ORIGINAL ARTICLE

THE INFLUENCE OF INDUCING EARLY REPRODUCTIVE ACTIVITY IN YOUNG HENS AND ROOSTERS OF EGG-LAYING TYPE BREED

INFLUENȚA DECLANŞĂRII TIMPURII A ACTIVITĂȚII DE REPRODUCȚIE LA PUICUȚELE ŞI COCOŞEII PĂRINȚI PENTRU OUĂ CONSUM

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ABSTRACT

A Fsample of young 60 hens and 40 roosters of Rhode Island breed were divided in groups L1, L2 and L3. Individuals in group L1 were transferred at the age of 16 weeks from 8 h light/ day to 16 h light/ day and fed with a normal adult poultry feed. The same switch was performed with groups L2 and L3 at the age of 18 and respectively 20 weeks. Modifications of the sexual traits were recorded including: phenotype, weight and length of the genital tract, laying intensity, average egg weight and semen parameters. Furthermore, blood samples were analysed for FSH and LH. From photo stimulated roosters -kept under an 8 h light/ day programme at the age of 18, 22 and 24 weeks- histology samples from testis and deferens ducts were analysed.

In young hens reproductive parameters are influenced both by light length and the age when this photo stimulation occur. All data shows that starting photo stimulation at 18 weeks has positive effects on the egg production in the analysed population.

In young roosters inducing sexual stimulation before the age of 20 weeks will prolong the time length to typical reproductive activity and also will affect the semen quality. Thus, it seems that age is more important for young roosters in achieving reproductive maturity than in young hens. Therefore, inducing early sexual development is rather detrimental for males.

KEY WORDS: reproduction, young hens and roosters, precocity, genitalia morphology, gonadotrophines, histology, production

REZUMAT

In efectiv de 60 puicuțe şi 40 cocoşei din rasa Rhode Island a fost structurat în loturile L_1, L_2, L_3. Păsările lotului L_1 au fost trecute la vârsta de 16 săptămâni de la 8 la 14 ore lumină şi furajate cu nutreț caracteristic păsărilor adulte. Aceaşi intervenție s-a realizat la vârstele de 18 şi 20 săptămâni formându-se loturile L_2 şi L_3. Modificările din sfera genitală s-au evidențiat bisăptămânal urmărindu-se caracteristicile fenotipice, greutatea şi lungimea aparatului genital, intensitatea de ouat, greutatea medie a ouălor, caracteristicile materialului spermatic. De asemenea s-a recoltat sânge pentru determinarea nivelului FSH şi LH, iar la 18, 22 şi 24 săptămâni în cazul cocoşilor fotostimulați şi întreținuți la 8 ore lumină au fost recoltate probe din testicul şi canalele deferente pentru analize histologice.

La puicuțe indicii de reproducție urmăriți sunt influențați de durata programului de lumină şi vârsta păsărilor la care acestea se aplică. Datele obținute arată că începerea stimulării luminoase la 18 săptămâni este favorabilă obținerii de producții ridicate şi corespunde particularităților materialului biologic analizat.

Declanşarea activității de reproducție, la cocoşei înaintea vârstei de 20 săptămâni măreşte durata de timp în care aparatul genital ajunge să funcționeze la nivelul caracteristic şi să producă material spermatic de calitate ce poate fi folosit pentru reproducție. La cocoşi vârsta are o influență mult mai mare asupra reproducției decât la femele, iar ca urmare precocizarea reproductivă nu dă rezultate.

CUVINTE CHEIE: reproducție, puicuțe, cocoşei, precocizare, morfologia aparatului genital, gonadotropine, histologie, producție

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DETAILED ABSTRACT

The current study was based on 60 females and 40 males, juvenile Rhode Island parents of the Romanian auto-sexable egg-type broiler. At the age of 16 weeks 20 females and 16 males were selected and moved from 8h lighting regime to 16 h light (Group 1). Feed provided was the one used for adult hens. The group remaining under a 8L:16D photoperiod was fed with a grower type ratio. Group 2 and 3 were divided by the time the first one reached the age of 18 and respectively 20 weeks and transferred under a regime of 8L:16D and adult hen feed.

In order to highlight the reproductive transformations weekly assessments were made on: phenotype changes, internal genitalia weight and length, egg laying intensity, average egg weight – in females; internal genitalia measurements and spermogram – in males. Furthermore from the males testicular and duct deferens tissue samples were prepared for histology study. In both sexes weekly blood samples were collected in order to assess the FSH and LH levels.

Results reveal that photo-stimulation of the juvenile hens have a positive impact on all recorded traits. There seems to be as well a strong relationship between the phenotype transformations and the morphology features of the internal genitalia. However, the age is the one who influence the most all genital transformation, according to the light: dark program. FSH and LH levels in the blood stream depend both on the age and photoperiod in juvenile hens. Thus, Group 1 juvenile hens need 4 weeks to trigger the laying while Group 2 females only one week. All data demonstrate that starting photo-stimulation at the age of 18 weeks is the most appropriate for the used genotype.

In juvenile cockerels increasing light at the age of 16 weeks has rather minor influence on the growth of internal genitalia when compared with females. The 8L:16D regime up to the age of 20 weeks has little influence on genital development. Duct deferens spirals appear only after 6 weeks of photo-stimulation in Group 1 and after 5 weeks in groups 2 and 3, thus delaying spermatozoa maturation. Juvenile cockerels subject to 14 h light regime from the age of 20 weeks demonstrate active spermatogenesis after only 3 weeks of photo-stimulation and semen output is improved. Histology study confirm the above findings as well. Thus, it seems that modifying light: dark regime for juvenile males before the age of 20 weeks delays the moment when the genitalia is able to produce high quality sperm cells.

INTRODUCTION

The age when poultry reach sexual maturity continuously decreased both in males and females. It is well known that this process depends on several factors as genetics, reaching the typical body weight [3], the age [3, 10], environment [5], chemical composition of the carcass [10, 20].

Ovary growth and maturation is correlated to the LH level in blood plasma. When daylight remains constant LH level in plasma will grow as well from 6 to 16 weeks of age [17, 19]. Increasing the light length in week 17 will lead to a higher level of LH secretion and thus the egg lying in 3-4 weeks [19]. If light length remains further constant LH will grow as well until before the first egg and will achieve the maximum value 2-4 weeks before the actual laying is starting [17, 19]. The response to light stimulation is correlated in poultry with specie, breed and the sex. Working on Leghorn young hens Lesson (1988) got an increase of the LH despite the fact that photo stimulation started at the age of 92 days. Lerner (1945) consider that achieving the sexual maturity is not as difficult as its correlation to the skeleton development due to calcium use in the egg laying process. Leeson and al.[10] demonstrate that Leghorn young hens photo stimulated in order to start the laying earlier will have an increased egg production despite that the size of eggs decrease and laying period length gets shorter. Females which start laying later will be heavier at the end of the productive cycle, produce bigger eggs and also consume more feed when compared to the those which were the classic technology was applied. Body weight of the lightweight breed hens will never be recovered through a compensatory weight gain in animals with early-induced sexual maturity. This demonstrates that egg laying has the priority when it is about feed nutrient usage [10].

Most studies reveal that hens can be induced to an early egg laying despite the fact that problems will occur regarding egg weight and a reduced body weight at the end of productive cycle. However, reduced egg weight seems to be compensated by the total egg mass in a positive way as it was demonstrated by Robinson F.E. (1996). He also observed that the mortality in experimental groups was due to uterine prolaps, abdominal ovulation, haemorrhagic fat liver syndrome and other reproductive dissfunctionalities. All these problems occurred more frequently in groups with higher average body weight (with minimum 3 g in Leghorn hens). Thus we can conclude that decreasing body weight through inducing early laying has positive effects on low mortality rates.

Table 1: Main reproductive indexes in young hens (average values)

Group	Age (week)	Weeks of photosti-mulation	Plasma level of gonadotrophines (ng/ml)		Genitalia weight (g)	Oviduct length (cm)	Egg -laying intensity (%)	Egg weight (g)
			FSH	LH				
L 1	16	Start	0.05 ±0.002	0.77 ±0.014	2.17 ±0.085	11.35 ±0.340	-	-
	18	2	1.41 ±0.161	2.31 ±0.316	7.63 ±1.980	20.02 ±3.563	*	*
	20	4	1.19 ±0.064	2.35 ±0.384	68.50 ±28.86	52.87 ±9.751	53.57	47.50
L 2	18	Start	0.36 ±0.135	1.29 ±0.125	3.00 ±0.204	11.00 ±0.353	**	**
	20	2	1.25 ±0.065	2.14 ±0.408	45.37 ±26.35	44.62 ±8.970	30.95	48.50
	22	4	0.18 ±0.051	1.32 ±0.443	91.50 ±2.783	64.00 ±1.870	87.05	49.00
L 3	20	Start	1.20 ±0.089	1.88 ±0.273	9.00 ±2.041	15.50 ±3.122	2.67	44.50
	22	2	0.45 ±0.089	1.64 ±0.600	71.62 ±2.041	56.55 ±10.44	36.66	47.50

* Begin laying at 19 weeks, intensity 26.19%, average egg weight 46.40g
** Begin laying at 19 weeks, intensity 4.46% , average egg weight 48.00g

MATERIAL AND METHOD

The study was done on a young Rhode Island population with 60 white type hens and 40 red type roosters, parents of the Romanian egg-type hybrid. Birds were kept on the ground (with bedding) using the known rearing and feeding technology.

At 16 weeks of age 20 females and 13 roosters were transferred from 8 h to 14 h light conditions. The feed was the one used in adult laying hens. This was considered group 1. Remained birds were kept under 8 h light length and rearing feed. Groups 2 and 3 were formed when young birds reached the age of 18 and 20 weeks with the same light programme. In order to have similar developed groups body weight

and the colour of head structures were used as selection criteria. Thus, according to this experimental model both hen and rooster groups - coded as L1 and L2- received both feed and light typical for adult birds in advance with 4 and respectively 2 weeks when compared to group 3. The later was considered as control group as usually the photo stimulation starts at 20 week of age in normal conditions. By this way we could observe both the influence of early egg laying and reproductive development induced by light length and feed in young hens and roosters.

Table 2: Main reproductive indexes in young roosters (average values)

Group	Age (week)	Weeks of photosti-mulation	Plasma levels of gonadotrophines (ng/ml)		Testis weight (g)		Deferens ductus length (cm)	
			FSH	LH	Left	Right	Left	Right
	16	Start	0.08 ±0.008	0.44 ±0.064	1.00 ±0.204	1.00 ±0.204	10.93 ±0.131	10.36 ±0.838
L1	18	2	0.45 ±0.194	1.37 ±0.070	3.83 ±0.589	3.66 ±0.824	11.93 ±0.526	11.50 ±0.353
	20	4	1.07 ±0.230	2.84 ±0.747	6.33 ±0.942	6.66 ±0.623	10.66 ±0.117	11.60 ±0.883
	18	Start	0.20 ±0.002	1.57 ±0.102	1.66 ±0.235	1.66 ±0.117	11.33 ±0.235	11.30 ±0.308
L2	20	2	1.08 ±0.011	1.37 ±0.102	4.33 ±0.235	5.33 ±0.235	12.66 ±0.942	11.00 ±0.408
	22	4	0.41 ±0.054	2.94 ±0.407	11.5 ±1.968	11.00 ±1.224	12.06 ±0.493	11.00 ±0.081
	20	Start	0.98 ±0.034	1.50 ±0.106	2.33 ±0.964	2.46 ±1.265	11.83 ±0.117	10.50 ±0.204
L3	22	2	0.21 ±0.021	1.59 ±0.106	5.66 ±0.235	7.33 ±0.471	12.53 ±1.228	11.26 ±0.379
	24	4	-	-	10.00 ±0.00	10.66 ±0.235	12.66 ±0.471	10.33 ±0.471

Sexual changes were monitored by recording twice a week the phenotype features, weight and length of the genitalia, egg-laying intensity, average egg weight and semen analyse. Blood samples were analysed by fluorometry with standard kits.

Testis and deferens ducts were used for histology investigations at the age of 18, 22 and 24 weeks of age with and without photo stimulation. Samples were fixed in formaline and then in potassium dichromate 3% for 2-3 days. Staining was made with haematoxylin – eosin.

Table 3: Semen features

Group	Age (week)	Weeks of Stimulation	Volume (ml)	pH	Mot*	Conc**	Dead (%)	Abnormalities (%)		
								Head	I.p.	P.p.
	20	4	0.23	7.00	2.66	2.33	58.66	33.33	1.33	0.33
L 1	22	6	0.26	7.13	4.00	28.16	28.66	5.00	1.33	1.33
	23	7	0.26	7.16	2.66	21.03	31.66	6.66	0.66	0.33
	22	4	0.20	7.06	2.66	18.46	55.66	15.66	0.33	0.33
L 2	23	5	0.21	7.00	3.00	21.36	31.00	28.33	1.00	0.33
	24	6	0.25	7.10	2.33	52.90	58.00	31.00	0.33	0.66
	23	3	0.40	7.00	3.00	23.20	62.00	22.00	0.00	1.00
L 3	24	4	0.23	7.00	3.00	39.33	50.66	5.66	2.66	1.66
	25	5	0.42	7.07	3.00	118.75	5.75	3.25	2.00	2.00

Mot* = motility; Conc** = concentration (x 10^9/ml); Head = head abnormality;
P.i. = intermediary piece abnormalities P.p. = principal piece and tail abnormalities

RESULTS AND DISCUSSIONS

Young hens at 16 weeks and kept under 8 h light conditions show differences regarding the development and colour of the head structures (comb, wattle and face). Thus the group can be divided in early, medium and delayed developing sub-groups.

The early phenotype has above average size and pink-red colour head structures. The medium phenotype has smaller and pale colour in all head structures. Even smaller and paler structures were found in delayed phenotype.

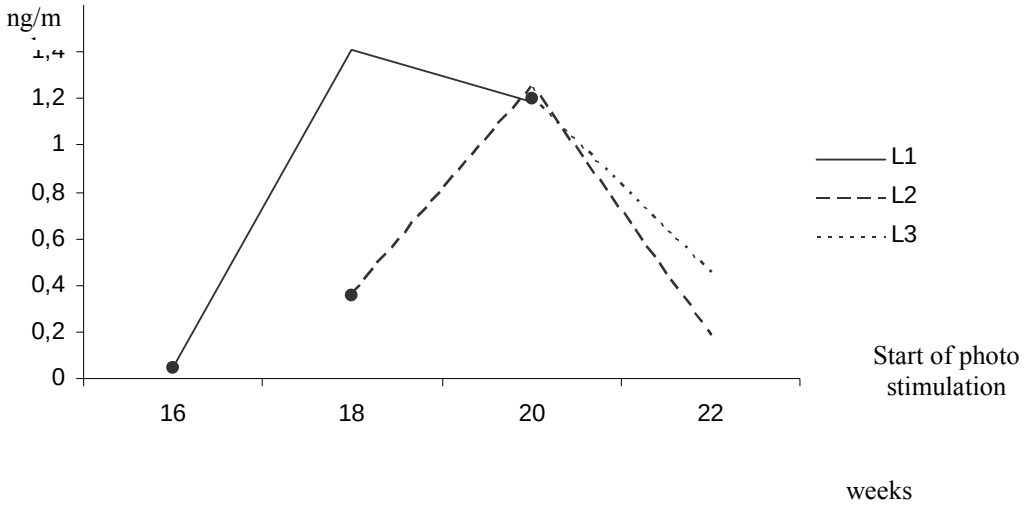
Light stimulation at 16 weeks of age will induce a faster growth of the head structures and a more

intense colour. The rhythm of these modifications depends upon the above-described phenotypes. Thus the medium phenotype will show up one week earlier than in the delayed phenotype but one week after the early phenotype. All phenotype changes depend on the reproductive development.

Delayed photo stimulation at 18 (Group L1) and respectively 20 (Group L3) weeks seems to accelerate all transformations and reduce the differences among the phenotype groups. This demonstrates improved function of the reproductive structures.

The weight of genitalia (ovary + oviduct) at the start of photo stimulation in groups L1 and L2 is the same. This demonstrates that in young hens kept under 8 h light conditions the genitalia are not changing morphologically. Although this changes appear in group L3 after the age of 18-20 weeks. After 2 weeks of photo stimulation the average weight of genitalia is growing in a similar way in groups L1 and L2 and faster in group L3. The same pattern was observed regarding the oviduct length. However, the oviduct length was higher in-group L1 (41 cm) after 2 weeks of photo stimulation when compared to group L1 (25.25 cm). Thus it can be seen the correlation between the phenotype dynamics and genitalia morphology. The age is the factor which induce the rhythm of both phenotype and morphology features of the genitalia. The egg-laying preparation seems to be more intense in-group L2 when compared to L1.

Figure 1: Plasma levels of FSH in young hens



Serum gonadotrophine levels are usual both for FSH and LH. The lightening programme and the age influences these levels. In-group L1 FSH reach a maximum level 2 weeks after the start of photo stimulation, than goes down gradually. The FHS level after 4 weeks of photo stimulation is still high. Keeping young hens under 8 h light conditions up to the age of 18 weeks induce a growth of FSH multiplied with 7. In-group L2 the FSH level decrease under the start level after 4 weeks of photo stimulation. Same pattern was observed in-group L3. LH levels in plasma are higher and have a similar dynamics. However, the minimum-maximum ratio is lower in the case of LH levels.

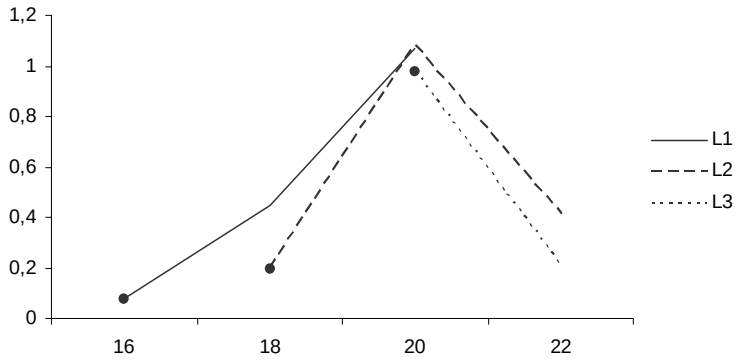
It is well known that before the time of complete sexual maturation hormonal mechanisms are working through a long, positive feedback. According to this theory in group L1 -due to the insufficient morphological development of genitalia- there is no oestrogen production high enough to stop the pituitary production of FSH.

Young hens in groups L2 and L3 reach the adult like hormonal control of reproduction at the age of 22 weeks, 4 and respectively 2 weeks following the start of photo stimulation.

Egg production was delayed until after 4 weeks of photo stimulation in group L1. Increasing the light length at the age of 18 weeks will induce the start of

laying after only one week, reaching the intensity of 87.05% at the age of 22 weeks. Thus, it seems that current used technologies recommending the start of photo stimulation at 20 weeks is not efficient in the new genetically improved lines which lay the first egg much earlier.

Figure 2: Plasma level of LH in young hens



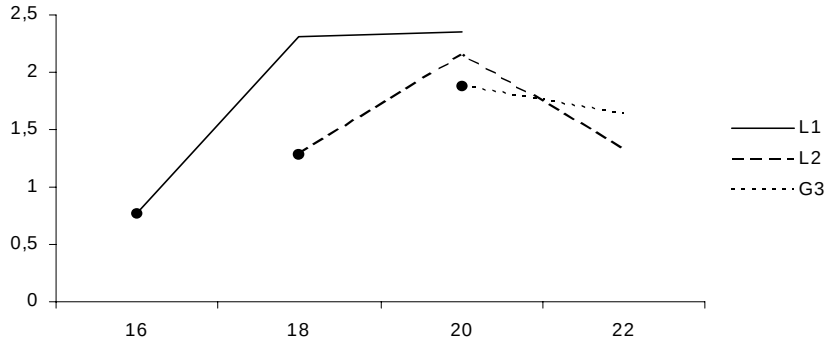
As a conclusion it can be considered that starting photo stimulation in young hens at 18 weeks of age is favourable for the total egg production and reducing the rearing length with 2 weeks. However, we have to remember that the egg weight is still low (49 g) when laying intensity reaches the 87.05%, especially in group L2 after 4 weeks of photo stimulation.

Phenotypic ally, the internal reproductive changes are expressed by the morphology and colour of the head structures.

The same as in young hens, roosters at the age of 16 weeks, reared under 8 h light conditions, can be divided in phenotype sub-groups as early, medium and delayed ones, according to the development and colour of the head structures. Roosters with induced photo stimulation at the age of 16 weeks respond to this process by an increased development and accelerated colouring of the head structures. The same pattern is observed in groups L2 and L3 although occurring much faster. Differences are due to the age which means that at 8 h light length the genitalia development is rather slow when compared with photo stimulated groups.

Testis average weight in control group has a sow growth as well. Thus, at 20 weeks of age the average value of the testis weight is only 2.33-2.46 g, which demonstrate the differences among sexes regarding the precocity. The photo stimulation influence is reduced in group L1 and much more intense in groups L2 and L3. In the later ones the testis weight is rather similar after 2 and 4 weeks of photo stimulation. The length of the ductus deferens has no relevance as it grows according to the body size.

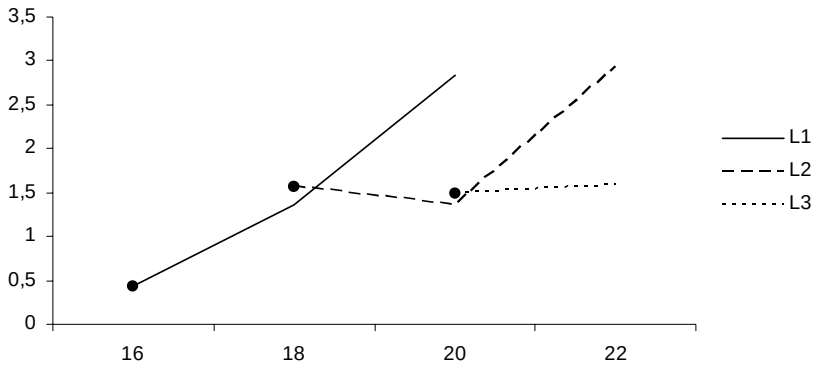
Figure 3: Plasma level of FSH young roosters



Sinuosity of the deferens ducts is considered as being the last process in the morphological features of the sexual mature rooster. Here is the place for sperm maturation. The spiral shape of the ductus deferens became obvious after 6 weeks of photo stimulation in group L1 and 5 weeks respectively in groups L2 and L3. The lack of spiral shape before these ages reveals that testis does not produce enough testosterone and the sperm cells are rather immature.

Serum gonadotrophine levels have different patterns in roosters, mainly regarding LH. FSH grows constantly in group L1, decrease in L2 after 4 weeks and in L3 after 2 weeks of photo stimulation. Considering that FSH acts mainly on the seminiferous tubuli we consider that at age of 20 weeks -when FSH reaches the maximum levels in all groups- seminiferous epithelia is formed, Sertoli cells are functioning and secrete inhibine. This process will start even without increasing photo stimulation. This observation relies on the fact that the value of FSH in L3 group is similar to the other groups at the same age and it will decrease at 22 weeks of age.

Figure 4: Plasma level of LH in young roosters



LH has a higher plasma level than FSH. LH grows constantly irrespective to the age when photo stimulation is induced. However, light influence upon the LH secretion is higher than in FSH. After 4 weeks of photo stimulation LH reaches peak levels in groups L1 and L2. It is well known that LH modulates mainly testosterone production of the Leydig cells. In our case it seems that these cells did not produced enough hormones in order to induce the negative feedback. Deferens ducts are not prepared yet for their basic biologic role, issue confirmed by the morphology assessment.

In group L1 semen production could be collected only after 4 weeks of photo stimulation. Semen parameters are common with the exception of the sperm abnormalities. Out of those, sperm head abnormalities are higher, consisting in large volume. Dead sperm percentage it is extremely high - 58.66%- showing the functional imperfections at the ductus deferens levels. After 6-7 weeks of photo stimulation the quality of the semen output is improving, still with high percentages of dead sperm cells. Not even after 8 weeks of photo stimulation the semen parameters do not reach the normal levels.

In group L2 the patterns of semen output is similar as in group L1. However, it is worth mentioning the increase of sperm concentration from 18.46 x 10^9/ml at 4 weeks of photo stimulation in comparison to 2.33 x 10^9/ml in control group. This means that seminiferous tubuli have an improved function at 22 weeks in comparison with 20 weeks of age.

Roosters in group L3 start spermatogenesis after only 3 weeks of photo stimulation. Sperm concentration is higher than in group L2 and the volume is double (0.4 ml). In the next weeks qualitative semen parameters are improving reaching adult levels at 25 weeks of age. The volume is now up to 0.42 ml and the concentration 118.75 billion/ml while the dead sperm percentage is going down to 5.75%.

Figures 5a and 5b: Structure of young rooster testis at the age of 18 weeks in experimental (5a) and control (5b) groups

Figure 5a

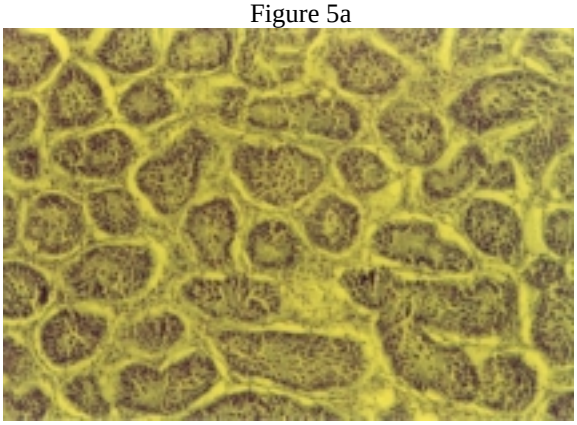
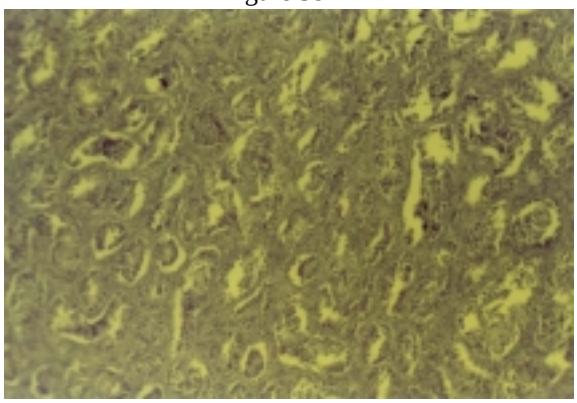


Figure 5b



Histology assessments on testis of roosters kept under 8 h light and fed with grower feed showed a reduced number of germinal layers and an intense colour of the nuclei and cytoplasm granules in Sertoli cells. This intense colour was correlated by Rosenstranch and al. [16] to a low spermatozoa output. The same situation was observed in testis from roosters at 18 weeks of age, following 2 weeks of photo stimulation. However, in this sample there is an increased number of seminiferous tubuli per square millimetre and with increased diameter. (Figure 5a and 5b).

At the age of 20 weeks -after 4 weeks of photo stimulation- differences between groups became more obvious. The diameter of seminiferous tubuli is increased while spermatide-spermatozoa-Sertoli cells complexes appear. Then the intra-tubuli degenerescence demonstrate the beginning of decay at this level [16](Figure 6a and 6b).

After 6 weeks of photo stimulation the number and diameter of seminiferous tubuli increases further and the

Figures 6a and 6b: Structure of young rooster testis at the age of 20 weeks in experimental (6a) and control (6b) groups

Figure 6a

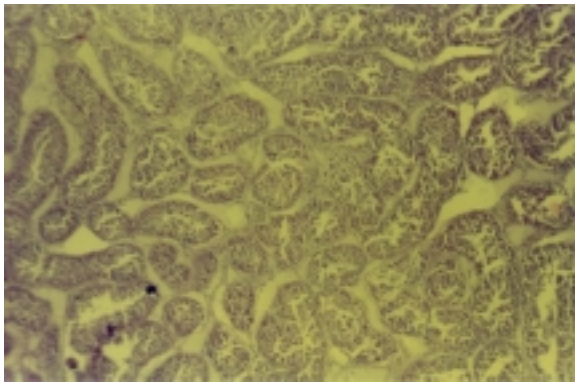
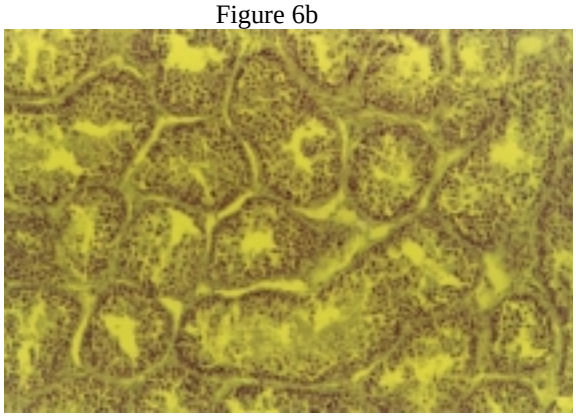


Figure 6b



SSS complexes multiply. Mean while the intra Sertoli structures loose the intense colour. All these demonstrate that at the age of 24 weeks the testis tends to reach the adult structure and function.

In control group at 22 and 24 weeks testis have less seminiferous tubuli per square millimetre and smaller diameter while the SSS complexes are less than in photo stimulated groups. However, in control group in both ages there are large numbers of spermatozoa binding to Sertoli cells (Figure 7a and 7b).

Figures 7a and 7b: Structure of young rooster testis at the age of 22 weeks in experimental (7a) and control (7b) groups

Figure 7a

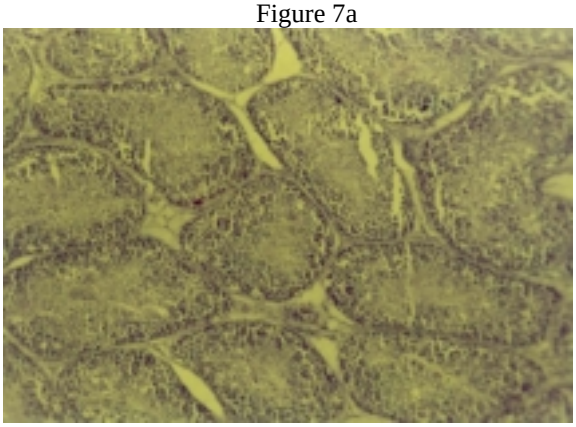
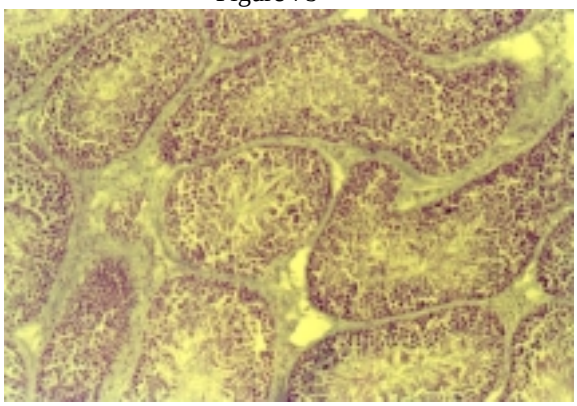


Figure 7b



Histology samples from ductus deferens reveals that irrespective of the photo stimulation patterns up to 20 weeks of age the epithelia has a reduced number of cubic cell layers, with high nucleus activity (Fig. 8a and 8b). After this age the cells become cylindrical and layers multiply. The presence of secretions within this tubuli confirms the maturation of these structures.

Thus, it seems that in birds as well as in mammals maturation of seminiferous tubuli and start of spermatogenesis occurs later than the ovary development, consequent to a longer puberty.

In young roosters increasing the light length at the age of 16 or 18 weeks of age has no beneficial effects. Even if semen output is higher the quality is not good enough for a high fertility. There are too many dead and abnormal sperm cells.

Therefore, inducing early reproductive activity in young roosters before the age of 20 weeks will rather delay the time of full reproductive functions. Thus, it seems that in males the age have higher influence on reproduction than in females and our stimulation model will not be appropriate for roosters.

Figures 8a and 8b: Structure of young rooster ductus deferens at the age of 18 weeks (8a - control group) and 22 weeks (8b - experimental groups)

Figure 8a

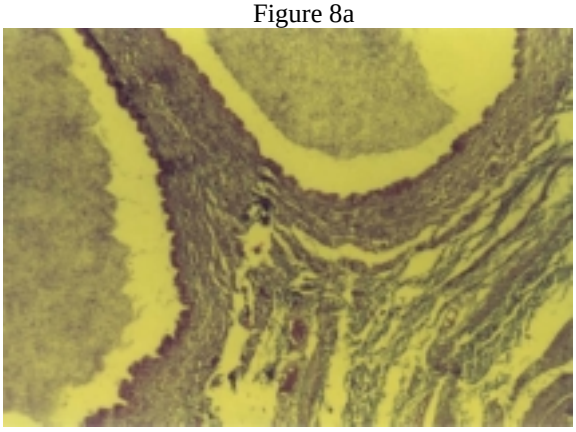
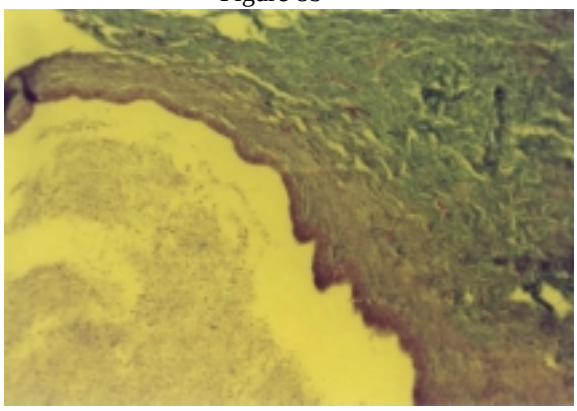


Figure 8b



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