Predicting hatching success by testing egg fertility

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Introduction

High fertility, and subsequent high hatchability, is fundamental in chick/poult production. Therefore, monitoring fertility is a common practice in commercial breeder facilities. Fertility can be monitored in different ways: by breaking and examining eggs in distinct points of incubation (unincubated, candled, and residual eggs); by isolating and examining the germinal disc of incubated eggs; by counting sperm trapped in the outer perivitelline layer of laid eggs; and by counting holes in the inner perivitelline layer of laid eggs. The description of each one of these ways of monitoring fertility will be presented and discussed below.

Predicting fertility by egg breakout

Eggs breakout is usually performed in commercial facilities and gives information of true fertility as well as of hatchery performance. It can be done in four points of chick/poult production: before incubation; after 7-12 or 10-14 days of incubation (for chicken and turkey eggs, respectively); during transfer from the incubator to the hatcher; and after hatching (Hulet, 1995).

In the first procedure, fresh unincubated eggs are broken and the germinal disc is examined for fertility. To decrease the use of settable turkey eggs, the evaluations can be performed in cracked or cracked eggs (Bakst et al., 2001). If the egg is fertile, the germinal disc (also called blastoderm) has a donut-like shape; it appears as a white disk with a clear region in the center. If the egg is infertile, the germinal disk (also called blastodisc) is condensed; it appears as a compact and irregular disc, containing some vacuoles (Wilson, 2010). The breakout of unincubated eggs is recommended when a fast evaluation of true fertility is needed.

The second procedure is performed with incubated eggs classified as “clear” during candling at 7-12 days (chicks) or 10-14 days (poults). With the breakout, it is possible to identify if the clear egg is infertile or if it contains an early-dead embryo. Infertile eggs will have similar appearance as the infertile unincubated eggs, while early-dead embryos (dead before 24 hours of incubation) will have the germinal disc more spread and less distinguishable than the fertile unincubated egg. If the embryos died during the first week of incubation, blood vessels could be present (Wilson, 2010). The breakout at this point is more accurate than the breakout of unincubated eggs, since it is easier to visualize the differences between the germinal disc of infertile and fertile eggs.

The breakout also could be done later in the incubation, when the eggs are candled during transfer from the incubator to the hatcher (Hulet, 1995). The characteristics of infertile eggs will be the same described before for unincubated eggs, however eggs with early-dead embryos (dead before the appearance of blood vessels) could be confused with infertile eggs, since the dead embryo starts to degenerate and some vacuoles could appear, turning it difficult to make the distinction.

The fourth breakout can be performed in residual eggs. At this point, it is possible to determine the stage of embryo mortality; they are usually classified as early-dead (1 to 7 days), middle-dead (8 to 14 for chicks and 8 to 21 days for poults), or late-dead embryos (15 to 21 for chicks and 22 to 28 for poults) (Wilson, 1995). Further details on the classification can be found on Wilson (2010). This last breakout is important in determining if the eggs did not hatch due to low fertility or due to high embryo mortality, helping to establish the cause of low hatchability and giving indications if there is a problem in flock
Predicting fertility by isolation of germinal disc

As pointed before, when egg breakout is performed late in the incubation, it is difficult to differentiate infertile eggs from eggs with early-dead embryos. In this case, examination of the germinal disc under a stereomicroscope could be helpful.

For detailed protocol of germinal disc isolation, see Bakst (2010). Briefly, filter rings with approximately 5mm inside diameter and 10mm outside diameter are prepared with filter paper. The eggs shelves are cut with scissors, the albumen is removed, and the yolk is rotated to bring the germinal disc into view. The filter ring is placed over the yolk; the germinal disc should be located in the center of the ring. The perivitelline layer will adhere to the filter and should be cut around the outer edge of the ring. The ring with the germinal disc is then transferred to a Petry dish containing saline solution, and observed under stereomicroscope. The characteristics of infertile eggs are the same as described in the eggs breakout session; the early-dead embryo may appear as an irregular cluster of cells surrounded by albumen and vacuoles.

If examination under stereomicroscope is not enough for differentiating infertile from early-dead embryos, the germinal disc can be aspirated and stained with a fluorescent probe. Blastodermal cells nuclei will be stained and easily identified under fluorescent microscope (Bakst, 2010).

Predicting fertility by counting sperm in the outer perivitelline layer of laid eggs

The perivitelline layer of the laid egg is composed of two membranes; the inner and the outer perivitelline layers. When the egg is ovulated, the inner perivitelline layer (IPVL) is already present; sperm binds to the IPVL, undergoes acrosome reaction, and penetrates the egg. Shortly after ovulation, the outer perivitelline layer (OPVL) is laid down around the egg surface, preventing further sperm binding. Therefore, by the time the egg is laid, the perivitelline layer has two membranes; the inner contains holes made by the sperm during egg penetration, and the outer contains trapped sperm that were present at the site of fertilization (Bakst and Howarth, 1977).

Sperm trapped in the outer perivitelline layer of laid eggs can be counted, providing information about the number of sperm present in the infundibulum at the time of fertilization. The procedure for counting sperm was first described by Wishart (1987), who found correlation between the number of sperm embedded in the OPVL and duration of fertility in hens. Later, Brillard and Bakst (1990) and Brillard and Antoine (1990) found strong correlations between the number of sperm trapped in the OPVL and the number of sperm recovered from the hen’s sperm storage tubules (SST). Therefore, counting sperm in the OPVL can give an estimate of duration of fertility, as well as of the sperm population in the SST.

For counting sperm in the OPVL, a piece of the whole perivitelline layer (containing both OPVL and IPVL) is cut, washed from residual yolk, and spread out on a microscope slide (for details on the technique, see Wishart and Bakst, 2010). The unstained sperm can be visualized on a microscope with darkfield optics, or they can be stained with fluorescent probes and observed on a fluorescent microscope.

The distribution of sperm in the OPVL is uniform around the egg; it is possible to count up to 50 sperm/mm² in chicken, and 10 sperm/mm² in turkey OPVLs. There is a decrease in OPVL sperm of eggs laid in consecutive days; therefore it is possible to predict the duration of fertility of a hen based on the number of sperm found in the OPVL of a single laid egg. For chicken, the average daily decrease is of 30%, and for turkeys, 10%. When there are less than 0.2 sperm/mm² of OPVL, the egg is likely to be infertile, for both chicken and turkey eggs (Wishart, 1995).

Predicting fertility by counting holes in the inner perivitelline layer of laid eggs

The inner perivitelline layer contains glycoproteins where the sperm binds, initiating the fertilization process. The attachment of sperm to these glycoproteins triggers the acrosome
reaction; acrosomal enzymes are then released and start to hydrolyze the IPVL, giving passage to the spermatozoa, which penetrates the egg (Okamura and Nishiyama, 1978).

The hydrolysis holes made by the sperm on the IPVL can be visualized under microscope and counted; their number is linearly correlated with the number of sperm trapped in the OPVL (Wishart, 1995), and both methods are considered to be a better approach for quantifying fertility than the traditional ways (Wishart and Staines, 1999). Between the two tests, counting IPVL holes is a more convenient technique for checking breeding efficiency and monitoring flock fertility than counting OPVL sperm (Staines et al., 1998).

The technique of counting IPVL holes is a valuable tool for predicting fertility; in commercial breeder flocks, it was shown that the transfer of sperm into eggs begins to decline some weeks before there is a noticeable decline in fertility (Wishart et al., 2004). Furthermore, the number of IPVL holes may affect embryonic survival (Christensen et al., 2005); excessive IPVL holes may be responsible for increased embryo mortality.

The procedure for counting holes in chicken eggs was developed by Bramwell et al., (1995), and further modified for use with turkey eggs (Donoghue, 1996). Briefly, a piece of the whole perivitelline layer (containing both OPVL and IPVL) is cut, washed from residual yolk, and spread out on a microscope slide (for details on the technique, see Bramwell and Donoghue, 2010). The IPVL holes can be observed using darkfield optics, if the membrane is unstained, or using brightfield, if the membrane is stained.

IPVL holes are more concentrated in the region around the germinal disc (Bramwell and Howarth, 1992), where it is possible to observe up to 50 holes/mm² of IPVL in chicken, and up to 100 holes/mm² in turkey eggs. In the other regions of IPVL, distribution of holes is uniform, but the number is lower than in germinal disk region; for both chicken and turkey eggs, an average of 5 holes/mm² can be counted. Similar to OPVL, there is a decrease in IPVL holes of eggs laid in consecutive days; when there are less than 0.2 and 0.4 holes/mm², respectively for chicken and turkey IPVL (region outside the germinal disc), the egg is likely to be infertile (Wishart, 1995). For both chicken and turkey, the eggs have 50% probability of being fertile if they have 3 holes in the germinal disc area, and maximum fertility is obtained when 6 holes are present (Wishart, 1997).

Applications

As the main objective of a poultry breeder is to produce chicks/poults, any drop in fertility or hatchability will bring economic losses. Therefore, monitoring fertility is mandatory in these facilities.

Eggs breakout is the common choice for commercial breeders, since it can be easily done with their own personnel; the amount of training will vary depending on the time eggs are collected (unincubated, candled or residual eggs) and the degree of accuracy necessary.

Breakout of unincubated eggs gives fast information about true fertility, since the procedure can be performed as soon as the eggs are collected (before incubation). In its turn, eggs breakout performed after candling at 7-12 or 10-14 days of incubation (for chicken and turkey eggs, respectively), gives more accurate information of fertility than the evaluation of unincubated eggs, because it is easier to differentiate the germinal disc of fertile and infertile eggs. However, breakout performed in eggs candled during transfer from the incubator to the hatcher can give a false information about the number of infertile eggs or with early dead embryos (dead before 24 hours of incubation), since they are harder to differentiate at this point. In this situation, the examination of the germinal disc can be done by microscopy; however it will demand more laboratory equipment, more time, and more training than the simple visual examination, which can increase the costs of evaluation.

Residual eggs breakout give information about the whole incubation process and helps to determine the causes of low hatchability. However, identification of the various stages of embryo mortality requires more training than only checking the germinal disc of unincubated eggs. The main advantage of breaking residual eggs is to allow the differentiation between problems in fertility and problems in embryo mortality; the first may be due to flock performance, while the last is usually related to hatchery practices. Measures to correct those problems are addressed in different ways, turning the residual eggs breakout a powerful diagnostic tool.

Counting sperm or holes in the perivitelline layer of laid eggs is a procedure that can be easily applied in a commercial facility; they are time consuming, but they can be performed after a minimum investment in equipment and personnel training. The great advantage of these techniques is
that they can predict the duration of fertility, since they are correlated with the number of sperm in the sperm storage tubules and this number decreases in a known rate in eggs laid in consecutive days. Therefore, it is possible to take actions to increase the number of sperm in the SST (change males if they are too old, or include more males, or increase the dose or frequency of artificial inseminations) in order to keep high fertility. Furthermore, both techniques (counting sperm and holes in the perivitelline layer) are performed with unincubated eggs, allowing the manager to search for solutions immediately after the diagnosis, in contrast with the residual eggs breakout, which provides information only after hatching.

Every method for monitoring fertility has its advantages and disadvantages, therefore the choice of only one, or a combination of them, will depend on various factors: the type of institution (i.e., if it is a commercial breeder or a research institution), the resources available (facilities and personnel), the urgency to have the results, and the accuracy of evaluations, among others.

References


