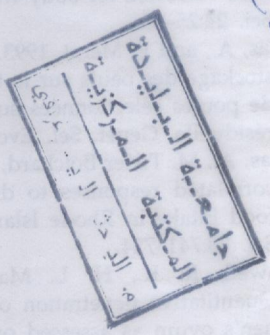
183

183 AGRO

N° 33101
Agronomie
Kandjaou N.



Pre-Order Your Copy Today!

Techniques for Semen Evaluation, Semen Storage, and Fertility Determination

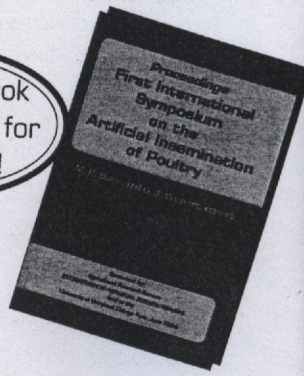
M. R. Bakst and H. C. Cecil, editors

PSA is excited about its newest publication - a lab manual! This manual is a companion volume of the book, *First International Symposium on the Artificial Insemination of Poultry*, and workshops held during that symposium (University of Maryland-College Park, 1994). It is a comprehensive, 100-page manual that provides a step-by-step approach to the commonly used semen evaluation and storage procedures. The more novel techniques that assess semen quality and fertility are also described in detail.

The Lab Manual contains sections on the following topics:

- Semen Collection and Dilution
- Determination of Sperm Concentration
- Sperm Viability
- Sperm Motility And Metabolism
- Evaluation of Semen From Nondomestic Birds
- Semen Cryopreservation
- Predicting Fertility

Buy the Book and Manual for only $40!



It is ideal for use in the classroom and laboratory, and is a must for educators, commercial breeders, research scientists, geneticists, managers, and producers.

The manual can be purchased for $12, and there is a 25% discount for orders of 10 or more. Buy the book and manual for only $40! (a savings of $7). Pre-order your copy of the manual by completing the following form and sending it to:
PSA, 1111 North Dunlap Avenue, Savoy, IL 61874, phone 217-356-3182, or fax 217-398-4119.

Item	Price	Qty.	Subtotal
Techniques for Semen Evaluation, Semen Storage, and Fertility Determination	$12		
First International Symposium on the Artificial Insemination of Poultry	$35		
Both Publications	$40		
		Shipping and Handling *	
		TOTAL	

*Shipping and Handling Charges:
Within the US, Canada, and Mexico - first book included with price, $5.00 each additional book.
Outside the US, Canada, and Mexico - add $10 for first book surface mail; $5.00 each additional.

Name _____

Phone _____ **Fax** _____

Univ/Company _____

Address _____

City _____

Zip/Postal Code _____

Country _____

Payment Information *(Checks must be drawn on a U.S. bank, in U.S. funds.)*

❒Check enclosed ❒Visa ❒ Mastercard ❒American Express # _____ Exp. Date_____

Signature_____

Associated Effects of Divergent Selection for Residual Feed Consumption on Reproduction, Sperm Characteristics, and Mitochondria of Spermatozoa

M. MORISSON,*[,1] A. BORDAS,* J. M. PETIT,† C. JAYAT-VIGNOLES,‡ R. JULIEN,† and F. MINVIELLE*[,2]

*Laboratoire de Génétique Factorielle, Institut National de la Recherche Agronomique, 78352 Jouy-en-Josas Cedex, France, †Institut de Biotechnologie, and ‡Service Universitaire de Cytométrie en Flux, Université de Limoges, 123 avenue Albert Thomas, 87060 Limoges, France

ABSTRACT Eighteen generations of divergent selection for residual feed intake have been completed in two Rhode Island Red lines of domestic fowl. The high intake R+ line and the low intake R- line cocks used to sire Generation 19 of the selection experiment have been compared for associated responses on fertility, hatching, and sperm quality. Evaluations of sperm samples were based on volume, cell concentration, biochemical parameters (pH, uric acid and protein concentrations), and motility and morphology of spermatozoa. Finally, individual spermatozoa were analyzed by flow-cytometry (FCM) using Rhodamine 123 (Rh123) and nonyl-acrydine-orange (NAO) specific fluorochromes to assess, respectively, overall mitochondrial activity and overall mitochondrial content. Hatchability of incubated eggs was 20 points higher for the R- line, mainly because unfertilized eggs were only 6 vs 30% in the R+ line. Early embryo mortality was also twice as high in the R+ line (21%). The ratio of Rh123 to NAO fluorescence was identical for both lines. This result suggests that there was no difference in the energy producing potential of the individual mitochondria. Therefore, the difference seen for both dyes between the two lines might be attributed to a difference in the quantity of mitochondrial inner membranes present in the cell (with 17% less for the R+ line). In the R+ line, the poor performance at fertilization and during early embryonic development was associated with lower production of motile spermatozoa, possibly in relation to a lower quantity of mitochondria in spermatozoa from R+ cocks. Although the female contribution to the differences between lines was not explored separately, results suggest that selection for residual feed intake may have altered some cellular function related to the production of energy in the R+ line.

(*Key words*: residual feed intake, fertility, spermatozoa, mitochondria, egg lines)

1997 Poultry Science 76:425–431

INTRODUCTION

To study the efficiency of feed utilization in laying hens, a divergent selection for residual feed intake was started in 1976 in a Rhode Island Red population (Bordas and Mérat, 1984; Bordas *et al.*, 1992). Eighteen generations of selection have now been completed. The high feed intake R+ line and the low feed intake R- line had previously shown line differences for correlated responses on traits related to heat production and dissipation (Geraert *et al.*, 1991), to lipid metabolism (Zein-el-Dein *et al.*, 1985; Tixier *et al.*, 1988; El-Kazzi *et al.*, 1995), and to fertility and embryo viability, with a drop of hatchability in the R+ line due to an increase of both unfertilized eggs and embryo mortality (A. Bordas, unpublished data). At the 17th generation (1992), hatching rate was 31% in R+ line as compared to 63% in R- line, whereas embryo development was delayed in the R+ line, with about 10 h difference in time of hatching (Bordas and Mérat, 1993).

These differences are likely to be correlated responses to selection, but their underlying mechanisms are unknown. In an attempt to explain the decrease in hatchability in the R+ line, inbreeding (Bordas *et al.*, 1992), chromosomal abnormalities (Ladjali *et al.*, 1995), porosity, shell microsplits, and response to long-term storage of the eggs (Bordas and Mérat, 1993) were studied; however, no significant interaction was found between these factors and line for hatchability. Degradation of R+ cocks' semen characteristics could also explain part of the observed differences between the two lines. Among possible responsible factors, uric acid forms an extraseminal pollution, modifying motility and fertilizing ability of spermatozoa (Hammade, 1986) and, on the other hand, Thurston *et al.* (1992) reported that high protein concentration in seminal plasma was related to reduced fertility in turkeys. Also, optimal pH for survival of fowl spermatozoa is close to 7 (Lake and

Received for publication February 29, 1996.
Accepted for publication October 30, 1996.

[1]Present address: Laboratoire de Génétique Cellulaire, INRA, BP27, 31326 Castanet Tolosan, France.

[2]To whom correspondence should be addressed.

TABLE 1. Average performances (± SE) of the 9 R⁻ and 10 R⁺ cocks selected for reproduction after the 28-d feeding trial[1]

Trait	R⁻ line	R⁺ line	Value of *t* and significance of difference
Body weight, g	3,454 ± 138	3,648 ± 116	1.1
Weight change on test, g	−102 ± 24	−101 ± 28	0.03
Feed intake on test, g	2,017 ± 133	3,798 ± 81	11.7***
Residual feed consumption on test, g	−746 ± 61	870 ± 62	18.5***

[1]R⁻ = low intake line, R⁺ = high feed intake line.
***$P < 0.001$.

Ravie, 1979) and deviations from this value may be examined. Moreover, the motility of spermatozoa is primarily dependent on mitochondrial function for the required energy for generation and propagation of the flagellar wave (Auger *et al.*, 1989; Folgerø *et al.*, 1993). Mitochondrial contents can be evaluated indirectly by using the 10-N-nonyl acridine orange (NAO), which spontaneously penetrates cells and specifically gets incorporated into the inner mitochondrial membrane by interaction with cardiolipin independently of transmembrane potential, even with dead or fixed cells (Maftah *et al.*, 1989, 1990; Leprat *et al.*, 1990; Petit *et al.*, 1992, 1994, 1995). Activity of mitochondria may be assessed by using Rhodamine 123 (Rh123), a cationic fluorochrome accumulated by mitochondria in response to the electrochemical gradient set up by the mitochondrial membrane potential. It is a responsive probe of membrane potential, providing information on the functional state of mitochondria in both intact cells (Darzynkiewicz *et al.*, 1981; Johnson *et al.*, 1981; Nadakavukaren *et al.*, 1985) and isolated mitochondria (Emaus *et al.*, 1986; O'Connor *et al.*, 1988). It has been used to assess ram (Windsor and White, 1993), turkey (McClean *et al.*, 1993), and human (Auger *et al.*, 1989, 1993; Folgerø *et al.*, 1993) sperm mitochondrial function.

In the present work, to study further the fertility and hatching rate decrease of the R⁺ line, Lines R⁺ and R⁻ have been compared for their performances of reproduction in relation to sperm quality, using 9 R⁻ and 10 R⁺ cocks, the sires of generation 19 of the divergent selection experiment. Quantitative evaluation of ejaculates was based on volume and concentration of spermatozoa, whereas qualitative evaluation was obtained for motility and morphology of spermatozoa. Biochemical characteristics of seminal plasma were also studied, including pH and uric acid and total protein concentrations. Finally, mitochondrial content and activity of spermatozoa were assessed by flow-cytometry analysis.

MATERIALS AND METHODS

Lines and Experimental Animals

A complete description of the selection procedures has been given previously (Bordas *et al.*, 1992). Briefly, two lines of the same Rhode Island Red origin have been divergently selected for residual feed intake since 1976. males, residual feed intake (R) is feed consumption (F adjusted to equal BW and BW gain (ΔW), from individu results of a 28-d feeding trial conducted at each generatio on all candidates to selection. The equation was R = FI 91.0 $BW^{0.5}$ − 3.67 ΔW + 1,907 for males (in females, fee consumption is adjusted also to equal eggmass to evalua R). Animals used in this work were hatched in early sprin 1993 and were housed in individual battery cages at th age of 18 wk. They consumed *ad libitum* a commercial di containing approximately 160 g total protein and 2,65 kcal ME/kg. The lighting regimen was 14 h light/d an the average ambient temperature was 22 ± 2 C. Thes males were also the sires of generation 19. The performances shown in Table 1 reflected the diverger selection for R:FI were significantly different betwee lines, whereas both BW and ΔW were quite similar in R and R⁺ cocks. Matings were done within line but full-si pairing was avoided. Hens and cocks belonged to th same generation.

Fertility and Hatchability

Semen was collected from the 19 selected males at th age of 11 to 12 mo, to inseminate by artificial insemina tions (AI) 5 hens per cock. Semen was not pooled. Thre inseminations were carried out before starting eg collection, and then twice per week thereafter. Semen wa diluted 1:1 in a 7.5 g/L NaCl solution and each femal received 0.2 mL diluted semen. Time between semer collection and AI did not exceed 30 min. Eggs wer collected for 3 wk and stored at 12 to 14 C befor incubation. Two hatches were set up and performance were registered for each sire and dam. Traits such a unfertilized eggs and early embryo mortality (observed a the 5th d of incubation), late embryo mortality (before th 18th d of incubation), and dead in shell eggs (mortalit between the 18th d of incubation and hatching) were noted and the ratios unfertilized eggs:incubated eggs hatched eggs:incubated eggs, early embryo mortality fertilized eggs, late embryo mortality:fertilized eggs, and dead in shell eggs:fertilized eggs were calculated. The ratio hatched eggs:incubated eggs represents the overal reproductive performance. Line differences may arise from problems at fertilization evaluated by the ratio unfertile eggs:incubated eggs. Among fertilized eggs normal development to hatching is measured by the ratio

hatched eggs:fertilized eggs. Failure to hatch may result from early or late embryo mortality evaluated by the ratios embryo mortality at 5 d:fertilized eggs and embryo mortality between 5 and 18 d:fertilized eggs, respectively, or from difficulties near the time of hatching measured by the ratio dead in shell eggs:fertilized eggs.

Ejaculates

A study of the sperm was performed immediately after the period of reproduction, when cocks were nearly 13 mo old. Sperm was collected by massage according to Burrows and Quinn (1937). Care was taken to avoid any contamination of semen with the cloacal products and particularly with the transparent fluid excreted from the lymph folds of the cloaca during ejaculation. Ejaculate volume of each cock was measured by weighing eight samples collected over 4 wk. The concentration of spermatozoa was estimated on four of the eight samples. Right after collection, the semen was diluted (20 μL in 2 mL of the 7.5 g/L NaCl solution), optical density was read using a spectrophotometer[3] at a wavelengh of 535 nm and the concentration of spermatozoa was estimated according to a standard curve.

Motility

Motility was assessed on seven of the eight ejaculates. Each one of the seven samples per cock was diluted 1:1 in BPSE (Beltsville Poultry Semen Extender) diluent (Sexton, 1977) and stored on ice until the motility measurement was achieved (no later than 2 h after sampling). Motility was observed under a light microscope and subjectively noted from 0 to 6 according to the following scale: 0 = no movement; 1 = spermatozoa moving on spot; 2 = spermatozoa moving on spot but more rapidly; 3 = preliminary waves; 4 = slow waves; 5 = rapid waves with preliminary eddies; 6 = very rapid waves with eddies.

Morphology of Spermatozoa

Spermatozoa staining (adapted from Blom, 1950) was performed on ice with semen diluted 1:1 in BPSE diluent. Forty microliters of diluted semen were added to 150 μL of the staining solution (eosin 16 g/L; nigrosin 60 g/L in BPSE). Two minutes later, two smears were performed for each sample and spermatozoa were observed by microscopy with an oil immersion objective. Dead spermatozoa (eosin-permeable, stained in pink), live ones (eosin-impermeable, still white) and live but abnormal ones were counted. Three hundred spermatozoa per sample were observed on four of the collected ejaculates (i.e., 1,200 spermatozoa per cock).

[3]Model DU20 spectrophotometer, Beckman, 93220 Gagny, France.
[4]Model ACR 1500, Bruker Spectrospin, 67160, Wissembourg, France.

Seminal Plasma Contents

Seminal plasma was separated from spermatozoa as described by Blesbois and Hermier (1990). To ensure separation without release of intracellular components into seminal plasma, four centrifugations were carried out. The first one was performed on the whole semen within 10 min after semen collection. It lasted for 10 min at 500 $\times$ *g* and 20 C. The resulting supernatant was harvested and a second centrifugation was performed under the same conditions. The second supernatant was submitted to a third centrifugation performed at 3,000 $\times$ *g* and 2 C for 30 min. The last centrifugation was carried out for 1 h at 10,000 $\times$ *g* and 2 C to eliminate cellular fragments. The seminal plasma was stored at –20 C for subsequent analysis. Four samples of seminal plasma per cock were pooled, and uric acid and total protein concentrations were determined with commercial kits, by spectrophotometry[3] at a wavelength of 590 and 570 nm, respectively.

Sperm Mitochondria

Spermatozoa were analyzed by flow cytometry[4] (FCM) after staining of their mitochondrial contents by fluorescent dyes. As two cocks could not be collected at the time of the flow cytometry experiment, it was carried out with semen of eight R^- and nine R^+ cocks diluted 1:1 in BPSE and stored on ice for 5 h. Staining with NAO (5×10^{-7} *M*) was conducted on 2×10^7 sperm cells for 15 min at room temperature. For Rh123 staining (10^{-6} *M*), 2×10^6 sperm cells were incubated for 30 min at room temperature, and they were counterstained by adding 50 mg/mL of propidium iodide (PI) 2 min before FCM analysis to distinguish dead (PI stained with specific red fluorescence) from living cells. The excitation wavelengths were selected using a band-pass filter centered at 485 $\pm$ 20 nm. The green fluorescence emitted by NAO or Rh123 was collected through a band-pass filter centered at 530 $\pm$ 30 nm. The red fluorescence of PI was collected at wavelengths greater than 600 nm (600 nm long-pass filter). Cellular debris and aggregates were gated out from the initial cytogram Forward Angle Light Scatter (FALS) vs Wide Angle Light Scatter (WALS). Due to the fluidity of the ACR 1500, all cells were appropriately aligned and only one population appeared in the cytogram FALS vs WALS. Electronic windows were placed around regions of interest and at least 10,000 cells included in the window were analyzed at a flow rate of 150 events per second.

Statistical Analysis

In all cases, proportion variables were subject to the arc sine square root transformation before analysis, but only least squares mean values of untransformed data are given for ease of interpretation.

For fertility and hatchability criteria, results from each mating were summed over the two hatches and the subsequent ratios were calculated for each set of sires and

TABLE 2. Least squares (LS) means and coefficients of variation of reproduction performances in R⁻ and R⁺ lines[1]

Rate	LS mean[2]		Value of F and significance of effect[3]		
	R⁻	R⁺	Line	Cock/line	CV[2] (%)
Hatched eggs:incubated eggs	0.55	0.35	13.8***	1.3	37
Unfertilized eggs:incubated eggs	0.06	0.30	21.8***	2.8**	58
Hatched eggs:fertilized eggs	0.58	0.48	4.9*	0.8	39
Early embryo mortality[4]	0.09	0.21	43.3***	0.4	61
Late embryo mortality[4]	0.09	0.09	0.2	1.2	86
Dead in shell	0.22	0.18	1.0	1.2	47

[1]R⁻ = low feed intake line, R⁺ = high feed intake line.
[2]Untransformed variables.
[3]Arc sine square root transformed variable.
[4]As a proportion of fertilized eggs.
*$P < 0.05$.
**$P < 0.01$.
***$P < 0.001$.

dams. All variables were then analyzed by nested analysis of variance with the model:

$$Y_{ijk} = \mu + l_i + s_{ij} + e_{ijk},$$

where Y_{ijk} is the observation from the k^{th} hen inseminated by the j^{th} cock of the i^{th} line, μ is the average over all effects, l_i is the fixed effect of the i^{th} line, s_{ij} is the random effect of the j^{th} cock in the i^{th} line, and e_{ijk} is the random error assumed to be N $(0, \sigma^2_e)$.

As sperm quality and contents were assessed repeatedly on the same cocks over 4 wk, split-plot analysis of variance was carried out on all corresponding variables with the model:

$$Y_{ijk} = \mu + l_i + s_{ij} + d_k + (ld)_{ik} + e_{ijk},$$

where Y_{ijk} is the observation on the k^{th} day from the j^{th} cock of the i^{th} line; μ is the average over all effects; l_i is the fixed effect of the i^{th} line, s_{ij} is the random effect of the j^{th} cock in the i^{th} line and the corresponding mean square is the error term to test the line effect; d_k is the fixed effect of the k^{th} day; $(ld)_{ik}$ is the interaction between the effects of the i^{th} line and of the k^{th} day; and e_{ijk} is the random error assumed to be N $(0, \sigma^2_e)$.

For all other variables, measured or calculated only once for each cock, the two lines were compared by using Student's t test.

RESULTS

Fertility and Hatchability Measurement

The differences between the two lines (Table 2) were highly significant ($P < 0.001$) for the hatched eggs:incubated eggs and unfertilized eggs:incubated eggs ratios. In the same way, early embryo mortality was higher ($P < 0.001$) in the R⁺ line than in the R⁻ line. On the other hand, late embryo mortality and dead in shell rates were not different in the two lines. Correspondingly, a small difference ($P < 0.05$) was found for the ratio of hatched eggs:fertile eggs between the two lines. The ratio of unfertile eggs:incubated eggs differed between cocks within line ($P < 0.01$), but there was no significant effect of the sire on the other traits.

Ejaculates and Spermatozoa Evaluation

Ejaculate volume was similar in the two lines (Table 3), but mean spermatozoa concentration was higher in R⁻ cocks. Therefore, the mean spermatozoa number of an ejaculate was almost half in R⁺ than in R⁻ cocks. The differences between the two lines for spermatozoa number and concentration were highly significant ($P < 0.001$). Spermatozoa motility mean value was higher ($P < 0.001$) in the R⁻ line, and the rate of dead spermatozoa was nearly twice higher ($P < 0.05$) in the R⁺ line. There were significant differences within line between cocks for all traits except the proportion of dead spermatozoa. Time had a large effect on the volume of the ejaculate ($P < 0.001$), but had little or no effect on other measures. Motility was the only trait significantly affected by all main effects of the model, and also had a significant interaction between line and time.

Seminal Plasma Traits

No difference was found between the two lines for any of the three seminal plasma traits (pH, uric acid, and total protein concentrations). Values of pH and of total protein concentration were, respectively, 8.8 and 5.86 g/L in both lines. Concentration of uric acid was 91.5 and 100.8 mg/L, respectively, in the R⁻ and R⁺ lines.

TABLE 3. Least squares (LS) means and coefficients of variation of sperm composition and spermatozoa variables

Trait	LS mean[1]		Value of F and significance of effect[2]				
	R⁻ line[3]	R⁺ line[4]	Line	Cock/line	Time	Line × time	CV[1]
							(%)
Volume, μL	435	444	0.05	7.7***	8.6***	1.4	20
Concentration, spermatozoa/mL	2.98×10^9	1.54×10^9	24.5***	2.6**	1.7	0.7	35
Number of spermatozoa	1.33×10^9	0.70×10^9	18.5***	2.6**	2.4	2.3	38
Motility	4.4	2.3	41.2***	4.0***	2.2*	3.2**	28
Proportion of dead spermatozoa	0.036	0.061	7.1*	1.5	2.6	1.1	64
Proportion of abnormal spermatozoa	0.043	0.052	0.6	5.3***	1.7	2.0	44
Proportion of living spermatozoa	0.92	0.89	3.8	3.7***	2.9*	0.2	4

[1]Untransformed variables.

[2]Arc sine square root transformed variables (for variables expressed as a proportion).

[3]R⁻ = low feed intake line.

[4]R⁺ = high feed intake line.

*$P < 0.05$.

**$P < 0.01$.

***$P < 0.001$.

Sperm Mitochondria Evaluation

Mean values (± SE) of the relative green fluorescence, assessing the overall mitochondrial mass of the cells, were 76.54 ± 3.46 for R⁻ cocks and 63.95 ± 2.64 for R⁺ cocks, with a significant line difference ($P < 0.01$). Mean values (± SE) of Rh123 green fluorescence were 133.33 ± 2.33 for R⁻ cocks and 110.60 ± 6.34 for R⁺ cocks; this difference was significant ($P < 0.01$). However, the ratio of Rh123 to NAO fluorescence was similar in both lines (1.74 and 1.73, respectively).

DISCUSSION

The present work intended to explain why R⁺ cocks, having a high caloric demand, also have poor reproductive capabilities when compared to R⁻ males with similar body weight but lower caloric demand. For that purpose, cocks of the two lines have been compared for their reproductive performance in relation to sperm quality.

Both lines showed rather poor overall reproductive performance, possibly due to the level of inbreeding and to the duration of egg storage before incubation. Indeed, inbreeding level was high, but it was similar (about 0.3) in both lines (Bordas *et al.*, 1992). Concerning egg storage, longer (3 wk) storage, as in this experiment, is known to depress fertility and hatchability in the R⁻ and R⁺ lines, to the same extent in both lines (Bordas and Mérat, 1993). Inbreeding and duration of egg storage therefore cannot explain why the results of reproduction were different in the two lines, with a much larger proportion of unfertilized eggs and with higher losses during early embryo development in the R⁺ line. As late embryo mortality and dead in shell eggs were similar in both lines, observed differences in hatching rate as well as those in proportion of fertilized eggs appear to stem from events that took place around the time of fertilization. These results confirm a previous study (Ladjali *et al.*, 1995), in which embryo development was observed, and which showed that the percentage of unfertilized eggs was significantly higher in the R⁺ line. Within line, the only difference between sires was for the rate of unfertilized eggs. It is interesting to note that in the early generations of the divergent selection, losses at hatching were higher in the R⁺ line because duration of incubation was longer in this line (Bordas and Mérat, 1993). This line difference was then eliminated by having the eggs incubate 12 h longer ever since.

As no significant difference was found between seminal plasma in the two lines for pH, uric acid, and protein concentrations, these characters were certainly not involved in the reduced fertility of the R⁺ line. Of course, total protein concentration may not reflect possible variation in the content of specific, potentially vital proteins. However, Thurston *et al.* (1992) showed that total protein could be used as an indicator of semen quality in turkeys because a high level of total protein in the seminal plasma was associated with reduced fertility and hatchability.

Line differences could then be explored further, on the male side, by considering sperm and spermatozoa contents and activity. The mean spermatozoa number of an ejaculate was almost 50% lower in R⁺ the number in R⁻ cocks. Because each ejaculate was distributed to five hens during artificial insemination, each R⁻ female received an average total number of 266 million spermatozoa as compared to 141 million for a R⁺ female. However, hens have been inseminated twice a week, so even the lower figure for the R⁺ line was well within the range of the number of spermatozoa required for successful artificial insemination (Brillard and de Reviers, 1989). Indeed, Bramwell *et al.* (1995) found less than a 0.3 percentage difference in fertility (from 97.4 down to 94.7) 4 d after AI, for doses of 100 and 50 million spermatozoa. Therefore, it is questionable

whether and to what extent the large 24-point difference in the proportion of fertilized eggs between the two lines can be explained by the lower number of spermatozoa available for fertilization in the R^+ line.

However, as spermatozoa motility was lower and the ratio of dead or abnormal spermatozoa was higher in the R^+ line, those traits might also be involved in the reduced fertility observed in R^+ cocks, as shown by the correlation observed between the abnormal spermatozoa ratio and the unfertile eggs:incubated eggs ratio (+0.45, $P < 0.10$) and between the abnormal spermatozoa ratio and the hatched eggs:incubated eggs ratio (–0.51, $P < 0.05$).

In other respects, the results clearly showed that mitochondrial content of sperm cells were lower in the R^+ line, as they confirmed unpublished observations of NAO fluorescence (data not shown) on five males each, made in a preliminary trial, with mean values of 96.6 and 54.6 respectively for R^- and R^+ cocks. In the present work, both NAO and Rh123 incorporations were lower in the R^+ line and they remained in the same relative proportions than in the R^- line. Moreover, line difference in Rh123 incorporation did not depend on cell viability, because the same low proportion of dead cells (4.9%) was observed in both lines after PI staining. Therefore, the two lines had the same mitochondrial activity per mitochondrial inner membrane present in the cell, but the R^+ line appeared to have less mitochondria than the R^- line (17% less, when assuming a perfect correspondence between the fluorescence of mitochondrial inner membranes and the number of mitochondria). Indeed, in spermatozoa, adenosine triphosphate (ATP) produced by mitochondria is essential for motility, and mitochondrial dysfunction is known to affect sperm motility (Auger *et al.*, 1989, 1993; Folgerø *et al.*, 1993).

Overall, the accumulation of inferior results for several sperm and spermatozoa variables measured in the present work indicates that some detrimental physiological condition appears to prevail in R^+ males, directly in relation to the decreased reproduction performances of the line at fertilization. The primary cause is unknown but observations on mitochondria contents and activity indicate that, beyond the depressing effect of inbreeding common to both R^+ and R^- lines, selection might have affected some cellular function involving the production of energy in the R^+ line. If it is the case, decrease of mitochondrial contents as a genetic consequence of selection to increase residual feed consumption could have taken place in any cell or tissue. Mitochondrial content should be studied next in other tissues of R^+ and R^- animals, including hens as line differences at fertilization reported here may also have been due to line differences on the female side, at least to some extent. Indeed, sire-related line differences for mitochondrial contents and quality of sperm were clearly associated with differences of reproduction performances between the two lines. On the other hand, possible female contribution to line differences obtained for reproduction traits around the time of fertilization could not be separated from male-related differences, as all matings were among individuals belonging to the same line.

ACKNOWLEDGMENTS

The authors are very grateful to Elisabeth Blesbois and Isabelle Grassot for helpful comments and advice and wish to express appreciation to Baudouin Rivet for his technical assistance and to Jean-Louis Monvoisin for the analysis of seminal plasma contents.

This work was supported by a grant from the A.I.P. "Variabilité génétique de la viabilité embryonnaire chez les animaux domestiques", Institut National de la Recherche Agronomique.

REFERENCES

Auger, J., X. Ronot, and J. P. Dadoune, 1989. Human sperm mitochondrial function related to motility: a flow and image cytometric assessment. J. Androl. 10:439–448.

Auger, J., S. Leonce, P. Jouannet, and X. Ronot, 1993. Flow cytometric sorting of living, highly motile human spermatozoa based on evaluation of their mitochondrial activity. J Histochem. Cytochem. 41:1247–1251.

Blesbois, E., and D. Hermier, 1990. Effects of high-density lipoproteins on storage at 4 °C of fowl spermatozoa. J. Reprod. Fertil. 90:473–482.

Blom, E., 1950. A one minute live-dead sperm stain by means of eosin-nigrosin. J. Fertil. Steril. 1:176–177.

Bordas, A., and P. Mérat, 1984. Genetic variation and phenotypic correlations of food consumption of laying hens corrected for body weight and production. Br. Poult. Sci. 22:25–33.

Bordas, A., and P. Mérat, 1993. Durée d'incubation et effet du stockage des oeufs sur le taux d'éclosion dans les lignées de poules sélectionnées sur la consommation alimentaire résiduelle. Genet. Sel. Evol. 25:397–402.

Bordas, A., M. Tixier-Boichard, and P. Mérat, 1992. Direct and correlated responses to divergent selection for residual food intake in Rhode Island Red laying hens. Br. Poult. Sci. 33:741–754.

Bramwell, R. K., H. L. Marks, and B. Howarth, 1995. Quantitative penetration of the perivitelline layer of the hen's ovum as assessed on oviposited eggs. Poultry Sci. 74:1875–1883.

Brillard, J. P., and M. de Reviers, 1989. L'insémination artificielle chez la poule. Bases physiologiques et maîtrise du taux de fécondation des oeufs. Prod. Anim. 2:197–203.

Burrows, W. H., and J. P. Quinn, 1937. The collection of spermatozoa from the domestic fowl and turkey. Poultry Sci. 16:19–24.

Darzynkiewicz, Z., F. Traganos, L. Staiano-Coico, J. Kapuscinski, and M. R. Melamed, 1981. Interactions of rhodamine-123 with living cells studied by flow cytometry. Cancer Res. 42:799–806.

El-Kazzi, M., A. Bordas, G. Gandemer, and F. Minvielle, 1995. Divergent selection for residual food intake in Rhode Island Red egg-laying lines: gross carcase composition, carcase adiposity and lipid contents of tissues. Br. Poult. Sci. 36:719–728.

Emaus, R. K., R. Grunwald, and J. J. Lemasters, 1986. Rhodamine-123 as a probe of transmembrane potential in isolated rat-liver mitochondria: spectral and metabolic properties. Biochem. Biophys. Acta 850:436–448.

Folgerø, T., K. Bertheussen, S. Lindal, T. Torbergsen and P. Øian, 1993. Mitochondrial disease and reduced sperm motility. Human Reprod. 8:1863–1868.

Geraert, P. A., S. Guillaumin, A. Bordas, and P. Mérat, 1991. Evidence of a genetic control of diet-induced thermogenesis in poultry. Pages 380–383 *in*: Proceedings of the 12th symposium on energy and metabolism in farm animals, Kartause Ittingen Switzerland, 1–7 September 1991, European Association of Animal Production Publications 58.

Hammade, H., 1986. Effet des gènes majeurs (Na, dw) sur les caractéristiques zootechniques et de la reproduction chez les coqs élevés à deux températures. Ph.D. thesis, University of Rennes, Rennes, France.

Johnson, L. V., M. L. Walsh, B. J. Bockus, and L. B. Chen, 1981. Monitoring of relative mitochondrial membrane potential in living cells by fluorescence microscopy. J. Cell Biol. 88: 526–535.

Ladjali, K., A. Bordas, E. P. Cribiu, and M. Tixier-Boichard, 1995. Contribution de la cytogénétique à l'étude de la fertilité de 2 lignées de poules pondeuses sélectionnées sur la consommation alimentaire résiduelle. Genet. Sel. Evol. 27:313–322.

Lake, P. E., and O. Ravie, 1979. Effect on fertility of storing fowl semen for 24 h at 5°C in fluids of different pH. J. Reprod. Fertil. 57:143–155.

Leprat, P., M. H. Ratinaud, A. Maftah, J. M. Petit, and R. Julien, 1990. Use of nonyl acridine orange and rhodamine 123 to follow biosynthesis and functional assembly of mitochondrial membrane during L1210 cell cycle. Exper. Cell Res. 186:130–137.

McClean, D. J., N. Korn, B. S. Perez, and R. J. Thurston, 1993. Isolation and characterization of mitochondria from turkey spermatozoa. J. Androl. 14:433–438.

Maftah, A., J. M. Petit, M. H. Ratinaud, and R. Julien, 1989. 10-N nonyl acridine orange: a fluorescent probe which stains mitochondria independently of their energetic state. Biochem. Biophys. Res. Commun. 164:185–190.

Maftah, A., J. M. Petit, and R. Julien, 1990. Specific interaction of the new fluorescent dye 10-N-nonyl acridine orange with inner mitochondrial membrane. FEBS Lett. 260: 236–240.

Nadakavukaren, K. K., J. J. Nadakavukaren, and L. B. Chen, 1985. Increased rhodamine-123 uptake by carcinoma cells. Cancer Res. 45:6093–6099.

O'Connor, J. E., K.J.L. Vargas, B. F. Kilmer, J. Hernandez-Yago, and S. Grisolia, 1988. Use of rhodamine-123 to investigate alterations in mitochondrial activity in isolated mouse liver mitochondria. Biochem. Biophys. Res. Commun. 151: 568–573.

Petit, J. M., A. Maftah, M. H. Ratinaud, and R. Julien, 1992. 10N-Nonyl acridine orange interacts with cardiolipin and allows the quantification of this phospholipid in isolated mitochondria. Eur. J. Biochem. 209:267–273.

Petit, J. M., O. Huet, P. F. Gallet, A. Maftah, M. H. Ratinaud, and R. Julien, 1994. Direct analysis and significance of cardiolipin transverse distribution in mitochondrial inner membranes. Eur. J. Biochem. 220:871–879.

Petit, J. M., M. H. Ratinaud, E. Cordelli, M. Spanò, and R. Julien, 1995. Mouse testis cell sorting according to DNA and mitochondrial changes during spermatogenesis. Cytometry 19:304–312.

Sexton, T. J., 1977. A new poultry semen extender. 1. Effect of extension on the fertility of chicken semen. Poultry Sci. 56: 1443–1446.

Thurston, R. J., R. A. Hess, and N. Korn, 1992. Seminal plasma protein concentration as a predictor of fertility and hatchability in Large White domestic turkeys. J. Appl. Poult. Res. 1:335–338.

Tixier, M., A. Bordas, and P. Mérat, 1988. Divergent selection for residual feed intake in laying hens: effect on growth and fatness. Pages 129–132 *in*: Leanness in Domestic Birds. B. Leclercq and C. Whitehead, ed. Butterworths, London, UK.

Windsor, D. P., and I. G. White, 1993. Assessment of ram sperm mitochondrial function by quantitative determination of sperm rhodamine 123 accumulation. Mol. Reprod. Dev. 36:354–360.

Zein-el-Dein, A. Bordas, and P. Mérat, 1985. Sélection divergente pour la composante résiduelle de la consommation alimentaire des poules pondeuses: effets sur la composition corporelle. Archiv. Geflügelkd. 49:158–160.