that contact multiple eggs are also under evaluation.