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Artificial Insemination in Poultry

Birkett Howarth, Jr
University of Georgia
U. S. A.

家禽의 人工授精

Summary

1. Diluted chicken semen can be preserved at 2 to 5°C for 24 to 48 hr with resultant fertility of greater than 90% of that of fresh semen. Turkey semen can be preserved at 10 to 15°C for 6 to 24 hr and provide economical fertility.
2. Frozen chicken semen has given variable results; a 21 to 93% fertility range as compared to 92 to 94% expected with fresh semen. Highest fertility levels obtained with frozen turkey semen intravaginally inseminated have been 61 and 63% using DMSO and glycerol, respectively, as cryoprotectants.
3. The use of glycerol as a cryoprotectant requires that its concentration in semen be reduced to less than 2% either by dialysis or centrifugation after thawing and before intravaginal insemination if optimal fertility is to be obtained.
4. The temperature at which cryoprotectants are added to semen and the time allowed for equilibration are important for subsequent fertility pre- and post-freezing.
5. The type of container used for packaging the semen, freeze or cooling rates, thaw rates and level of cryoprotectant all interact in affecting cell survival.
6. Plastic freeze straws as a packaging device for semen offers the following advantages: easy to handle, require minimal storage space, offer a wide range of freeze and thaw rates, and insemination can be made directly from them upon thawing.
7. Controlled slow cooling rates of 1 to 8°C/min have thus far provided the best results for cooling chicken semen through the transition phase change (liquid to solid) or critical temperature range of +5 to -20 or -35°C.
8. Highest fertilities have been achieved with frozen chicken semen where a slow thaw rate (2° to 5°C) has been used regardless of the freeze rate.
9. To maintain a constant high level of fertility throughout a breeding season with frozen semen, a higher absolute number of spermatozoa must be inseminated (2 to 3 times as many) as compared to fresh semen since approximately 50% are destroyed during processing and freezing.
10. The quality of semen may vary with season and age of the male. Such changes in sperm quality could be accentuated by storage effects. Thus, the correct number of spermatozoa may very well vary during the course of a breeding period.
11. As to time of insemination, it is best to avoid inseminating chicken hens within 1-2 hr after or 3-5 hr before oviposition; and turkey hens during or 7-10 hr before oviposition.
12. The physiological receptiveness of the oviduct at the time of insemination is a very important biological factor influencing fertility levels throughout the breeding season.

I. Introduction

As with many other industries today, agriculture is faced with rising costs of production. Increasing costs of labor, feed and energy are forcing the poultry industry to seek more efficient ways of producing poultry meat and eggs. One means of reducing production costs

is to increase reproductive efficiency. On a worldwide basis we are far from realizing the reproductive potential of poultry especially broiler breeders and turkeys. Gerrits, et al. (1979) estimated that an 84 million dollar savings could be realized due to improvement in the reproductive efficiency of chickens and turkeys.

Artificial insemination (AI) represents one of the most important techniques available for improving the reproductive efficiency of the male breeder and for improving the efficiency of genetic selection for production traits. From a commercial standpoint, however, AI is practiced most extensively by the turkey industry. Also, in the Far East, meat-type (mule) ducks are produced by breeding the Muscovy male with the Pekin female, primarily accomplished through the use of AI (Huang and Chow, 1974; Tan, 1980a, b). In both cases emphasis has been placed on selective breeding either for rapid growth or for heavy breast muscle weight for meat production. Physical incompatibility between the sexes has resulted in poor fertility from natural mating and AI has become obligatory.

AI with other types of poultry (commercial egg and broiler type chickens) is currently used on a limited basis by primary breeders for maintaining lines and for screening and evaluating males for semen quality. Development of the dwarf broiler female has also necessitated the use of AI in certain circumstances. While recent declines in fertility and hatchability in broilers (as a consequence of continued intensive selection for growth rate) has prompted breeders to begin seeking alternatives to current breeding practices, there has been no important trend by the broiler industry toward the use of AI.

The purpose of this presentation is to review some of the more recent developments in AI of poultry with special reference to methods of liquid and frozen semen preservation and to factors affecting the efficiency of producing fertile hatching eggs via AI. The author wishes to acknowledge several excellent reviews on AI and semen preservation which have contributed greatly to the preparation of this presentation (Lorenz, 1969; Lake, 1978, 1983; Lake and Stewart, 1978a; Sexton, 1979, 1980a, 1983).

II. Short-term Liquid Semen Preservation Above 0°C

As soon as semen (chicken and turkey) is ejected from the male, a proportion of spermatozoa begin to lose their integrity and the process continues during storage in vitro with a resultant decline in fertility after insemination (Lake and Stewart, 1978a; Harper, 1955; Carter et al., 1957; Burke et al., 1973). Diluents and semen storage techniques are designed to minimize this loss in viability of spermatozoa in vitro. In general, if semen is to be held for longer than 30 to 45 minutes from the time of collection to insemination, it should be suspended in a synthetic diluent and kept at a low temperature (2 to 5°C for chicken semen; and 10 to 15°C for turkey semen).

In addition to preserving semen viability, diluents are used to lower the cost of AI by allowing diluted semen to be inseminated into more hens than would be possible with undiluted semen. With dilution - one part semen to two parts diluent - and holding insemination dose (1/30 ml) constant, the number of hens inseminated is increased threefold. Likewise the number of males needed for AI can be reduced to one-third. Considering that production costs may be as high as $30 per rooster and $60 per tom (Sexton, 1983), the requirement for fewer males or any improvement in the reproductive efficiency of the male breeder can result in substantial savings in production costs.

Additional savings can be realized from short-term preservation of semen prior to AI through reduced labor costs and increased pro-

duction efficiency. Advantages would include the transport of semen over short distances, the establishment of breeder tom farms, better utilization of the AI labor force, and the insemination of hens late in the afternoon, a time found to be most desirable for this purpose (Johnston, 1977).

It is now general knowledge that diluted chicken semen can be preserved at 2 to 5°C for up to 24 hr and provide satisfactory fertility; 90% of that of fresh semen as shown in Table 1. Turkey spermatozoa on the other hand are more difficult to maintain in vitro than those of the chicken. Current information indicates that turkey semen can be preserved at 15°C for up to 6 hr and provide economical fertility as shown in Table 2. Recently, Wishart (1981) evaluated the effect of continuous aeration on the fertility of chicken and turkey semen stored at 10°C. His results demonstrate that chicken and turkey spermatozoa can be preserved for 48 and 24 hr respectively with resultant fertility of greater than 90% of that of freshly-diluted semen.

Some general criteria of diluents used for short term semen preservation are shown in Table 3. The diluent generally provides an exogenous source of energy in the form of glucose, fructose, inositol or some other alcohol sugar. The composition of the diluent should provide osmotic balance for the sperm cells. The freezing point depression ($\triangle$) of chicken seminal plasma is about 0.64°C. Both chicken and turkey spermatozoa retain their full fertilizing ability in diluents having a fairly wide range in $\triangle$, 0.455 to 0.736°C. Slightly hypertonic diluents however are often best for the preservation of chicken spermatozoa (Van Wambeke, 1977).

Since spermatozoa are metabolically active cells, the diluent must have buffering capacity to neutralize the noxious effects of metabolic end products. Thus most diluents contain substances such as glutamate, phosphates of sodium and potassium or biological buffer salts (e.g. TRIS, MES, BES, TES). The pH is generally held at some point between 6.8 and 7.4.

Most diluents contain a chelator, such as glutamate, albumen or milk, to protect against toxic ions. An antibacterial agent may also be added such as gentamycin, penicillin or streptomycin.

III. Frozen Semen Preservation

Prolonged storage of semen at sub-zero temperature enables the creation of banks of semen from selected (progeny tested) proven sires to be used long after their death. With suitable precautions to avoid the concentration of adverse genes, frozen semen preservation could increase the rate of genetic improvement through facilitating the transportation, between and within countries, of semen of desirable sires.

While frozen semen preservation and AI have been applied commercially as a mode of reproduction to maximize the utilization of superior sires by many animal industries, these practices have found limited use in the poultry industry. A primary reason for this is the lowered fertility of frozen-thawed chicken and turkey semen as compared to unfrozen semen or fertility from natural mating. Success with the use of frozen-thawed chicken and turkey semen, furthermore, depends upon producing a prolonged fertile period in the hen (i.e. a daily succession of fertile eggs over a period of several days or weeks) after a single insemination. This is unlike the case with other domestic livestock, where only a single egg or several eggs ovulated over a period of approximately six hr are required to be fertilized. Thus many more fowl spermatozoa need to be revived in a fully viable state after freezing than is the case with the mammal, i.e. more than a 60 to 70% revival rate is necessary (Lake, 1978).

A summary of the fertility results obtained

with the current methods for chicken semen preservation are shown in Table 4 and reveal that frozen semen has given a 21 to 93% range of egg fertilization rate during days 2-8 after insemination as compared with an average fertility level of 92-94% expected with fresh semen. The highest level of fertility (93%) was obtained by Lake et al. (1981) from hens over an 11 day period after the first of four inseminations at 3-day intervals with frozen-thawed chicken semen. The cryoprotectant used in this case was glycerol (7-8% V/V in diluted semen); its concentration being reduced to less than 2% after thawing and before intravaginal insemination.

Polge, et al. (1949) discovered that glycerol would preserve the motility of frozen chicken spermatozoa. However, subsequent fertility trials demonstrated that spermatozoa frozen in glycerol were infertile unless the glycerol was removed by dialysis (Polge, 1951) or centrifugation (Clark and Shaffner, 1960) prior to insemination. Other work revealed that glycerol if not removed prior to insemination reduced fertility in chickens when it was added to spermatozoa at concentrations greater than 2% whether the spermatozoa were frozen or unfrozen (Allen and Bobr, 1955; Clark and Shaffner, 1960; Neville et al., 1971; Sexton, 1973). Westfall and Howarth (1977a) noted a decreased fertility in hens when glycerol was deposited vaginally five minutes before, after, or concurrently with spermatozoa. The contraceptive action of glycerol is due to its induction of osmotic damage to sperm cells which disrupts normal sperm transport in the vagina and thus reduces the number of spermatozoa stored in the uterovaginal sperm host glands (Marquez and Ogasawara 1977b). In addition to a direct effect on the spermatozoa, glycerol may produce a shortterm inflammatory response in the vagina which could also interfere with sperm transport. Thus one can reduce to some extent the harmful effects of glycerol by surgically inseminating the thawed semen into the uterus or magnum. Watanabe and Terada (1976) reported fertility as high as 74% when glycerol treated frozen-thawed chicken semen was deposited into the uterus. Similarly, Marquez and Ogasawara (1977b) reported fertility of 70 to 74% for the first week after depositing frozen-thawed semen into the turkey hen's magnum. Surgical insemination or removal of glycerol after thawing are certainly not practical however from a commercial standpoint. Researchers continue to seek for and test other cryoprotective agents. Sexton (1976) reported 59% fertility following intravaginal A.I. every seven days with cock semen frozen and thawed in the presence of 4% dimethylsulfoxide (DMSO). Modifications by Williamson et al. (1981) in the time and temperature at which DMSO is added to semen have brought about further increases in fertility (71%) and offered technical simplicity for the use of this cryoprotective agent in the poultry industry. The Beltsville Method using DMSO (Sexton, 1980a) has been tested and incorporated into the breeding programs of commercial breeders with some success. Another cryoprotectant, di-methyl acetamide (DMA), looks promising (Lake and Ravie, 1982a). Frozen thawed chicken semen in DMA inseminated intravaginally into hens for 3 consecutive days provided 76.5% fertility during days 2 to 6 after the first insemination.

The preservation of turkey semen in a frozen state (Table 5) has not been as successful as the experienced with chicken semen. Schefels (1978) using glycerol as a cryoprotectant reported 63% fertility when hens were inseminated at 4-day intervals with frozen-thawed turkey semen. Sexton (1981) reported 61% fertility from hens early in their laying season inseminated on two consecutive days with frozen-thawed turkey semen in DMSO.

1. Methods for Freezing Semen:

Freezing procedures vary but can generally

be broken down into pre-freeze, freeze and thaw steps as shown in Table 6. The rationale for or the effects of these procedural steps in some cases are well documented while others require additional evaluation.

1) **Pre-freeze Steps:**

The pre-freeze procedures mimic in many respects those used for liquid semen preservation above 0°C. The main differences being the addition of the cryoprotectant and the packaging of semen for freezing. In Table 6 the temperature of the semen following collection but prior to the addition of cryoprotectant is reduced to 5°C. In the procedure by Lake, et al. (1981) this reduction in temperature is rapid (3 to 5 min.). However, we generally assume that chicken spermatozoa are not harmed by the rapid decrease in environmental temperature referred to as "cold shock." Excellent fertility can be obtained with chicken semen that has been abruptly cooled (DeSilva, 1963). Turkey spermatozoa, however, may be susceptible to rapid cooling to temperatures above 0°C. Brown (1966) observed the number of bent and damaged spermatozoa increased any time the temperature of turkey semen held at 30°C experienced a sudden drop of 15°C or more. Sexton (1981) subsequently studied the effect of pre-freeze chilling rate before the addition of DMSO on the fertility of processed unfrozen and frozen-thawed turkey semen. Weekly fertility levels (88 to 96%) were highest with processed, unfrozen semen collected directly into Beltsville poultry semen extender (BPSE) maintained at 25°C and chilled to 15°C at a slow rate of .3°C/min.; 4% DMSO was added when the semen temperature was 25°C. The fertility level of hens inseminated two consecutive days during the early stages of egg production with semen processed and frozen to -20°C was 61%.

Perhaps the single most-researched area involving the development of a procedure to freeze poultry semen has been the selection and use of a cryoprotectant. A detailed discussion on the action of cryoprotective agents has been published by Meryman (1971). Maintenance of cell structure and the survival of cells depends on the avoidance of intracellular ice crystallization and salt damage due to increased osmotic concentration during freezing. The so called penetrating cryoprotective agents such as glycerol and DMSO function by depressing the freezing point of water and by reducing cell dehydration to a tolerable degree. There are two immediate obstacles to the general use of penetrating cryoprotectants. First, the penetration of cells must be uniform and achieved without the imposition of osmotic stresses. Drevius (1971) noted that the permeability of bull spermatozoa to glycerol is so great that they swell and lyse as a result of the increase in intracellular osmolarity. The rate of DMSO penetration into most cells is also rapid, and the rate of influx is temperature dependent (Meryman, 1971). In studies conducted by Harris, et al. (1973) and Westfall and Howarth (1977b), the addition of glycerol did not cause any ultrastructural changes in chicken spermatozoa, although in the latter study glycerol caused a significant decrease in sperm magnesium and potassium concentrations. Upon removal of glycerol by both centrifugation and dialysis, extensive ultrastructural changes were observed and the cation concentrations were further distorted from the values obtained with untreated spermatozoa (Bakst and Howarth, 1977; Westfall and Howarth, 1977b). The changes in morphology indicate that the removal of glycerol induces osmotic shock. Marquez and Ogasawara (1977a) also attributed the membrane irregularities of turkey spermatozoa treated with glycerol and then frozen and thawed to osmotic effects.

The second obstacle to the use of penetrating cryoprotectants is their toxicity especially at the high concentrations necessary to achieve

protection. Both DMSO and glycerol produce changes in cell electroyte distribution at concentrations comparable to those required for cryoprotection. Whether the electroyte changes are manifestations of the toxicity of these cryoprotectants or arise secondary to osmotic induced damage is difficult to assess. However, Bakst and Sexton (1979) observed the sequential decrease in fertility and motility of fowl and turkey spermatozoa before, during and after freezing and storage at -196°C to be correlated with structural damage of the spermatozoa of both species. The cryoprotectant used in their study was DMSO.

Prefreeze steps such as holding and cryoprotectant equilibration times and temperatures in many studies lack validation. Sexton has published several reports which support the rationale for some of the procedural steps of the Beltsville method for freezing chicken semen. Optimal holding (2 hr) and equilibration (2 hr) periods at 5°C with 4% DMSO were reported by Sexton (1981) for chicken semen. There is a discrepancy however between these results and the results of Williamson et al. (1981). Fertility levels of semen equilibrated in BPSE and 4% DMSO for 1 hr were higher than samples equilibrated for 2 hr. Williamson et al. (1981) also reported that the fertility of frozen semen was slightly higher if the DMSO was added when semen temperature was 15°C rather than 10°C. As mentioned earlier, the rate of influx of DMSO is temperature dependent. At a higher temperature, DMSO may penetrate and reach equilibrium faster thereby reducing cellular injury (Wolstenholme and O'Connor, 1970). This may explain partly why DMSO is less damaging to sperm cells when added at a higher temperature for a shorter period of time as was shown by Williamson, et al. (1980) for chiecken spermatozoa and as was reported by Sexton (1981) for turkey spermatozoa. Optimal equilibration conditions with glycerol have been reported to be 60 to 90 minutes at 2° or 5°C (Watanabe, et al. 1970). A much shorter period of time (1 to 15 min) however now appears to be adequate to equilibrate the interior of chicken spermatozoa with glycerol prior to freezing (Watanabe et al., 1975).

The packaging of both mammalian and avian spermatozoa for freezing has been investigated (Mortimer et al., 1976; Sexton, 1978). The three most common packages for frozen spermatozoa are the ampule, the straw, and the pellet. With glass ampules, the thickness of the glass can make high cooling and thawing rates difficult. Furthermore, the use of glass ampules creates an additional loss of spermatozoa associated with the act of transferring the semen to a pipette before insemination. Plastic freeze straws have been shown to be easier to handle, require less storage space than the conventional glass ampules, allow the use of a wide range of freeze and thaw rates, and insemination can be made directly from them upon thawing (Graham, 1978). Where very high consistent cooling rates are required, pellets can be formed by placing a drop of extended semen directly on the surface of dry ice or on liquid nitrogen. With this method, some variation in cooling rate can be achieved by varying the pellet volume.

2) **Freezing and thawing semen**:

In general, controlled slow cooling rates of 1 to 8°C/min have thus far provided the best results for cooling chicken semen through the transition phase change (liquid to solid) which has been determined as -10°C (Sexton, 1980b). An average cooling rate of 1 to 2°C/min from +5 to -15°C corresponds to the "standard" rate for freezing bull spermatozoa used in the early fifties (Polge, 1953, 1957; Smith and Polge, 1950 : Parkes, 1956).

Data published with mammalian and avian semen indicate that factors such as type of container used for packaging the semen, cooling methods, cooling rates, thaw rates and level

of cryoprotectant interact in affecting cell survival. An optimum combination of these variables must be considered for satisfactory recovery of frozen semen. Sexton (1978) showed that the rate of cooling through the transition phase could be altered by the type of packaging container used (i.e. glass ampule or plastic straw). Also, the initiating temperature for the heat of fusion was lower with all cooling rates for semen in straws than for semen packaged in ampules. Semen frozen in plastic straws produced significantly better fertility than the same semen frozen in ampules (Sexton, 1978). Three factors which should be influenced most by a shift in packaging unit geometry are the optimums for freeze rate, thaw rate, and level of cryoprotective level. Since it is quite likely that these factors interact with one another, studies should be designed to determine simultaneously the influence of such factors on sperm survial and to establish the optimum combination of these variables for semen preservation.

Where frozen chicken semen has provided the highest fertilization rates following AI (Harris, 1968; Sexton, 1980a; Lake, et al., 1981) the method of cooling used has been controlled at least through the critical temperature range of +5 to -20 or -35°C. With controlled cooling, the sample temperature follows the bath temperature until it crystallizes. During the freezing plateau the bath continues to cool. Once the heat of fusion has been removed the rate of sample cooling speeds up until the sample temperature catches up with the bath. Sexton (1980a, b) uses an alcohol bath for maintaining a cooling rate of 1°C/min between +5 to -20°C. Lake, et al. (1981) use a programmable minifreezer (Type R202/200R; Planer Products Ltd., Sunbury, England) to lower semen temperature by 1°C/min from +5 to -35°C. In this system, vaporized liquid nitrogen is injected at a controlled rate into a closed chamber and circulated.

Uncontrolled cooling methods of freezing involve plunging the semen sample into a bath (alcohol, liquid nitrogen vapor or liquid nitrogen) fixed at a temperature colder than the sample. This method generally results in nonlinear cooling curves which can vary greatly from sample to sample within a freeze and especially between freezes when employing liquid nitrogen vapor. Uncontrolled high cooling velocities for chicken semen through the critical temperature range of +5 to -20 or -35°C has generally not provided satisfactory fertilization rates (Brown, et al., 1963). Bull spermatozoa, likewise, are sensitive to high cooling velocities above -27 or -30°C, but are very resistant to high velocities below 30°C (Davis, et al., 1963; Polge, 1957). Plunging into liquid nitrogen from -20°C resulted in practically no survival but plunging from -27°C allowed maximal survival (Luyet and Keane, 1955). In glycerol, chicken spermatozoa can be plunged into liquid nitrogen from -35°C with good recovery (Lake, et al., 1981). Using DMSO as the cryoprotective agent, Sexton (1980 b) observed maximal fertility when chicken semen was cooled from +5 to -20°C at a rate of 1°C/min in an alcohol bath, then transferred to liquid nitrogen vapor and cooled to -80°C at 30°C/min before being plunged into liquid nitrogen and cooled to -196°C.

When survival data for several cell types (mouse marrow stem cells and bovine red cells) were plotted against cooling rate or temperature, the curves produced resembled an inverted "U" (Mazur, 1977). Data obtained by Sexton (1980b) indicates that for a given cooling rate their is likewise an optimum temperature range or temperature point for maximal recovery. In addition, Mazur (1977) has shown that the optimal cooling rate, based on cell survival, changes as the level of cryoprotectant changes. Data accumulated on bull sperm, furthermore, indicate an interaction between the level of cryoprotectant and the optimal thaw rate (Robbins, et al., 1976). Highest fertilities have been

achieved with frozen chicken spermatozoa where a slow thaw rate (2 to 5°C) has been used regardless of the freeze rate (Sexton, 1980a, b; Watanabe and Terada, 1976; Lake et al., 1981). Harris (1968) obtained highest fertility with intraperitionally inseminated semen cooled at a rate of 6°C/min. and thawed rapidly at 41°C. Upon reflecting on the many variables and their interactions which influence the survival of frozen semen, it is obvious that only throughcontinued systematic examination can optimum combinations of these variables be obtained.

2. Factors affecting the efficiency of producing fertile hatching eggs via AI.

In order to achieve the maximum level of fertility and hatchability in birds (particularly the chicken and turkey) using AI, attention must be paid to insemination procedures as well as biological factors associated with the hen's oviduct (age and seasonal effects).

3. Fertility as affected by number of spermatozoa inseminated into the oviduct:

To maintain a constant high level of fertility throughout a breeding season, a minimal number of good quality spermatozoa must be inseminated at regular intervals. While the minimum number of spermatozoa required per insemination for undiluted fresh semen has been determined to be 50 million (Kim et al., 1974) it is generally recognized that about 80 to 100 million are necessary to maintain high fertility over an entire breeding period. Sexton (1978) determined the minimum number of chicken spermatozoa required per insemination for frozen-thawed semen to be 300 million indicating that sperm are being damaged by freezing and thawing. Lake (2983) estimated that 50% of fowl spermatozoa are normally destroyed during freezing. In a more recent study by Sexton (1981), it was determined that chicken spermatozoa can also be damaged during dilution and processing for freezing. Considering the above losses, higher absolute numbers of frozen-thawed spermatozoa must be inseminated to produce high levels of fertility.

4. Fertility as affected by timing of insemination:

It is generally recognized that fertility is decreased when hens (chicken) are artifically inseminated within 1-2 hr after or 3-5 hr before oviposition (Lee, 1968a&b), 1970, 1973; Parker and Arscott, 1971; Johnston and Parker, 1970; and Giesen and McDaniel, 1980). Fertility is likewise decreased when turkey hens are inseminated in the last 7-10 hr that the egg is in the uterus and during the approximate time of ovulation (Christensen and Johnston, 1975, 1977). Recent evidence does not support previous explanations that the presence of a hard-shelled egg in the uterus of the hen at or near the time of insemination decreases levels of fertility. Lee (1968a), Parker and Arscott (1971), Johnston (1967) and Giesen and McDaniel (1980) reported that inseminating chicken hens at 2100-0700 hr, when the incidence of uterine hard-shelled eggs is highest has resulted in comparable levels of fertility when compared with hens inseminated from 1500 hr to sunset. What then is the primary cause for the decreased fertility when inseminations from 1500 hr to sunset. What then is the primary cause for the decreased fertility when inseminations are made at or close to the time of oviposition? Is it due to the physical expulsion of the egg and loss of spermatozoa, is it related to the endocrine status of the bird at this time which affects oviduct motility, or is it related to changes in the oviductal environment which may be hostile to spermatozoa? Lee (1973) reported that in high producing and highly fertile flocks of chickens, a fairly high fertility could be obtained by inseminating only 2 to 3 or 1 to 2 hr before oviposition. In other words, a very distinct

difference existed in the relationship of interval from insemination to oviposition and fertility between high producing flocks.

5. Fertility as affected by biological factors associated with the hen's oviduct (age and seasonal effects):

Fertility levels after AI may be influenced by the effect of oviduct environment on transport of spermatozoa, incorporation and retention of their fertilizing ability by the sperm-host glands, and on their activation prior to ovulation and fertilization. An interesting recent observation by McIntyre et al. (1982) indicates that artifically inseminating turkey hens before laying commences resulted in a 7% increase in fertility over an 8 week production period. The difference in fertility may be related to the physiologic receptiveness of the oviduct at the time of insemination as indicated by an increased desire on the part of hens to mate prior to their first oviposition. If this is true, greater numbers of spermatozoa would be expected to be stored and or maintained initially. This same physiologic receptiveness of the oviduct at the time of insemination would appear to be associated in some measure with age or seasonal declines in fertility. In this respect it is interesting that Ogasawara and Fuqua (1972) and Christensen (1981) found fewer utero-vaginal glands filled with spermatozoa in turkey hens during seasonal declines in fertility. In support of the view that the oviduct is less capable of sustaining spermatozoa, Sexton (1977) was unable to alleviate a late season decline in turkey fertility by multiple inseminations.

Many seasonal declines in fertility may be due to a seasonal decline in sperm quality with advancing age of the male, which is accentuated by storage effects (Giesen and Sexton, 1983b). The net result of this possibility would be a reduction in the numbers of viable sperm inseminated late in the season, which would ultimately lead to lower fertility levels. Thus a practical breeder must pay proper attention to the quality of semen and the correct number of spermatozoa for insemination which may very well vary during the course of a breeding period.

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Table 1. Short-term chicken semen preservation

Storage			No. of sperm		
Time	Temp.	Diluent	inseminated	Fertility	Reference
(hr)	(C)		($X10^6$)	(%)	
24	0-2	Diluent A	0.1 ml of a semen mixture diluted 1 : 3 (semen : diluent)	64	Lake (1960)
48	0-2	〃	〃	47	〃
24	2-5	Diluent 1 - without egg albumin	0.10-0.12 ml of a semen mixture diluted 1 : 1 (semen : diluent)	83-95	Van Wambeke (1967)
24	2-5	Diluent 2 - with egg albumin	〃	92-95	〃
24	5	Diluents buffered at pH 6.8-7.1	120-180	87	Lake and Ravie (1979)
48	5	Diluent with glucose buffered at pH 7.1-aerated	159	48-92	Wishart (1981)
48	5	Diluent without glucose buffered at pH 7.1-aerated	159	56-93	〃

Table 2. Short-term turkey semen preservation

Storage			No. of sperm		
Time	Temp.	Diluent	inseminated	Fertility	Reference
(hr)	(C)		($X10^6$)	(%)	
24	10	Diluent with glucose buffered at pH 7.1-aerated	143	81	Wishart (1981)
6	15	Modified BPSE			
		pH 6.5, 280 mosm	275	92	Sexton and Giesen
		pH 6.5, 355 mosm	275	95	(1982)
24	10	Diluent with glucose buffered at pH 7.1	170	62	Lake and Ravie (1982b)
6	15	BPSE	365	91	Giesen and Sexton (1983a)
6	15	MTGA	365	96	
6	15	French	365	94	
6	15	Universal	365	83	
18	5	BPSE	250	82	Giesen and Sexton, (1983b)
18	15	BPSE	250	41	

* BPSE-Beltsville Poultry Semen Extender ; MGTA-Minnesota Extender, Minnesota Turkey Growers Assoc., St. Paul, MN 55114 ; French-I.M.V.-French Extender, I.M.V. International Corporation, L'Aigle, France ; Universal -Universal Medium, Applied Genetics Laboratory, Omaha, NE 68127.

Table 3. Common Constituents of Semen Diluents

Primary Function	Constituent
Energy source	Glucose, fructose, inositol
Osmotic balance	Insured by a variety of ions (i. e. magnesium chloride and acetate; potassium citrate and chloride; sodium acetate and chloride)
Buffer-pH regulation	Phosphates of sodium and potassium, biological buffer salts (i. e. TRIS, MES, BES, TES)
Chelator	Glutamate, albumen, milk
Antibacterial	Gentamycin, penicillin, streptomycin

Table 4. Current status of frozen (−196℃) chicken semen preservation

Cryoprotectant	No. sperm inseminated	AI frequency	Fertility (Days 2-8)	Reference
	($X10^6$)		(%)	
DMSO	50	Weekly	21	Sexton, 1976
DMSO	100	Weekly	32	
DMSO	300	Weekly	59	
Glycerol	−	Single (intra-uterine AI)	74	Watanabe and Terada, 1976
Glycerol	−	Single	80 (Days 2-6)	Lake and Stewart, 1978b
Glycerol	−	Single	65	
Glycerol	−	Every 3 days	93	Lake et al., 1981
DMSO	200	2 consecutive inseminations	71	Williamson et al., 1981
DMSO	100	Weekly	55	Bakst and Sexton, 1979
DMSO	−	2 consecutive inseminations	67 (Days 3-7)	Sexton, 1980a (frozen by a commercial breeder)
DMA	225	3 consecutive insemination	76	Lake and Ravie, 1982a

DMSO − Dimethylsulfoxide ; DMA − Dimethylacetamide.

Table 5. Current status of frozen turkey semen preservation

Cryoprotectant	No. sperm inseminated	AI frequency	Fertility	Reference
	($X10^6$)		(%)	
Glycerol	200 – 250	Single	70 – 74	Marquez and Ogasawara, 1977b
Glycerol	170	Every 4 days	63	Schefels, 1978
DMSO	200	Every 5 days	26 – 50	Sexton, 1980a
DMSO	200	2 consecutive inseminations.	61	Sexton, 1981

DMSO-Dimethylsulfoxide.

Table 6. Methods for freezing chicken semen

Pre-freeze steps	Sexton, 1980a	Lake, et., 1981
1. Diluent	BPSE	Glycerolised diluent (Lake & Stewart, 1978)
2. Dilution rate (semen : diluent)	1 : 5	1 : 3
3. Cooling time and temperature	Cool at 5℃ for 2 hrs.	Cool to 5℃ in 3 to 5 min.
4. Cryoprotectant	DMSO	Glycerol
5. Equilibration time & temperature	2 hrs. at 5℃	Short time for packaging prior to freezing
6. Packaging	5 cc plastic straw	1 cc glass ampule containing .6 cc semen mixture
Freeze steps		
7. Alcohol bath temperature rate	+5° to −20℃ at 1℃/min.	
8. Liquid nitrogen vapor temperature rate	−20° to −125℃ at 50℃/min.	+5° to −35℃ at 1℃/min.
9. Liquid nitrogen temperature rate	−125° to −196℃ at 160° C/min.	−35° to −196℃ at 160℃/min.
Thaw steps		
10. Bath temperature	+2℃	+2° to 5℃