# Sperm Penetration in Broiler Breeder Strains

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***Introduction***

Fertility of broiler breeders is of utmost importance to the overall success of the poultry industry. Through intense genetic selection and improved nutritional management, there has been a steady increase in the growth rate of broiler breeders and their progeny. Consequently, a few years ago some people believed that in order to maintain acceptable fertility levels, artificial insemination would find its place in the broiler industry much like it has in modern turkey breeding programs. Artificial insemination, however, is currently not commonly accepted by the broiler industry.

It is apparent that the broiler industry is going to continue with the use of natural mating systems as their primary means of reproduction. Consequently, much of the work involving artificial insemination programs which were designed to correct fertility problems, are not directly applicable in the field. The primary goal of the breeder manager is to maximize the economic return from each breeder flock; consequently, this must occur through the management of flocks under natural mating conditions. Obviously, the ultimate goal of the breeder producer is to produce the maximum number of quality chicks per hen housed, and to achieve this goal it is necessary to maintain optimum fertility in the breeder flocks. Without producing fertile eggs, the best incubators and hatchery management procedures can not produce chicks. Therefore fertility is essential to the broiler breeder. In order to more effectively manage flock fertility, it is beneficial to have a better understanding of the series of events which occur prior to and at the site of fertilization in the avian egg.

***Anatomical Structure of the Membrane Overlying the Hen's Ovum***

The vestments surrounding the recently ovulated hens ovum consist of the original vitelline membrane, or oolemma, and an overlying perivitelline layer (Bellairs *et al.*, 1963; Bakst and Howarth, 1977b; Bakst, 1978). Anatomical observations made by Bakst and Howarth (1977a) indicated that the perivitelline layer from the germinal disc region was structurally different from the remaining ovum. The area over the germinal disc is the preferred binding site of spermatozoa. This is understandable in that the female pronuclei resides in this area of the ovum. Aside from the germinal disc region, the perivitelline layer from the other areas of the ovum have been found to be similar in their anatomical structure.

The membranes overlying the ovum do not remain constant from the time of ovulation throughout its passage in the hens oviduct. Following engulfment of the recently ovulated ovum into the infundibulum of the oviduct, an outer perivitelline layer is added which remains in contact with the egg albumen (Bellairs *et al.*,1963; Jensen, 1969). It has been reported that the addition of this outer perivitelline layer prevents additional sperm from penetrating the ovum due

to the presence of a trypsin inhibitor (Howarth, 1971; Fujiwara *et al.*, 1973; Bakst and Howarth, 1977b). This trypsin inhibitor prevents the digestive enzymes in the sperm from creating a hole, or pathway, in the perivitelline layer. Observations have been recorded which indicate that fertilization occurs in the infundibulum of the oviduct within 15 minutes following ovulation (Fofanova, 1965; Bakst and Howarth, 1977b), although personal observations suggest that this time frame may be between 5- 10 minutes after ovulation. The addition of this outer layer acts as a mechanism which prevents a detrimentally large number of accessory sperm from penetrating the ovum. Although physiological polyspermy is the rule in avian species, evidence suggests that their may be a physiological limitation to excessive polyspermy (Van Krey *et al.*, 1966; Lorenz and Ogasawara, 1968; Bekhtina, 1968).

Based on the function and location of the avian inner perivitelline layer, this structure is believed to be analogous to the mammalian zona pellucida. It is therefore interesting to follow the many observations made in reference to the mammalian zona pellucida. (Bleil and Wassarman, 1980; Kido and Doi, 1988).

***Sperm Penetration of the Perivitelline Layer***

Bellairs and co-workers (1963) suggested that the avian sperm was capable of passively traversing the perivitelline layer without breaking down this vestment. More recently, work performed by Fujii (1976) using scanning electron microscopy supported the theory of a non-digestive method of sperm penetration of the perivitelline layer as suggested by the previous authors. Fujii claimed to have observed sperm in the open spaces between the perivitelline layer fibers of infundibular ova. Fujii then suggested that these sperm were in the act of penetrating the inner layer and that there was no evidence of the occurrence of digestion of the perivitelline layer. He subsequently questioned the idea of sperm digesting a pathway or hole in the perivitelline layer in order to gain access to the female pronucleus. This was in contrast to reports regarding the mammalian system. Since these observations were recorded, the belief of a non-digestive mode of passage of avian spermatozoa through the perivitelline layer has been challenged by many in the literature.

In mammalian species, passive sperm entry into the ovum is not believed to exist. Following binding of the mammalian sperm to the zona pellucida, an acrosome reaction is initiated (Bleil and Wassarman, 1983; Wassarman, 1987) in which the sperm's plasmalemma and outer acrosomal membranes fuse and vesiculate exposing the inner acrosomal membrane . The release of acrosin which, apparently, is responsible for the digestion of a pathway through the zona pellucida for the penetrating sperm, is associated with the inner acrosomal membrane of the spermatozoon (McRorie and Williams, 1974). Following the acrosome reaction, the mammalian spermatozoon then penetrates the zona pellucida.

In avian species, Okamura and Nishiyama (1978) reported that as fowl spermatozoa come in contact with the perivitelline layer they, too, undergo an acrosome reaction. This contact, much like that found in mammals, results in a fusion of the sperm outer acrosomal membrane and the plasmalemma, their vesiculation, and the subsequent release of a trypsin-like enzyme (Palmer and Howarth, 1973), or more precisely, acrosin (Ho and Meizel, 1976; Froman, 1990). The digestive enzymes released by the acrosome of the sperm, are then responsible for the digestion of a pathway or hole through the perivitelline layer through which the spermatozoon may pass (Bakst and Howarth, 1977b; Okamura and Nishiyama, 1978; Howarth, 1984).

Work published by Howarth (1984), in which the use of scanning electron microscopy

was employed, shows photomicrographs of holes present on the surface of the perivitelline layer. These holes occurred as a result of trypsin like enzymes (acrosin) being released by the bound spermatozoa onto the intact perivitelline layer. One photo shows spermatozoa partially penetrating through a hole in the otherwise intact perivitelline layer. These photomicrographs offered additional strong support to the digestive method of passage of fowl spermatozoa through the avian perivitelline layer. After passage through the perivitelline layer, the sperm plasma membrane fuses with the vitelline membrane of the ovum and syngamy occurs, thus completing the avian fertilization process.

***Supernumerary Sperm at the Germinal Disc***

Since the turn of the century, researchers have shown a special interest in the germinal disc area of the ovum. The germinal disc of the hens ovum overlies the female pronucleus and passage through the perivitelline layer over lying the germinal disc region is required for successful fertilization of the egg (Romanoff, 1960). Considering the small area occupied by the germinal disc in relation to the entire ovum, it was believed that physiological polyspermy (or the presence of supernumerary sperm) was necessary to ensure syngamy. Although only one sperm is required to fertilize the egg, multiple sperm would increase the likelihood of a single sperm coming in contact with the ovum and penetrating its vestments at precisely the correct place and time. Historically, the presence of supernumerary sperm at the germinal disc region of the hen's ovum has been well documented. Multiple sperm pronuclei have been measured in the germinal disc region of the pigeon, the turkey, and chicken (Perry, 1987; Nakanishi *et al.*, 1990). The presence of multiple sperm 'trapped' in the outer perivitelline layer (Wishart, 1987; Brillard and Antoine, 1990) and multiple sperm penetrating the perivitelline layer in the germinal disc region of the chicken ovum (Bakst and Howarth, 1977b; Bramwell and Howarth, 1992a; Bramwell *et al.*, 1995) have also been well documented. However, the number of sperm reported to be present in the area of the germinal disc by these authors varies considerably, primarily due to the different measurement techniques used. Trapped spermatozoa may or may not possess the ability to bind to and undergo an acrosome reaction, and may have still been in transit to a future site of attachment, binding, and/or penetration or death. The sperm cells that penetrate the perivitelline layer possess all the criteria necessary to reach the female pronuclei.

***Preferential Attraction to the Germinal Disc***

Romanoff (1960) stated that sperm cells must enter directly into the germinal disc region of the ovum to ensure fertilization of the egg. Due to the large surface area of the hen's ovum, this led many to believe that there must be a preferential attraction to the germinal disc area in order to increase the likelihood of the occurrence of successful fertilization. This preferential attraction, then, was thought to be responsible for the large numbers of sperm reported to be present in the germinal disc region. In support of the concept of a preferential attraction of sperm to the germinal disc region of the ovum, several reports have been published (Howarth and Digby, 1973; Bakst and Howarth, 1977b; Ho and Meizel, 1975; Bramwell and Howarth, 1992a). Bramwell and Howarth (1992a) reported a significantly greater number of sperm penetrating the perivitelline layer from the germinal disc region as compared to the perivitelline layer from adjacent areas of ova exposed to spermatozoa *in vitro*.

*Fertilization*

In 1968, Van Tienhoven suggested that three specific conditions must be met in order to achieve successful fertilization of the avian egg. First, there needed to be a long fertilizable life span of the ova and/or sperm. We now know that the sperm cell does maintain a long fertilizable life span inside the hens body. Second, there must be accurate synchronization between copulation and ovulation. This is not entirely true due to the sperm storage capacity in the uterovaginal junction of the hens oviduct. However, there does need to be some synchronization of sperm presence in the infundibulum of the oviduct at the time of ovulation. Third, copulation must occur frequently enough to ensure that somewhat fresh and viable sperm are available at the time of ovulation.

In addition to the conditions mentioned above, successful fertilization is the result of the completion of several other steps. Following successful insemination sperm cells must be properly stored within the sperm storage tubules in the uterovaginal junction. Sperm then must be transported up the oviduct to the infundibular region which is the site of the actual fertilization of the ova. Once the sperm cells are located in the infundibulum, they must recognize the sperm binding sites on the perivitelline layer overlying the ovum. Following recognition and binding of the sperm cell to these sites, viable sperm are induced to acrosome react to digest a portion of the perivitelline layer through which the sperm cell may pass through. At this point the male gamete has gained access to the female pronuclei and syngamy, or the joining of the male and female gametes can occur. Following these steps, the avian egg has then been successfully fertilized and given the proper incubational conditions, additional embryonic development can occur.

*In vitro Sperm Penetration Assay*

In 1992 (a), Bramwell and Howarth developed a method in which they were able to quantitatively analyze the sperm-egg interaction *in vitro*. The perivitelline layer used in these studies must be removed from a recently ovulated ovum prior to their use in the *in vitro* assay. Following co-incubation with spermatozoa, the perivitelline layer is stained with a Schiff's reagent, after which it takes on a dark maroon color. As light passes through the clear distinct holes digested in the perivitelline layer by sperm which have undergone an acrosome reaction, the holes are then able to be easily counted under a light microscope. This *in vitro* method of quantifying sperm penetration has since been successfully utilized as an accurate and useful measure of sperm activity at the site of fertilization (Bramwell and Howarth, 1992b; Bramwell and Howarth, 1992c; Howarth, 1992).

The primary disadvantage of the *in vitro* method of analyzing sperm penetration mentioned above, is that it requires hens to be killed in order to obtain the recently ovulated ova from which the perivitelline layer is removed. Because hens must be killed at approximately 20 minutes post oviposition, these hens must be individually caged in order to identify the exact time in which a hen lays her egg. This limits the types of research for which this method can be utilized. For economical reasons, it limits its usefulness on a large scale basis; from a practical standpoint, essentially preventing its use in commercial breeder flocks. Also, having to kill at least one hen to obtain each perivitelline layer makes studies with time as one of the variables extremely difficult and costly to run. It would also prohibit observations to be made on individual hens over time. However, this method allows for comparisons to be made between sperm from individual males on the perivitelline layer from a single female. This type of test removes all female storage, transport and other physiological variation and only measures

different groups of semen in their physiological ability to bind and penetrate the perivitelline layer.

***Sperm Penetration Assay***

The *in vivo* sperm penetration assay is a relatively new technique that is used to determine the number of sperm that bind, acrosome react, and penetrate the outer layer of the ovum prior to fertilization. The sperm penetration assay was developed in 1990 at the University of Georgia primarily as a research tool and has been used with great success. Since that time, the assay has been modified as a research tool to include its use as an *in vivo* assay using laid eggs. However, possibly more important is its use to evaluate commercial flocks to predict their future fertility.

As previously discussed, following insemination, sperm cells are stored in sperm storage tubules located in the utero-vaginal junction of the oviduct. Sperm cells can be successfully stored for up to 2 to 3 weeks in the hens oviduct. After an egg is laid, sperm is released from these crypts, and it must travel up the oviduct to the infundibulum. It is in the infundibular region where sperm are able to come in direct contact with the outer layer of the recently ovulated ovum, or yolk. After coming in contact with the ovum, sperm must recognize and bind to sperm binding sites located on this perivitelline layer overlying the ovum. Following binding of the sperm to the perivitelline layer, they undergo an acrosome reaction releasing digestive enzymes which digest a hole allowing the sperm cell to pass through. Since the female genetic material is located in the germinal disc region of the ovum, sperm must penetrate the perivitelline layer overlying the germinal disc in order to maximize the chance of fertilizing the egg. Fertilization of the egg must occur within the first few minutes after ovulation as the digestive action of the sperms' enzymes are inactivated in the presence of egg albumen.

Evaluating *in vivo* sperm penetration can be accomplished using a sample of laid eggs taken from experimental birds, commercial flocks, or specifically selected lines. The protocol for completing the assay is included at the end of this paper. Briefly, the process of evaluating sperm penetration involves removing a portion of the perivitelline layer of the ova which lies directly over the germinal disc region. This section of the outer layer is rinsed, then straightened while on a microscope slide. The section of perivitelline layer is fixed with formalin and stained with Schiff's reagent to darken the intact portion of this outer layer. The digested holes in the perivitelline layer can then be easily seen and counted using a standard light microscope as light passes through the holes.

Although it does only take one sperm to fertilize a *single egg*, few numbers of sperm penetration holes within a flock are highly correlated with both low fertility and early embryonic mortality. In other words, an increased number of sperm penetration sites in the germinal disc region are desired. As sperm penetration values drop too low, in addition to reduced fertility, there is also a subsequent increase in early embryonic mortality and chick quality which further reduces hatchability. Low numbers of sperm holes represents a situation where mating is not taking place frequently enough to maintain a daily supply of a large number of fresh viable sperm for fertilization. Old and stale sperm in the oviduct have been associated with poor chick quality and early embryonic mortality (Nalbandov and Card, 1943; Dharmarajan, 1950; unpublished personal results). Also, uniformity of sperm penetration holes throughout the flock, eggs with increased numbers of sperm penetration holes will result in optimum fertility and hatchability from the entire flock.

It has been determined that an average of more than 30 sperm penetration holes in a *flock* are needed to maintain good fertility and embryo livability. Although values in excess of 30 will not necessarily result in increased fertility, values below this basal level of 30 holes will result in reduced fertility. Also, levels lower than this have also been associated with increased early embryonic mortality due to the conditions previously mentioned. Flock averages have ranged from less than 60 holes to more than 150 holes when they were at peak egg production. Each of these flocks had 'acceptable' fertility values at the time, but as the birds age sperm penetration values are dramatically reduced. These values eventually fall below the basal level of 30 holes in the germinal disc region therefore reducing fertility. Given this range of mean sperm penetration values, those flocks with consistently more sperm penetration holes are less likely to be affected by slight reductions in sperm penetration and can be considered 'more forgiving' or 'more resistant' to stresses which are normally depress fertility. Also, those flocks or birds with increased sperm penetration values at peak production will remain above the basal level of 30 holes longer thus they will remain more fertile later in life.

The sperm penetration assay is a powerful tool. These values are a more accurate predictor of reproductive fitness than fertility values alone because you can determine how susceptible your flocks may be to future fertility problems. A flock with sperm penetration numbers which average 100, is much less susceptible to future fertility problems (due to environmental stress, age, etc.) than a flock which averages 30 holes. In many cases you can identify flocks which may experience future reductions in fertility and hatchability and, with minor management changes, avoid a costly reduction in the number of quality chicks hatched. This can be accomplished because fertility values are based on a binary scale (an egg can only be fertile or infertile), and sperm penetration values are determined using a sliding or graduated scale. Consequently, flocks which are beginning to experience reduced sperm penetration values can be identified before the decreasing values negatively affect fertility and hatchability. Put in simple terms, sperm penetration values allow an individual bird, a flock of birds, or a particular strain of birds to be evaluated based upon their *potential* future reduction in fertility *before* fertility is actually reduced. Preventing birds from, or identifying birds which have the potential to produce eggs with reduced fertility is the objective behind this technique.

A very important evolvement of this assay has been its use in evaluating commercial flocks to predict their future fertility. As mentioned, three of the more popular commercial strains of broiler breeders as well as lines within one of those breeds have been evaluated and compared. It was discovered that there is great variation between breeds and lines within breeds. A sample of several flocks at peak production from each of three strains (A, B, and C) averaged 106, 91 and 79 holes. More alarming however, was that male and female lines from one of the strains of broiler breeders had averages of 106 and 51 holes respectively. This indicates a drastic difference in the fertilizing capacity of these two lines and also exhibits differences in the three strains examined.

A possible look into the future of todays broiler breeder stock can be seen by examining two quail lines selected for body weight gain for 100 generations. The control line had average sperm penetration values of 76 while the heavy line averaged 20 holes in the perivitelline layer. Average fertility in the heavy birds is around 30 percent and most of these birds had sperm penetration of less than 5 holes. The concern is that the capacity of todays modern broiler breeders to produce fertile eggs is being slightly reduced with each succeeding generation. It is becoming more difficult to manage many of these high producing birds for optimum fertility. It

would be beneficial to monitor these birds with each successive generation to determine what our selection practices are doing to the physiological ability of these broiler breeders to reproduce.

This assay has also been used to look at flocks of various ages to determine how age affected sperm penetration values in relation to fertility. It has also been used to determine the effects of elevated ambient temperature on sperm penetration and its potential effects on fertility. Projects are currently underway with commercial breeders to study the effects of spiking on sperm penetration values to assist in a determination of how many males per female is optimum for the different ages of flocks. We may also be able to spike certain flocks more heavily when sperm penetration values indicate it necessary in an effort to keep the mean number of holes high to *prevent* fertility, hatchability and chick quality problems. This technique is currently being used in a turkey operation to assist in a determination of how often to inseminate and with what concentration of sperm as well as to periodically monitor their breeding program. One of the primary breeders has also been using this tool to monitor the change in sperm penetration values of specific selected lines over time. Current efforts are concentrated on continually fine tuning and improving the commercial use of this assay as an evaluator of commercial flocks, breeds, and lines within breeds. Again, a gradual reduction in the ability of eggs to be fertilized as indicated by sperm penetration values can be determined using this technique long before fertility problems are realized. As an example, if with each generation of selection a specific line continues to experience a reduction in mean peak sperm penetration values, this may be corrected before the line actually experiences fertility problems. This would save several generations of back pedaling in order to improve the fertility of that line of birds. It may also allow geneticists to try and keep their selected lines away from the basal level of 30 sperm holes in an effort to maintain high sperm penetration lines and establish lines recognized for good reproductive traits with easier management requirements as well as performance characteristics.

This technique is simple, inexpensive and very effective in evaluating flocks with suspect fertility. The equipment required is minimal and it is relatively easy to train others on how to use this technique with the results available within minutes after beginning the procedure. At the primary breeder level, lines can be examined and monitored to try and prevent certain selected lines from becoming susceptible to fertility problems. At the hatchery, eggs can be examined to determine the level of sperm penetration to help determine what should be expected from the eggs of each flock. It has also been used to assist in management decisions such as when and which flocks to spike. In summary, the sperm penetration assay is a tool that can be used by many facets of the poultry industry to help maximize and improve the reproductive capabilities of todays breeders.

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# The Sperm Penetration Assay in Laid Eggs of the Chicken

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## PROCEDURE

1. Separate ovum (yolk) from the egg albumen either with a yolk separator or by decantation with egg shell halves.

2. Gently place the yolk on a smooth paper towel and gently roll the yolk or dab at the germinal disc (GD) until the GD area is free from adhering albumen.

3. Place the yolk in approximately 1% NaCl solution making sure the GD area is completely submerged for at least 5-10 minutes.

4. After incubation in the NaCl solution, orient the GD upwards and puncture the underside of the yolk to relieve internal pressure.

5. Carefully cut the perivitelline layer (PL) overlying the GD away from the yolk in a square with each side *at least* 1 cm in length. Vigorously shake the PL section in the NaCl solution to make sure it is free from adhering yolk material (adhering yolk or debris which causes cloudiness will interfere with counting holes after staining).

6. Place the PL section on a microscope slide (2-3 PL sections per slide can be used) and straighten with needles or small probes. (hint: if 1 or 2 drops of water are added it often makes straightening somewhat easier). It is important to keep the GD area as smooth as possible without wrinkles and overlapping.

7. Add a few drops of the prepared formalin solution to the PL sections, let sit for ~ 10-20 seconds.

8. Add a few drops of Schiff's reagent and allow ample tie to stain the PL section. The time required to stain properly is dependant upon the age and strength of the reagent. It can take as little as a few seconds to a minute or more. (Too dark a stain can make viewing holes and locating the GD difficult)

9. Gently place a coverslip over the PL section and carefully press on the coverslip to remove air bubbles. (note: do not move coverslip after placed on the slide, it may cause wrinkling of the PL section)

10. Place slide on the microscope and locate the GD on low power and count holes on higher power. Count only those holes in the circular GD region.

**MATERIALS NEEDED**

small dissecting scissors
small forceps
bowls
disposable pipettes
microscope slides & cover slips
paper towels
needles or probes (for straightening membrane)
yolk separator (optional)

**EQUIPMENT**

light microscope (4x and 10x objectives)

**CHEMICALS**

NaCl (1% solution)
Schiff's reagent (Sigma #S-5133)
Formalin (10-20% solution)

(Eggs can be stored in a refrigerator or egg storage room similar to hatching eggs for several weeks. Although, in eggs stored too long the PL begins to weaken and break easier during preparation)

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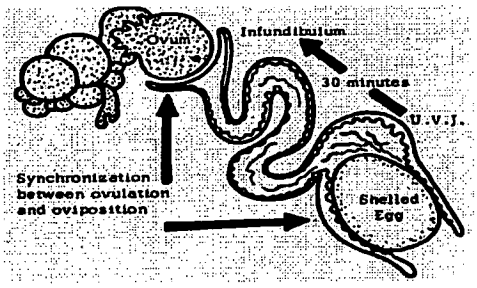
## Requirements for Successful Fertilization

- Van Tienhoven (1968) suggested that the following conditions must be met:
  - long fertilizable life span for ova and/or sperm
  - accurate synchronization between copulation and ovulation
  - copulation must occur frequently to ensure viable sperm at the time of ovulation

## Internal Sperm Storage and Transport




## Historical Background

- Spermatozoa must bind and penetrate the perivitelline layer prior to fertilization of the egg.
- Spermatozoa preferentially penetrate the perivitelline layer over the germinal disc of the hens egg.

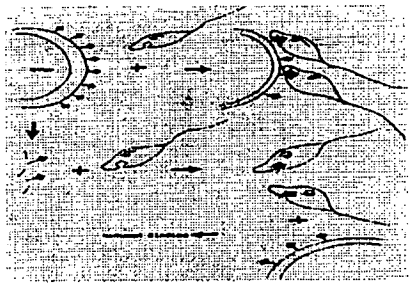
## Historical Background

- Reported numbers of sperm cells in oviposited eggs varies partly due to the technique used.
  - Staining PL < 5 sperm/egg
  - Fluorescent staining ~200 sperm/gd
  - Sperm penetration up to ~850 holes
    Avg = 100-120 holes/gd

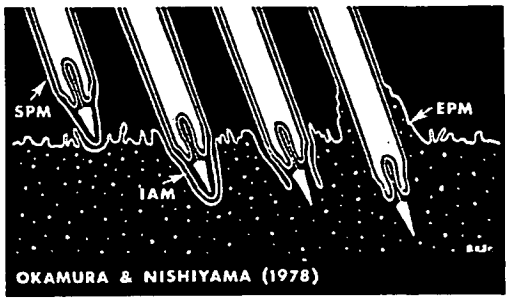
## Historical Background

- Sperm numbers average between 60-400 with an excess of 200 sperm resulting in abnormal embryonic development.
- Only one sperm is required to fertilize an egg.

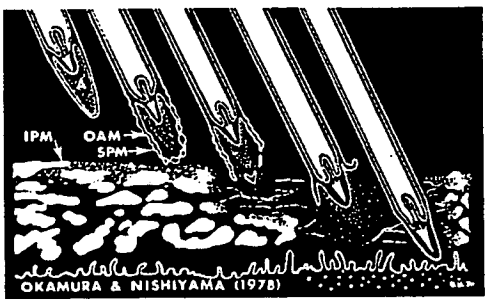
## Sperm Recognition and Binding to Perivitelline Layer



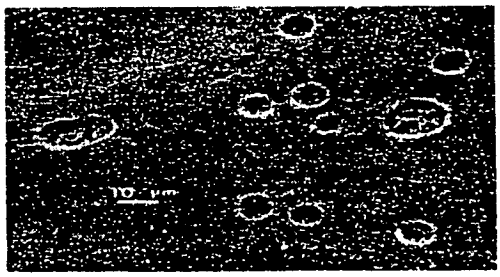
## Sperm Binding and Acrosome Reaction



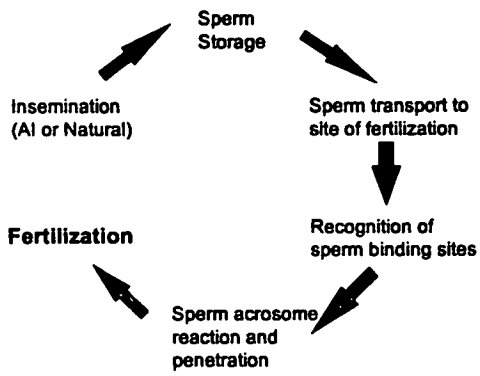

## Sperm Binding and Acrosome Reaction




## Sperm Penetration of the Perivitelline Layer



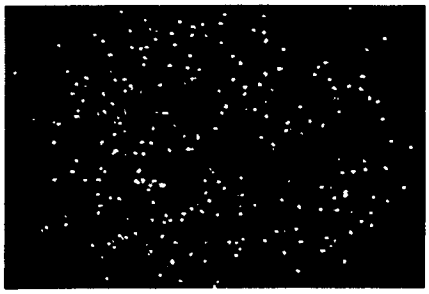
## Sperm Penetration of the Perivitelline Layer



## *In Vivo* Sperm Penetration Assay

- Separate yolk and albumen from a fresh egg and bath yolk in 1% NaCl solution.
- Remove PL from germinal disc area.
- Mount PL section on slide, fix with formalin and stain with schiff's reagent.
- Visually count holes in the PL from the germinal disc region.

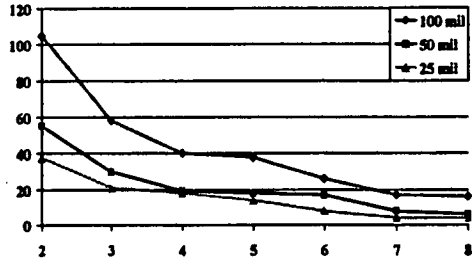
## Sperm Penetration of the Germinal Disc



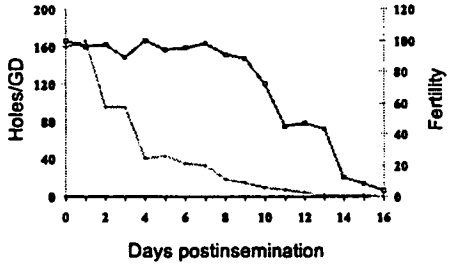
## Effect of Polyspermy on Reproduction

| Sperm Dose | Fert | H of Fert | Sperm Penetration |
|---|---|---|---|
| 400 mil | 91.5 | 87.3 | 109/21 |
| 200 mil | 88.3 | 80.5 | 59/10 |
| 100 mil | 75.0 | 81.5 | 49/9 |
| 50 mil | 71.1 | 85.1 | 29/7 |
| DPI | ++ | - | ++ |

## Sperm Penetration Over Days Postinsemination



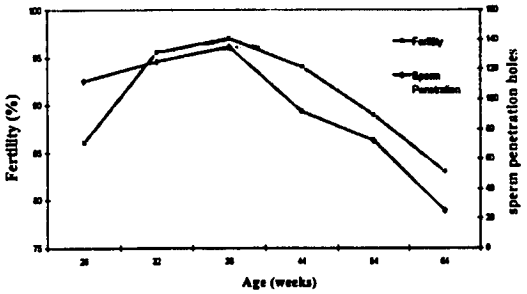

## Duration of Sperm Penetration Naturally Mated (ACRB)



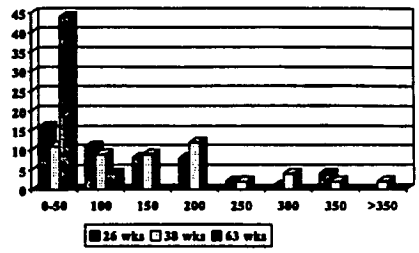

## Effect of Age on Reproduction in Broiler Breeders

- Libido and/or successful mating is reduced in older males
- Semen quality from older males is reduced
- Older broiler breeder hens do not store sperm as efficiently
- Sperm which are stored in the hens don't bind and penetrate the ovum as efficiently

## Sperm Penetration in Naturally Mated Broiler Breeder Flocks



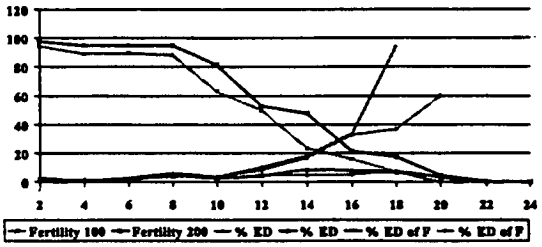

## Frequency Distribution of Sperm Penetration by Age




## Sperm Penetration by Days

| Dose (mil) | 2 | 4 | 6 | 8 | 10 | 12 | 14 | 16 |
|---|---|---|---|---|---|---|---|---|
| 200 | 69.2 | 50.6 | 18.9 | 14.1 | 10.5 | 8.7 | 7.0 | 3.6 |
| 100 | 35.9 | 20.4 | 10.5 | 8.8 | 7.3 | 4.8 | 3.7 | 2.5 |
| 50 | 24.6 | 14.1 | 7.8 | 6.2 | 5.0 | 3.6 | 2.8 | 0.7 |

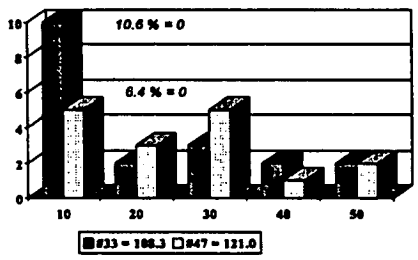
## Fertility & Embryonic Mortality



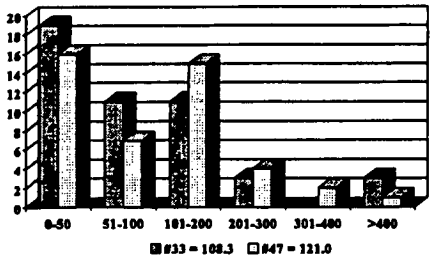

## Sperm Penetration Values in Commercial Flocks

| 26-63 wks | Flock # | Flock #642 |
|---|---|---|
| 0-10 | 16.7 (3) | 42.1 (2) |
| 11-30 | 23.3 | 36.8 |
| 31-60 | 16.7 | 15.8 |
| 61-100 | 10.0 | 5.3 |
| 101+ | 33.3 | 0 |
| mean | 77.1 | 19.8 |

## Frequency Distribution of Sperm Penetration



## Frequency Distribution of Sperm Penetration




## Sperm Penetration Values in Commercial Flocks

| 40 wks | Flock #112 | Flock #423 |
|---|---|---|
| **0-10** | 3.8 (1) | 0 |
| 11-30 | 3.8 | 7.7 |
| 31-60 | 19.2 | 7.7 |
| 61-100 | 11.5 | 11.5 |
| 101+ | 61.5 | 69.2 |
| mean (fert) | 107.4 (98.3) | 130.5 (95.0) |

## Sperm Penetration Values in Commercial Flocks

| 41 wks | Flock #15 | Flock #16 |
|---|---|---|
| **0-10** | 4.3 (1) | 23.3 |
| 11-30 | - | 10.0 |
| 31-60 | 21.7 | 16.7 |
| 61-100 | 17.4 | 6.7 |
| 101+ | 56.5 | 43.3 |
| mean (fert) | 99.8 (89) | 77.5 (84) |

## Sperm Penetration Values in Commercial Flocks

| 38-40 wks | Flock #454 | Flock #455 |
|---|---|---|
| **0-10** | 3.6 | 6.7 |
| 11-30 | 7.1 | 10.0 |
| 31-60 | 10.7 | 36.7 |
| 61-100 | 10.7 | 36.7 |
| 101+ | 67.9 | 10.0 |
| mean (fert) | 118.5 (94.8) | 65.2 (97.2) |

## Effect of Broiler Breeder Strain on Sperm Penetration

| Strain | Fertility | Sperm Penetration |
|---|---|---|
| Strain A | 95.0 % | 105.8 |
| Strain B | 87.1 % | 90.6 |
| Strain C | 87.7 % | 78.6 |

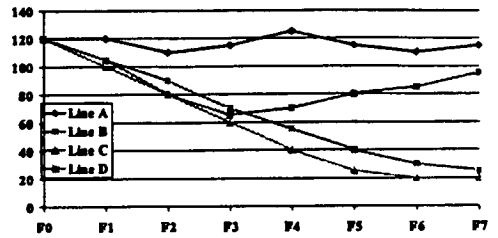
## Effect of Line of Broiler Breeder on Sperm Penetration

| Breeder Line | Fertility | Sperm Penetration |
|---|---|---|
| Male | 90.0 % | 106.4 |
| Female | 85.4 % | 50.8 |

## Sperm Penetration in Selected Quail Lines

| Holes | C-Line | P-Line |
|---|---|---|
| **0-10** | 0 | 58.3(6) |
| 11-30 | 12.1 | 16.7 |
| 31-60 | 27.3 | 11.1 |
| 61-100 | 30.3 | 11.1 |
| 101+ | 30.3 | 2.8 |
| Mean | 76.2 | 20.3 |

## Monitoring Selected Lines of Breeders




## Benefits of *in vitro* Sperm Penetration Assay

- Uses include testing the effects of extenders, diluents and storage on sperm cells.
- Can test the sperm from many males on the PL from a single female to determine its fertilizing capacity.
- Removes female variation (i.e.. mating efficiency, sperm storage, sperm transport, sperm binding etc..)

## Potential Benefits of the Sperm Penetration Assay in Breeders

- Selected lines could be monitored to determine the effects of selection on reproduction potential.
- Trends towards decreased sperm penetration could be addressed before fertility is reduced in a selected line.
- Keep selected lines away from the basal sperm penetration values which negatively affect fertility.

## Future Plans and Applications of Assay

- Used as a research tool to assess sperm membrane stability and capacity to fertilize
- Expanded use in trouble shooting unproductive commercial breeder flocks
- Use in artificial insemination programs

Questions:

#1 from Dr. Morteza Sadjadi: What mechanism explains the release of sperm from the U-V junction? I am not aware of any hard evidence which would suggest a specific mechanism responsible for the release of sperm from the storage crypts in the U-V junction. It is believed by some that sperm cells gradually trickle out of the storage sites on an almost continually basis with no regard for the timing of ovulation. I believe that although this probably does occur to some extent, that oviposition of an egg and/or the passage of an egg by this region of the oviduct stimulates sperm cell release. If the release of these cells was continual it would be biologically inefficient because most of these sperm cells would be either engulfed by the developing egg or blocked from passing up the oviduct.

#2 from Dr. John Tierce: Is a female receptive to a male if there is an egg in the oviduct? In a good producing hens there will be a developing egg in the oviduct for 24-25 consecutive hours. Following oviposition, a good producing broiler breeder hen will ovulate the ova which will be part of the next days eggs within about 25 minutes (20 minutes in commercial egg layers). Assuming she sits in the nest for a short time after oviposition, this wouldn't leave much time remaining for her to be mated if the only time she was receptive was when she had an empty oviduct. In my observations, if a hen is in the vacinity of a male and HE wants to breed her, she doesn't have a lot of choice. Although it does help if the hen is also in the mood.

#3 from Dr. Surinder Gill: What is the effect of spiking on sperm penetration? We are currently in the process of learning what the actually affects of spiking are in relation to sperm penetration values. Whether spiking flocks results in the less frequently mated hens to be mated more often or wether all the birds are mated a little more frequently will be determined. The sperm penetration assay can give us an idea of the effectiveness of spiking on the entire flock and their fertility. The ideal situation is to have the majority of the birds with large of numbers of sperm penetration values and a few others with less sperm. Most flocks that are getting spiked have two populations of hens, one with plenty of sperm, and one with very few sperm penetration holes which is generally a result of infrequent matings.

#4 from Dr. John Kirby: How many eggs would I need to break out and how long would it take for me to assess a flock of 16,000 to 20,000 breeder pullets? I generally will take a sample of at least two flats of eggs from a house with the eggs collected from various parts of the house. When checking eggs for sperm penetration values it will take roughly 1 hour per flat. Because of the time involved I generally will use this technique as a trouble shooting measure for problem flocks and not as a general test of every flock in the state of Georgia.

#5 from Neil O'Sullivan: How many hens were killed to determine the five minutes time estimate between ovulation and the previous oviposition? I have not set up a specific test to determine this 5 minute time period. Over the years we have sacrificed about 1,000 hens while collecting recently ovulated ova. These have been either for the *in vitro* sperm penetration assay, or to collect the recently ovulated ova to extract the perivitelline layer protein from the ova for other tests. The estimate of 5 minutes was taken from observation of when the albumen begins to be added around the ova during these collection periods and not from any specific test. I do feel that the 5 minutes is only an estimate but I am reasonably certain that it is within 1-2 minutes of the actual time period.