**SZENT ISTVÁN UNIVERSITY**

# EXAMINATIONS FOR THE ELONGATION OF PERSISTENCY OF THE REPRODUCTION CYCLE IN BROILER BREEDER FLOCKS

**Thesis of PhD dissertation**

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**2013**

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# 1. ANTECEDENTS AND OBJECTIVES OF THE WORK

## 1.1. Introduction, importance of the subject

The steadily increasing demand for broiler meat entails the need to increase the number and production level of broiler breeder parent flocks. The objectives of genetic work are primarily determined by the market demands, according to which the most important targets of selection are the excellent carcass yield and the highest possible breast meat yield. However, intensive selection for body weight has negatively affected the reproductive parameters, although the rate of this negative effect varies by breed (*Reddy and Sadjadi, 1990; Brillard, 2009*).

The various management technology manuals recommend to keep broiler breeder parent flocks in production until 61 to 64 weeks of age. In recent years, a drastic decrease in the fertility of eggs has been reported, with the ratio of infertile eggs reaching 15%, which makes it uneconomical to keep the flocks in production until the recommended age. This problem is a cause of concern for the specialists all over the world (*McDaniel, 1986; Creel et al., 1990; Walsh and Brake, 1997; Fragoso et al., 2012*).

The contribution of the male to the fertility of eggs is determined by the quality of sperm and the libido of cockerels, while on the female side the quality of eggs and the sperm storage capacity of the oviduct are the most important factors. In order to avoid excessive weight gain of the males and to stimulate their mobility, the technological specifications recommend a decrease in the number of males as the production cycle advances, thus increasing the sex ratio. Although the results of this technological recommendation fell short of the expectations, it is still in force. Therefore, production companies are forced to adopt the solution to replace some, or possibly all, of the ‘old’ broiler breeder males with younger ones at the middle of the production cycle (at around 40–45 weeks of age). This technique, termed ‘spiking’, extremely increases the production costs and is believed to cause severe distress and a resulting production drop in the flock; moreover, according to the experience gained so far, its success is only temporary, which makes its use questionable.

For the above reasons, it would be urgent to conduct research to determine whether or not it would be possible to prolong the persistence of fertility, i.e. to increase the number of fertile eggs per hen to an economical level, or whether the current genetic possibilities would limit these efforts. In our studies, we used novel-type fertility tests that provide a wider range of information than the standard methods.

## 1.2. Objectives

a) To study the effect exerted by different technological changes (sex ratio, replacement of males) affecting breeding efficiency on the persistence of fertility.
b) To investigate the effect of feed additives containing organic selenium + vitamin E on the reproductive functions of both sexes.
c) To study the effect of synthetic GnRH pretreatments on increasing the fertilising ability (libido, sperm quality) of males and on the fertility of the flock.
d) To study the effect of additional artificial inseminations on the persistence of fertility in the second half of the production cycle.

## 2. MATERIALS AND METHODS

Our experiments were carried out on the area of the current Centre for Farm Animal Gene Conservation, formerly called Institute for Small Animal Research and Co-ordination Centre for Gene Conservation, with a Ross 308 broiler breeder parent stock.

### 2.1. Study of the effect of different sex ratios and male replacements

The basis of these experiments was the assumption that the fundamental cause of fertility problems was the low sperm count or the deficiency of sperm in the female reproductive tract. In these experiments we used groups of different sex ratios, and thus we studied the effect of male number on fertility. The effect of male age was investigated by the replacement of old males with young ones at different ages and ratios ('spiking'), the phenomenon of 'losing interest' was studied by exchanging males between two groups and by decreasing the sex ratio. Fertility was checked by candling and by the examination of freshly laid eggs (*Perivitelline Sperm Penetration Assay, PSPA*) during the production cycle, between 27 and 59 weeks of age. We determined the 'true' fertility (TF%), i.e. the rate of very early embryonic mortality, and monitored the hatching data. By ethological observations we monitored how the age of males influenced the mating frequency.

### 2.2. Study of the effect of organic selenium and vitamin E in the male sex

For this experiment, 20 Ross 308 males were placed into individual cages. Males belonging to the experimental group (n = 10) received 0,3 mg Sel-Plex (Alltech Ltd.) and 200 mg Lutavit E 50 S (BASF) per kg of diet, while the control birds were fed an inorganic selenium source usually contained by premixes and 100 mg vitamin E per kg of diet. After training the males in semen collection, we took semen twice a week by the *dorso-abdominal* massage technique described by *Burrows and Quinn (1935)*. Semen production and sperm quality were tested once a week between 31 and 61 weeks of age. Qualification of semen was done by macroscopic inspection and microscopic, single-function tests. At the end of the reproduction cycle we took tissue samples from the testicles for histological examination.

### 2.3. Study of the effect of organic selenium and vitamin E in both sexes

Four experimental groups containing 80 females and 8 males were formed. In all groups, the sex ratio was changed according to the technological recommendations, i.e. the number of males was reduced in weeks 36, 40 and 44 of life. Thus, the control group produced with a male number corresponding to the technological specifications and was fed a diet without selenomethionine supplementation. In addition to the control group, three experimental groups were formed: in the first only the males, in the second only the females while in the third birds of both sexes received 0,3 mg/kg Sel-Plex® (*Alltech Ltd.*) and 200 mg/kg Lutavit E 50 S (*BASF*) mixed in the diet, from week 20 of life until the end of production. In addition to monitoring the production parameters and performing the examinations during the male manipulations, we collected histological samples from the uterovaginal and infundibular portions of the oviducts and from the testicles, at different ages. To demonstrate the protective effect of organic selenium against stress, we performed steroid analyses in faecal and egg yolk samples.

### 2.4. Study of the effect of synthetic GnRH pretreatment in the male sex

To mitigate the sperm quality deterioration occurring in the second half of the production cycle and to improve the reduced libido of males, we performed treatment with a synthetic GnRH product (*Ovurelin, Reanal*) in the sensitive phase of maturation. At 20 weeks of age, 40 ROSS 308 cockerels were placed into individual cabinets provided with artificial illumination. The time of the GnRH treatments was selected on the basis of the development of secondary sex characteristics rather than on the basis of age. Males of Groups *GnRH1*, *GnRH2* and *GnRH3* were treated with a synthetic GnRH analogue administered by intramuscular injection in a dose of 5 mg/male/day three times a week, every other day, at 23, 25 and 42 weeks of age, respectively. Training in semen collection was started at 26 weeks of age, and then the qualification of semen samples commenced at 28 weeks of age. Between 28 and 32 weeks of age, semen samples were qualified twice a week and then once a week, but semen samples were taken twice a week on a continuous basis. During the qualification of semen samples, the volume of semen, sperm concentration, sperm motility, and the ratios of live-intact sperm, morphologically abnormal sperm and dead sperm cells were determined.

### 2.5. Study of the effect of synthetic GnRH priming on fertility

The effect exerted by a synthetic GnRH analogue administered at different stages of sexual maturation on fertility and sexual behaviour was studied also in flocks using natural mating. Three experimental groups, each comprising 80 females + 8 males, were formed. Ross 308 males of the different experimental groups were treated with a synthetic GnRH analogue administered by intramuscular injection in a dose of 5 mg/male/day three times, every other day, at 21, 23 and 25 weeks of age, respectively. In addition to recording the production parameters and the examination of eggs (PSPA), ethological observations were also made.

### 2.6. Study of the use of additional artificial insemination

To confirm the hypothesis that the infertility problem of broiler breeders is primarily due to the reduced number of matings and the increased emptying of sperm from the oviduct of layers, we considered it necessary to study the effect of using additional artificial inseminations. The inseminations were started at the time when, based on the results of prior egg examinations by PSPA, sperm transport began to decrease in the females. Our objective was to try to counterbalance the faster rate of sperm emptying by the administration of extra sperm through additional artificial inseminations. A group comprising 60 females with the males belonging to them was used in this experiment. The group produced entirely according to the recommendations specified in the technology (amount of feed, light, number of males, etc.). Additional artificial inseminations were started in the layers' 45th week of life, using semen from young cockerels. Insemination was performed once a week, always on the same day, in the early afternoon hours. The inseminating dose was 200–300 million sperm cells/layer/week between weeks 45 and 55, and thereafter it was increased to 400–500 million/layer/week. The original 'old' males of the group remained in the flock; thus, these additional artificial inseminations indeed served only as a support to the natural matings.

We monitored the production parameters, the effect of stress caused by the insemination, and also the changes of sperm population in the layers' oviduct (PSPA).

# 3. RESULTS

## 3.1. Study of the effect of different sex ratios and male replacements

The objective of this experiment was to determine how the decrease of breeding efficiency occurring after 50 weeks of age could be prevented by changing the sex ratio or by the use of male replacement techniques (‘spiking’). We divided the study period into three parts (first part: weeks 27–37 of life, second part: weeks 38–48 of life, third part: weeks 49–59 of life), so that we could follow also the changes within the different groups.

The most intensive sperm transport and the highest fertility results were found between 31 and 40 weeks of age, and thus peak production also occurred in that period. In the period characterised by a downward trend of production, both the number of penetration (IPVL) holes and the fertility results decreased significantly in the technological control group, in the group with a 100% male replacement ratio and also in the groups between which the males were exchanged. In groups where 50% of the ‘old’ males were replaced by young ones and the number of males was increased, the sperm transport improved; however, this caused only a temporary improvement in the fertility results. In the last third of production, the control group showed a significantly more intensive sperm transport only in comparison to two groups (i.e. the groups between which the males were exchanged). In the third period, i.e. the period most interesting for us, the fertility results determined by candling and the ‘true’ fertility data showed a similar trend.

During the further examination of eggs discarded after candling, the samples were divided into three categories. The ‘truly’ infertile category included eggs in which embryonic cell division could not be detected at all. The second category comprised eggs with embryos detected by propidium iodide (PI) staining, those which had died already in the oviduct. We also determined the phenotypes of embryonic mortality taking place in the first week of incubation; however, the individual phenotypes occurred in low numbers, and there was no substantial difference between the groups. Therefore, we considered it better to combine these phenotypes and evaluate them collectively as embryonic mortality occurring in the first week of incubation.

Comparing the results obtained in the different groups by category to those of the control group, it can be stated that no difference was found in the ratio of embryonic mortality within the oviduct. However, in the groups between which the males were exchanged, the proportion of embryos dying in the first week was significantly ($p \leq 0,05$) higher (8,16% and

7,95%, respectively) than in the control group (5,07%). In the production phase most interesting for us, i.e. in the third period, eggs containing embryos that had died in the first week of incubation occurred in the highest proportion in the groups with male replacements. As compared to the control group, the group with 100% male replacement ratio contained significantly ($p \leq 0,05$) higher numbers of embryos dying in the oviduct.

During the ethological observations, we studied the number of matings per cockerel. In the 44th week of life, immediately after the male replacements, the highest number of matings was shown by the young cockerels added to groups with a 50% spiking ratio (13,3 matings/cockerel), which was probably induced by competition with the 'old' males that remained in the group. The second highest number of matings was observed in the group in which 100% of the 'old' males were replaced with young cockerels (8,6 matings/male). In terms of mating, the young cockerels in the group of mixed male age composition were the most active even after one week (15,3 matings/male); however, in this group the 'old' males also showed intensive mating activity (12 matings/male). As compared to the previous week, the sexual activity of young cockerels in the group with 100% male replacement ratio increased as well (14 matings/male). In the 52nd week, the lowest number of matings (4,3 matings/male) was recorded for the 'old' males of the group with 50% spiking ratio, which may be attributed to the fact that the so-called 'old' males got exhausted in the struggle for the hens and the feed. The highest mating activity (with 9 matings/male) were shown by the young cockerels of this group.

### 3.2. Results of the semen analyses

We studied the effect of 0,3 mg/kg Sel-Plex® (*Alltech Ltd.*) and 200 mg/kg Lutavit E 50 S (*BASF*) in the male sex. Like in the previous part of the work, the period of study was divided into three parts also in this case, so as to determine whether the quality of semen declines with the advancement of the production cycle in a degree that would explain the observed reduction in breeding efficiency.

In the control group, we did not observe a semen quality decline of an extent that could explain the severe decrease of fertility with the advancement of the production cycle. However, the administration of selenomethionine and vitamin E could still improve the values of the semen parameters, as it significantly increased the motility of spermatozoa ($p \leq 0,01$), the sperm cell concentration ($p \leq 0,05$) and the proportion of live, intact spermatozoa ($p \leq 0,01$). Parallel to this, the ratios of abnormal and dead spermatozoa were lower in the samples than

in those of the control males. A further non-negligible result was that the feeding of selenomethionine and vitamin E decreased the incidence of secondary abnormalities in the samples, indicating an increased resistance of spermatozoa to environmental factors.

On the basis of the histological results it can be stated that selenomethionine and vitamin E had a favourable effect on the histological parameters of the testis and thus also on spermatogenesis, which is in harmony with the findings of the spermatological examination.

### 3.3. Study of the effect of organic selenium and vitamin E in both sexes

The control group left without selenomethionine and vitamin E supplementation showed good fertility results, although at the end of the cycle the values of 'true' fertility and those of fertility determined by candling tended to diverge, indicating a slight increase in early embryonic mortality. In the group in which only the layers received selenium supplementation, sperm transport was on a relatively high level throughout the cycle, and thus the fertility values were also relatively high. In the other group, only the males received a diet supplemented with selenomethionine. Despite the fact that in the previous experiment we had demonstrated a significant improvement in semen quality for the treated males, this was not reflected in the results of the group study. On the contrary, all the parameters studied by us were the worst in this group. Although we replaced the males with young ones during the production cycle (in the 46th week of life), we could not improve the performance of the group. When both sexes received selenomethionine and vitamin E supplementation, the number of penetration holes in the membrane significantly decreased ($p \leq 0,01$) in the final third of the production period also in this case. We obtained good fertility results also in this group; however, the changes of fertility during the production cycle coincided with changes in the sperm transport. Comparing the groups, it can be established that at the beginning of production sperm transport was the most intensive in the control group, but subsequently, at the time of peak and declining production this difference was equalised, with the exception of the group in which only the males received selenomethionine. Regarding the two types of fertility measurement, it can be stated that at the beginning of production fertility was significantly higher in the control group than in the selenium-supplemented female ($p \leq 0,05$) and male ($p \leq 0,01$) groups; however, this difference was later levelled up for the selenium-supplemented layer group. Although the layers responded to the administration of selenomethionine and vitamin E to some extent (compensating for the better performance

observed in the control group), even so no significant improvement in fertility could be demonstrated.

By further examination of the eggs discarded during candling we found that the number of eggs truly not containing an embryo tended to increase in all groups during the production cycle. The incidence of embryonic mortality within the oviduct was on a very low level throughout the production cycle and it did not show a notable change. However, a significant ($p \leq 0,05$) increase in the number of embryos dying during the first week of incubation was demonstrated only in the control group in the last third of the production cycle. Therefore, like its beneficial influence on the fertility data, selenomethionine was found to exert a favourable effect of a certain degree by counteracting the early embryonic mortality that tends to increase at the end of the production cycle.

Based on the corticosterone values measured in the egg yolk, it can be stated that the proportion of eggs containing a dead embryo increased in parallel to the elevation of corticosterone level. However, in the last third of the production cycle there was a certain decrease in the embryo mortality as compared to the control group, despite the fact that high glucocorticoid levels could be measured in the egg yolk. According to our hypothesis, selenium entering the egg yolk may have a beneficial effect on embryogenesis; thus, the feeding of selenium at the end of the cycle decreases the rate of embryonic mortality by counterbalancing the adverse effects of the stress hormone. The testosterone content of the yolk is the highest in the last third of the cycle, when this elevated testosterone level may have an anti-stress effect, as higher androgen content is known to be capable of counterbalancing the effect of corticosterone to a limited extent.

### 3.4. Study of the effect of synthetic GnRH pretreatment in the male sex

According to our spermatological examinations, synthetic GnRH treatment performed at the beginning of maturation exerted a significant positive effect on sperm concentration ($p \leq 0,01$) and on the motility of spermatozoa ($p \leq 0,05$), and thus it resulted in higher overall sperm quality; however, GnRH treatments performed later did not have such effects. Thus, the studies performed on individual males have demonstrated that the application of synthetic GnRH analogues in the initial period of maturation (usually at 21–23 weeks of age in the case of broiler breeders), at the concentration and with the frequency used in this study, can influence semen quality, which was manifested in higher sperm concentration and better sperm motility. If synthetic GnRH treatment is used at a later stage of maturation or in a

sexually mature age, this positive effect will be less expressed or completely absent. The possible negative influence of elevated corticosterone level on spermatogenesis may explain the observation that the overall incidence of sperm abnormalities and, within the former, the incidence of primary sperm abnormalities of testicular origin was higher in the group that showed the best production in all respects (*GnRH treatment in the 21st week of life*).

### 3.5. Study of the effect of synthetic GnRH priming on fertility

The results of the previous experiment indicate that synthetic GnRH treatment resulted in the improvement of some spermatological indices but, despite this fact, both the sperm count and the fertility values significantly decreased ($p \leq 0,05$ and $p \leq 0,01$, respectively) by the end of the production cycle. By comparing the results of the different groups, it can be stated that the control group showed the highest values at the onset of production. In the second third of the production cycle, there was no significant difference in sperm transport as compared to the control group. Moreover, the area under the curve decreased the least (by 0,12%) in the group treated in the 23rd week of life. However, in the last third of the production cycle only the group treated in the 21st week of life was not significantly inferior to the control group in terms of the number of penetration holes. While in the first third of production there was no significant difference between the groups in terms of either of the two fertility indices, in the last third of the production cycle both fertility indices were significantly higher in the control group ($p \leq 0,01$).

By studying the embryonic mortality it can be established that the incidence of embryonic mortality in the oviduct was very low (0,05–0,19%) and did not change markedly during the production cycle. In the last third of the production cycle, the proportion of embryos dying in the first week of incubation significantly increased in the control group ($p \leq 0,05$), and it also tended to rise in the other groups, although this increase was not significant. At the beginning of production, embryo mortality showed the highest incidence in the groups, while by the end of the production cycle the proportion of absolute infertile eggs had increased markedly. Comparing the results of the treated groups to those of the control group, it can be established that in the critical last third of the production period the number of eggs not containing an embryo was significantly lower in the control group ($p \leq 0,01$).

Based upon the results presented above, we can state that the GnRH treatments used in this experiment, especially those given at later stages of maturation (in the 23rd and 25th weeks of life), did not have a positive effect in increasing the persistence of fertility. From the

50th week of life onwards, both the sperm counts in the eggs and the fertility values decreased drastically. Of the groups treated with GnRH, the group of males treated at 21 weeks of age showed the best results; however, a significant improvement as compared to the control group could not be achieved by this treatment either.

Our ethological observations indicate that the young cockerels showed the most intensive mating activity in the periods monitored by us. However, in the last period of the production cycle the mating activity increased again, but this was not, or only belatedly, reflected in improved fertility. Of the GnRH treatments, early GnRH treatment performed in the 21st week of life proved to be the most effective; however, this was not reflected in an improvement of the production parameters, as shown also by the results presented above.

### 3.6. Study of the use of additional artificial insemination

In this experiment our objective was to try to compensate for the accelerated emptying of sperm from the oviduct by the supply of extra sperm through additional artificial inseminations.

In the experimental group, sperm transport and, as a result, the fertility values were very low already in the first third of the production cycle. As opposed to the control group, in the experimental group the sperm transport and the fertility values did not decrease further in the second third of production as compared to the first one, but in the last third of the cycle they decreased significantly ($p \leq 0,01$) despite the additional artificial inseminations applied. As a result of the low initial sperm transport in the experimental group, at the beginning of the cycle the production results of the control group significantly ($p \leq 0,01$) exceeded those of the experimental groups given additional artificial inseminations. However, in the middle third of the production period the sperm transport not only did not decrease but even increased in the experimental group, and thus in this period we did not find a notable difference between the two groups. However, this was not reflected in the fertility results, which were significantly better in the control group ($p \leq 0,05$). In the last third of the production period, the fertility results of the experimental group were markedly lower than those of the control group.

Studying the incidence of early embryonic mortality and ‘truly’ infertile eggs, we found that changes in the number of eggs not containing an embryo at all coincided with the results of the membrane examinations and the fertility results. While in the control group embryonic mortality showed higher incidence, in the experimental group infertile eggs occurred in the highest number at the beginning of production. In the second third of the production cycle,

both the number of infertile eggs and the proportion of embryos dying in the oviduct decreased to some extent. In the last third of the production period, both the number of infertile eggs and the number of embryos dying in the first week of incubation increased significantly ($p \leq 0,05$) in both groups. By comparing the two groups, however, it can also be seen that while there was no significant difference between the two groups in terms of the embryonic mortality (9,15% vs. 9,63%), the number of truly infertile eggs was almost twice as high in the experimental group with additional artificial insemination (8,54% vs. 15,29%; $p \leq 0,01$). It was surprising even for us that the use of artificial inseminations did not improve either the fertility or the sperm transport, despite the fact that 300–500 million extra spermatozoa were introduced into the oviduct of layers weekly.

To study the potential stress caused by procedures associated with artificial insemination, after each insemination we collected faecal samples to determine the concentrations of the steroid hormone corticosterone. In addition, on the second day after insemination we determined the changes of steroid concentration in fresh egg yolk samples. A significant increase in corticosterone concentration ($p \leq 0,05$) occurred on day 2 after the first artificial insemination, then in weeks 46–47 no further increase was seen. The even so slightly elevated stress hormone level tended to decline from the 49th week of life and then became stabilised on a lower level; however, the periodic spikes seen on the concentration curve indicated that stress was continuously present and older birds found it difficult to adapt to it.

## 4. CONCLUSIONS AND RECOMMENDATIONS

### 5.1. Study of the effect of different sex ratios and male replacements

The replacement of 'old' males with young ones, the so-called 'spiking' technique, is widely used in the practice to increase fertility in Ross 308 broiler breeder flocks, despite the fact that the technological recommendations include only a reduction in male numbers. In previous studies we established that 17–25% replacements of 'old' males with young ones, as used by the producers in weeks 42–44 of production, failed to result in improved flock fertility (*Végi et al., 2007*).

During our work, we successfully applied for fertility monitoring the method developed by *Staines et al. (1998)*, which is based on studying the number of hydrolysed points produced by spermatozoa in the inner perivitelline layer over the germinal disc (IPVL holes) when they penetrate the ovum.

On the basis of the results, and in harmony with the findings reported by several authors earlier (*Bramwell et al., 1996; Hazary et al., 2000; Wishart et al., 2004*), sperm transport reaches its peak between the 30th and 40th week of life, and decreases thereafter. This decrease may be due to the age-related increase in the rate of emptying of spermatozoa from the sperm storage tubules (*Brillard, 2009*) or the decrease in sperm storage capacity, as supported by the findings of histological examinations (*Yoshimura et al., 2008; Barna et al., 2009*).

In the last, critical third of production, only in two cases could we achieve an increase in the number of spermatozoa in the egg: in the case of 50% male replacement and when the number of males was increased. In the case of *50% male replacement* this can be explained by the fact that, according to the ethological observations, the number of matings was the highest in this group, as the presence of young cockerels stimulated the old males to increased mating activity. However, the fertility rates did not improve despite the increasing sperm transport. *Increasing the number of males* also had a positive influence on sperm transport, which seems to be logical; however, producers tend to reject this practice in order to spare the layers and reduce the aggression of cockerels. In our own studies, we did not observe either increased male aggression or layer 'overstraining'. However, nor was there a significant increase in fertility rates as compared to the control; therefore, we cannot recommend this method either for use in the practice, although it would be a less expensive solution than the rearing of young cockerels. When *the number of males was kept on the same level*, neither the number

of penetration holes in the egg nor the fertility rate changed as compared to the technological control group. At the start of production, the most intensive sperm transport was observed in the group where 100% of the 'old' males were replaced with young ones, and this sperm transport appeared to be intensive despite the reduction of male numbers. When 100% spiking was performed in mid-production, we could not keep sperm count in the egg on a high level in the second and the last third of production, and thus the fertility rate also decreased. In the case of 100% and 50% male replacements, embryonic mortality was more common in the last third of production than were infertile eggs. From this result we concluded that the presence of higher numbers of spermatozoa in the female reproductive tract in the second half of production is not reflected in the hatching results. Consequently, the female is much more responsible for the fertility problems than the male, as has been suspected by others on the basis of earlier works. One of the first such studies was the work of *Pierson et al. (1988)*, who demonstrated in Hubbard broiler breeders that at 85 weeks of age spermatozoa were detectable in a higher number of sperm storage tubules and there were less tubules unsuitable for the storage of sperm than at 125 weeks of age. Subsequently, it was also established that the shortened persistence of fertility with age was not due to the reduced storage capacity of the sperm storage tubules but rather to the accelerated emptying of spermatozoa from the tubules (*Brillard, 1993*). In contrast, from their studies *Gumulka and Kapkowska (2005)* have drawn the conclusion that with the ageing of hens less sperm can be stored in the oviduct, and suggested this as a potential reason for reduced fertility. During their experiment with Arbor Acres broiler breeders, *Bramwell et al. (1996)* found more IPVL holes in the eggs when young hens were mated by old cockerels than when old hens were mated by young cockerels, with the same amount of sperm. As a result, the necessity of using male replacements was questioned already at that time (*Pierson et al., 1988; Brillard, 1993; Bramwell et al., 1996; Gumulka and Kapkowska, 2005*).

***Recommendations regarding male replacements***

If a farm insists upon the use of spiking at all events, the proper time and rate of spiking should be decided separately for each house. However, from the results presented above we could see that a more intensive sperm transport is not necessarily reflected in better hatching results. By counting the penetration holes made by the spermatozoa, i.e. by the perivitelline layer examinations used also by us, the decrease of candling fertility can be predicted about 6–8 weeks in advance. By the repeated examination of freshly laid eggs, the

expected time of candling fertility decrease can be determined. The results of our studies indicate that the increase of sperm transport occurs about 3 weeks after young cockerels are placed into the flocks, as that much time is needed for the adaptation and strengthening of the young cockerels and for the development of new harems. This allows us to accurately determine the proper time of spiking. In our experience, in a flock of average production level the amount of spermatozoa in the eggs starts to decrease from the 40th week of life; thus, a decrease in candling fertility can be expected at around week 48. Thus, if we replace at least 30% (!) of he old males with young cockerels in week 46, then an increase in the amount of spermatozoa, somewhat slowing down the decrease of candling fertility, can be expected from week 51. On the other hand, if we wish to maintain the hatching rate above 80–85% up to week 60, it would be better to wait until week 50 with the spiking. Further investigations should be conducted to support the above conclusions, as during our work it was impossible for us to test all variations.

It can be stated, however, that the 15–25% male replacements used in the practice, which are always performed before week 44, have no benefit at all. It is quite clear that it is useless to perform spiking before week 48 in an average flock.

### 5.2. Analysis of the results of studies aimed at the male sex

On the basis of control semen analyses performed at weekly intervals throughout the production cycle, we established that in the ROSS 308 hybrid the sperm concentration markedly decreased between weeks 40 and 45 while the proportion of live, morphologically intact spermatozoa between weeks 42 and 47. However, this decrease was transient, and after week 50 higher values were detected again. Contrary to this, the experiments conducted by *Fragoso et al. (2012)* with the Cobb 500 hybrid have shown that sperm production increases up to the 36th week of life and decreases continuously thereafter. In an experiment carried out by *Gumulka et al. (2005)* with Arbor Acres broiler breeders, ejaculate volume and sperm concentration decreased approximately by 15,7% with advancement of age. Our own earlier studies also demonstrated that genetically different breeds or hybrids might show differences in semen-producing ability and semen quality, as well as in the responsiveness of these traits to external influences (*Végi et al., 2007*). However, our results obtained with the ROSS 308 hybrid indicate that in the last third of the production cycle the volume of semen did not decrease or it even increased slightly, and sperm concentration decreased only by 8,4% on

average. These results do not show a deterioration in semen quality of such a high degree that would explain the marked decrease of fertility with advancement of the production cycle.

Numerous studies have demonstrated that selenium deficiency results in reduced motility of spermatozoa and an increased percentage of abnormal sperm cells (*Wu et al., 1979; Surai et al., 2006; Sanches-Gutierrez et al., 2008*). The lasting deficiency of selenium has a negative effect on sperm concentration and fertilising ability (*Edens and Sefton, 2009*). In the Hubbard hybrid, *Edens (2002)* demonstrated that the administration of selenium resulted in an increased percentage of live, morphologically intact spermatozoa and a decreased incidence of certain morphological abnormalities. In our own experiment, organic selenium administered in combination with vitamin E successfully maintained the sperm quality of ROSS 308 cockerels on the initial high level throughout the production cycle; in contrast, in Hubbard cockerels organic selenium and vitamin E supplementation could not produce further improvement in the parameters of semen which was of high quality already at the outset (*Végi et al., 2007*). Selenomethionine and vitamin E supplementation significantly improved the motility and concentration of spermatozoa and the percentage of live, morphologically intact sperm cells as compared to the control samples. The incidence of secondary abnormalities decreased, indicating that the spermatozoa became more resistant to external influences. The results of histological examinations indicated an increase in the intensity of spermatogenesis.

Synthetic GnRH analogues are commonly characterised by high receptor binding affinity, resulting in a longer duration of action as compared to non-synthetic hormones. Thus, their ability to induce a more intensive release of gonadotropic hormones and their behavioural and physiological effects make them suitable to treat reproductive hypofunction (*Myamoto et al., 1983; Skarin et al., 1984*). A GnRH analogue implant has been reported to increase sexual activity and fertility in the budgerigar (*Costantini, 2009*). *Alavi (2012)* successfully increased the motility of spermatozoa by GnRH treatment in sterlet. In mammalian species, GnRH treatment has produced variable results. In dogs, *Kawakami (2012)* applied GnRH for therapeutic purposes, and reported improved sperm concentration and motility. On the other hand, in ponies treated with GnRH *Boyle (1991)* did not find any significant change either in semen quality or in sexual activity. So far, the effect of GnRH treatment on semen quality and, through that, on fertility indices and sexual behaviour has not been studied in broiler breeders. We have established that GnRH treatment can improve the sperm parameters if it is performed at a given, initial stage of sexual maturation, within a

narrow time window, which should be determined carefully but can be judged on the basis of the external sexual morphological characteristics. In broiler breeders, this time window is mostly between weeks 21 and 23 of life. Naturally, the time of onset of sexual maturation can be markedly influenced by the rearing conditions and the subsequent management and feeding. Experiments with individual cockerels have shown that the use of a synthetic GnRH analogue at the given concentration and frequency in the initial period of sexual maturation can influence the quality of semen, which is reflected in higher sperm concentration and better sperm motility. However, if this intervention is applied in a later stage of maturation or at a sexually mature age, this favourable effect will be less expressed or entirely absent. The negative influence exerted on spermatogenesis by the corticosterone level elevated by the hormonal intervention may explain the observation that the overall incidence of sperm abnormalities and, within the former, the incidence of primary sperm abnormalities of testicular origin was higher in the group that showed the best production in all respects (*GnRH1*).

***Recommendations for improving the performance of males***

In our opinion, the administration of organic selenium (selenomethionine) and vitamin E supplementation in the concentration used in these studies is justifiable and advisable both in the rearing period and during the production cycle, as its beneficial effect on spermatogenesis is unquestionable. If there is interest from the producers, it would be worth testing the use of GnRH for the treatment of males in large flocks and, provided that the results are favourable, considering the use of GnRH as a substitute for spiking techniques.

### 5.3. Analysis of the results of studies aimed at the female sex

Research results published in the literature indicate that organic selenium and vitamin E supplementation increases not only the rate but also the persistence of fertility (*Agate et al., 2000; Breque et al., 2003; Surai et al., 2006*). It has also been established that a large proportion of selenium is present in the egg in the form of selenomethionine, and the embryo cannot produce it (*Surai et al., 2006*). This also underlines the importance of selenium in the feed. It should be emphasised that only organic selenium can be incorporated into the egg in an efficient way. Administering organic selenium supplementation to Ross 508 hybrid birds from 22 weeks of age, *Renema (2004)* found that egg production between weeks 49 and 58 of

life was about 8% higher than in the control group. The number of IPVL holes also increased, indicating the presence of a higher number of spermatozoa in the egg, while late embryonic mortality after the peak of egg production decreased in the group receiving selenium supplementation.

On the basis of our results, organic selenium and vitamin E supplementation provided from the 20th week of life did not improve either the egg production or the fertility rate, and did not result in the presence of more spermatozoa in the egg. The favourable effect of this supplementation was that in eggs originating from the last third of the production period the percentage of embryonic mortality in the first week of incubation was significantly lower and the hatching rate relative to fertile eggs was significantly higher in the treated group. In the last third of production, the number of unhatched eggs was about 3% lower, although this difference was not statistically significant. It is a known fact that in the yolk of eggs containing dead embryos elevated corticosterone concentrations can be measured, which hinder embryonic development (*Biczó et al., 2004; Janczak et al., 2006; Ferencziné Szőke, 2008*). This was demonstrated also during our present work; however, this deleterious effect on the embryos was partially compensated by the selenomethionine content of the egg. It has been established earlier that organic selenium fed to layers appears not only in the egg but also in the tissues of hatched day-old birds, and that flocks fed supplemental organic selenium had lower mortality rates than control flocks without such selenium supplementation (*Surai and Fisini, 2012*). Flocks receiving organic selenium supplementation are expected to perform on a higher level also later on.

The differentiation and structure of the sperm storage tubules during sexual maturation have been studied in detail (*Bakst, 1992; Holm and Ridderstale, 2002*). It is also a well-established fact that the sperm storage capacity of hens depends on their age (*Bakst et al., 1994*). To the best of our knowledge, the structural changes of sperm storage tubules with the advancement of age have not been studied yet. The results of our studies prove that the oviduct undergoes substantial morphological changes during the production cycle. Within these changes, a phenomenon of particular importance for our studies is that in weeks 54–60 of the cycle the sperm storage tubules become dilated, assume a looser structure, and a large volume of secretion is accumulated in many of them. This may indicate that with the ageing of layers not only the emptying of spermatozoa from the tubules is accelerated, which is known from data of the literature (*Brillard, 1993*), but the ability of the sperm storage tubules to accept and store sperm also decreases. This may be one of the possible explanations for the observation that even if we increase the sperm count at the end of the cycle, only a small

proportion of the extra spermatozoa can be deposited in the sperm storage tubules of the oviduct.

We studied the beneficial effects of GnRH treatment on male birds also in a production experiment conducted with groups of birds. Although GnRH treatment performed in week 21 of life tended to enhance the intensity of the weak sperm transport of the group, this was not reflected in improved fertility rates. According to our hypothesis, this is due to the fact that improvement of the 'sexual quality' of cockerels is in itself not sufficient for increasing the fertility rate, and that layers seem to have a more important role in influencing the fertility. It cannot be stated in general that GnRH treatment consistently increases the number of matings, as an increased mating activity could not be observed at all times of observation. In the control group, the number of matings was the lowest in week 44 of life, while in the treated group such a nadir did not occur, indicating the favourable effect of GnRH treatment. This difference was reflected in sperm transport in the following week, which resulted in a 2% improvement in fertility; however, that improvement was transient and failed to be translated into higher fertility rates in the last third of the production cycle.

The emptying of the sperm storage tubules is accelerated with the advancement of the production cycle (*Brillard, 1993*), which leads to the conclusion that more spermatozoa are needed for the maintenance of fertility. Therefore, we assumed that the amount of spermatozoa stored in the sperm storage tubules could be increased by the complementary use of artificial insemination, and thus the fertility of eggs could be maintained. Although *McCartney and Brown (1976)* obtained the highest fertility rates when they combined natural mating with artificial insemination, in our experiment the results obtained by the complementary use of artificial insemination fell short of the expectations. Instead of increasing, the sperm count radically decreased, and parallel to this the fertility rates also declined. The hormone analyses demonstrated that the use of artificial insemination caused a detectable stress in the birds. It has been proved earlier that exposure of the mother to stress elevates the corticosterone content of the egg yolk, which results in increased embryonic mortality (*Biczó et al., 2004; Janczak et al., 2006; Ferencziné Szőke, 2008*). In our study, in addition to an overall increase in embryonic mortality the percentage of eggs not containing an embryo at all also rose. *Koohpar et al. (2010)* did not obtain higher fertility and hatching rates by the use of artificial insemination than by natural mating, and attributed this finding to the stress occurring during the insemination. It can also be presumed that high corticosterone level affects the deposition of spermatozoa into, and their emptying from, the storage tubules. Naturally, further investigations would be needed to prove this hypothesis. It should also be

studied further whether organic selenium can counterbalance these adverse effects through its protective action against stress.

***Recommendations for improving the performance of females***

All the results obtained during our studies indicate it is much easier to influence the decreased sexual functions in males by different methods (organic selenium and vitamin E supplementation, GnRH treatments), and it is also much easier to examine these functions. However, according to the results of the present studies, these interventions alone are not sufficient for achieving a significant improvement of fertility.

As in males, supplementation with organic selenium and vitamin E is recommended also in the females not only during the production period of broiler breeders but already in the rearing phase.

It has been demonstrated clearly that the complementary use of artificial insemination has many negative implications in broiler breeder parent flocks, primarily because of the stress effects involved. The birds could not get used to the hassle associated with this procedure performed once a week (separation and collection of layers, eversion of the cloaca, insemination); therefore, the use of this practice is not recommended.

The favourable effect of GnRH experienced during earlier spermatological analyses did not manifest itself in the group studies. For lack of financial resources we could not repeat the experiment in a larger flock. However, we do not exclude the possibility that an intervention of this type may help increase the persistence of fertility.

All the studies performed by us indicate that the fertility decrease occurring in the last third of the production cycle can be attributed decisively to the impaired reproductive-physiological functions of the females. These latter cannot be influenced by external factors such as nutritional, hormonal or spermatological interventions. In our opinion, genetic selection aimed at increasing the number as well as the storage and holding capacity of the sperm storage tubules may be a possible solution to increase the persistence of breeding efficiency on the female side.

## 5. NEW SCIENTIFIC RESULTS

1. On the basis of the data obtained during my work, manipulation of the sex ratio, the male replacements and the complementary use of artificial insemination caused only a temporary or no significant fertility improvement in the critical period of production. As a conclusion, it cannot be demonstrated that the expensive and labour-intensive male replacements (spiking) would have any positive effect on the fertility results.

2. I performed the semen qualification of ROSS 308 cockerels during a complete production cycle, by analyses performed weekly on a continuous basis. So far, no data have been available about this in the literature. On the basis of the results, I established that the deterioration of semen quality in the last phase of production is not reflected in a fertility rate decline of an extent that would justify the replacement of either the cockerels or the entire flock.

3. I have demonstrated that, in addition to significantly improving sperm motility and concentration, selenomethionine and vitamin E supplementation provided to the males also reduced the incidence of secondary sperm abnormalities. Despite this fact, organic selenium and vitamin E supplementation did not result in a marked improvement of fertility. However, I could demonstrate the embryo-protective effect of this supplementation, attributable to the incorporation of selenium into the egg yolk, and potentially associated with the steroid hormone concentrations as well.

4. With the advancement of age, pronounced histological changes occur in the sperm storage tubules of the oviduct, which support the age-related decrease in sperm storage capacity. Selenium did not have a detectable effect either on morphological changes of the oviduct or on sperm storage.

5. Synthetic GnRH analogue treatment (5 mg/cockerel/day) performed at the beginning of sexual maturation had a favourable effect on semen quality; however, this was not reflected in an increased persistence of fertility.

## 6. PUBLICATIONS RELATED TO THE DISSERTATION

**Scientific paper published in reviewed international journals:**

**Végi, B.** Váradi, É., Szőke, Zs. and Barna, J. (2013) Effect of sex ratios, spiking and extra artificial insemination on the breeding efficiency of broiler breeders. *Acta Vet Hung* 61 (3) DOI: 10.1556/AVet.2013.016. *In press.*

**Passage of scientific book:**

Szőke, Zs., **Végi, B.**, Varga, Á., Lennert, L., Péczely, P. and Barna, J. (2006) Effects of artificial insemination as a handling stress on egg weight, yolk corticosterone content and embryonic mortality. In: *New insights into fundamental physiology and perinatal adaptation of domestic fowl.* (Ed.s: S Yahaw and B. Tzscheentke). Nottingham University Press. Nottingham. pp. 189-197.

**Scientific papers published in reviewed Hungarian journals:**

1. **Végi B**., Varga Á., Szőke Zs., Lennert L., Barna J.: (2005) A termékenység előrejelzése új *in vitro* technika alapján. / Predicition of fertility in broiler breeders by *in vitro* techniques. *Állattenyésztés és Takarmányozás* (Hungarian Journal of Animal Production) 54. (3): 208-215. (in Hungarian with English summary)
2. Szőke, Zs., **Végi, B.,** Varga, Á., Lennert, L., Péczely, P. és Barna, J. (2006) Tyúk mesterséges termékenyítésről, másképp. / Artificial insemination of domestic fowl, from different approach. *Állattenyésztés és Takarmányozás*. (Hungarian Journal of Animal Production) 55 (5) 483-491. (in Hungarian with English summary)
3. Váradi É., Szőke Zs., **Végi B.,** Péczely P., Barna J. (2008) A kakascsere mint stresszor endokrinológiai elemzése hústípusú szülőpárállományban. / Endocrinological analysis of spiking as a stressor in broiler breeders. *AWETH (Animal Welfare, Ethology and Technology)* 4 (2) (Különszám): 381-390. (in Hungarian with English summary)
4. **Végi, B.,** Váradi, É., Szabó, Zs., Szőke, Zs., Kőrösiné Molnár, A**.,** Barna, J. (2008) A hőkezelés, illetve hőstressz hatása hímivarú baromfifélék spermatológiai mutatóira. / Effects of heat treatment and heatstress on sperm parameters of various poultry species. *AWETH (Animal Welfare, Ethology and Technology) 4* (2) 401-408. (in Hungarian with English summary)

5. **Végi, B.,** Váradi, É., Ferencziné Szőke, Zs., Barna, J. (2008) Szerves szelén és E-vitamin kiegészítés hatása hústípusú szülőpárok spermatológiai mutatóira. / Effect of organic selenium and vitamin E supplementation on sperm traits of broiler breeders. *AWETH (Animal Welfare, Ethology and Technology) 4* (2) pp. 391-400. (in Hungarian with English summary)
6. **Végi, B.,** Váradi, É., Ferencziné Szőke, Zs., Péczely, P. és Barna, J. (2009) Szteroid hormon-változások és egyes spermatológiai mutatók összefüggéseinek vizsgálata hústípusú szülőpár kakasoknál. / Relationship between steroid hormone changes and sperm traits in broiler breeders. *Magyar Állatorvosok Lapja.* (Hungarian Veterinary Journal) 131. 489-493. (in Hungarian with English summary)

**Scientific paper published in referred Hungarian journals:**

Barna, J., **Végi, B**., Varga, Á., Szőke, Zs., Lennert, L., Török, T., és Kovács, T. (2005) Termékenységi problémák és előrejelzésüknek új vizsgálati módja brojler szülőpár állományoknál. / Fertility problems in broiler breeders and a new tool for its prediction. *Baromfiágazat /Poultry Sector.* 5.(1) 24-31. (In Hungarian)

**Full papares in proceedings of international conferences**

1. Barna, J., **Végi, B.,** Váradi, É., Szőke, Zs., Liptói, K. . (2007) Analysis of fertility in broiler breeder flocks. 5th Vietnamese-Hungarian Conference and MGE NEFE Major Conference, pp. 75-81. Can Tho, Vietnam. 12-16 Aug, 2007.
2. **Végi, B**., Váradi, É., Ferencziné Szőke, Zs. and Barna, J. (2008) Spiking or not? XXIII World's Poultry Congress. Brisbane, Australia. 30 June- 4 July 2008. World's Poultry Science Journal Vol. 64. Suppl. 2. p. 389.
3. Barna, J., **Végi, B**., Váradi, É., Ferencziné Szőke, Zs. and Péczely, P. (2009) Studies related to fertility in broiler breeders. Proc. XXI International Poultry Symposium PB WPSA. pp. 18-23. Wroclaw – Szklarska Poreba, Poland. 7-9 Sept 2009.

**Abstracts in proceedings of international conferences**

1. Szőke, Zs., **Végi, B.,** Varga, Á., Liptói, K., Várkonyi, E., Lennert, L., Péczely, P. and Barna, J. (2005) Effects of Artificial Insemination - as a Handling Stress - on the Egg

Weight, the Yolk Corticosterone Content and the Embryonic Mortality (Pilot study). The 2nd Combined Workshop of Fundamental Physiology of the European Working Group of Physiology and Perinatal Development in Poultry, September 23 – 25, Berlin 49.p.

2. **Végi, B**., Váradi, É., Szőke, Zs., Liptói, K. and Barna, J. (2007) Analysis of fertility in broiler breeder flocks – male side approaches. 29th Poultry Science Symposium, WPSA UK Branch, Edinburgh 23-25 July, 2007.
3. **Végi, B.**, Váradi, É., Szőke, Zs. And Barna, J. (2007) Comparative study on the effect of organic selenium and vitamin E on sperm characteristics in two lines of broiler breeder males. XIX International Symposium Polish Branch WPSA. „Science for poultry practice – poultry practice for science" Olsztyn, Poland. 10th – 12th September 2007
4. **Végi, B.,** Váradi, É., Ferencziné Szőke, Zs. and Barna, J. (2008) Changes in sperm quality of broiler breeder males supplemented with organic selenium. 16. International Congress on Animal Reproduction. Budapest, Hungary. 13-17 July 2008. pp. 1-4.

**Posters and presentations in Hungarian conferences**

1. **Végi, B**., Varga, Á., Szőke, Zs., Lennert, L., Barna, J. (2004) A termékenység előrejelzése új *in vitro* technika alapján hústípusú szülőpár-állományokban. / Predicition of fertility in broiler breeders by *in vitro* techniques. Előadás, Proc. 11. Szaporodásbiológiai Találkozó, /11th Meeting of Association of Hungarian Reproduction Biologists, Dobogókő, 2004. p. 18.
2. Barna, J., Varga , Á., **Végi, B**., Szőke, Zs, és Lennert, L. (2005) Spermium-pete interakció vizsgálatának módszerei madártojásokban. / Examinations on sperm – egg interaction in avian egg. Poster. Proc. "Vadállatok szaporodásbiológiája, állatkerti tenyészprogramok" konferencia, Zoo conference, Budapest, 2005. Márc. 18-20. pp. 53-55.
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4. Szőke, Zs, **Végi, B.,** Varga, Á., Lennert, L, Péczely P., Barna J (2005) Tyúk mesterséges termékenyítésről, másképp. / Artificial insemination of domestic fowl,

from different approach. Proc. 12. Szaporodásbiológiai Találkozó, /12th Meeting of Association of Hungarian Reproduction Biologists, Hajdúszoboszló, 2005. 11. 04-05. p. 32.

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6. Váradi, É., Szőke, Zs., Péczely, P., **Végi, B**., Barna, J. (2007) A kakascsere okozta stressz hatásának endokrinológiai elemzése fekális szteroid-analízissel hústípusú szülőpárállományban. / Endocrinologycal examination on the effect of stress caused by spiking by fecal steroid analysis. Proc. 14. Szaporodásbiológiai Találkozó, / 14thMeeting of Association of Hungarian Reproduction Biologists, Keszthely, 2007. október 5-6. p. 41.
7. **Végi, B**., Váradi, É., Ferencziné Szőke, Zs., Péczely, P. és Barna, J. (2009) Szteroid hormon-változások és egyes spermatológiai mutatók összefüggéseinek vizsgálata hústípusú szülőpár kakasoknál. / Relationship between steroid hormone changes and sperm traits in broiler breeders. Előadás. 15. Szaporodásbiológiai Találkozó, /15th Meeting of Association of Hungarian Reproduction Biologists, Eger, 2009. április 17-18. p. 21.
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