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Performance and proposal of reproductive management in European quails

Desempenho e proposta de manejo reprodutivo em codornas europeias

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Abstract: The objective of this study was to verify posture, fertility, and embryonic viability depending on the time and frequency use of the male for reproduction, as well as evaluate sperm storage time in the female after copulation in European quails. For this, 180 males were used (18 males mated with 10 females each). The experimental design was completely randomized in a 2x10 factorial scheme, with two genetic lineages and ten days of use for couples, with 14 replications. Males remained in copulation for ten consecutive days, mating each day with a different female. Information on egg laying, embryonic viability, and egg fertility was collected daily for fourteen days after copulation. The time males remained in copulation did not alter the egg fertility. In females, the presence and possible stress caused by males did not change the laying rates and eggs' embryonic viability. The fertility rate was higher than 70% only three to five days after copulation, not achieving the same performance on other days. Based on these observations, a more efficient reproductive management proposal for large populations may be implemented using three females for each male on alternate days, with egg collection starting on the third day after the first copulation, with the necessity of another copulation every three days per female, considering the mating period of 10 consecutive days. For smaller breeding stocks, the ratio of two females for one male may be used as an alternative, considering a rest day for the male.

Key-words: *Coturnix coturnix*; embryodiagnosis; fertility; posture; reproduction.

Resumo: Objetivou-se verificar postura, fertilidade e viabilidade embrionária em função do tempo e frequência de utilização do macho para a reprodução, assim como avaliar o tempo de armazenamento espermático na fêmea após a cópula em codornas europeias. Para isso, foram utilizados 180 animais (18 machos acasalados com 10 fêmeas cada). O delineamento foi inteiramente casualizado, em esquema fatorial 2x10, com duas linhagens genéticas e 10 dias de utilização dos casais, com 14 repetições. Os machos foram mantidos em cópula por dez dias consecutivos, acasalando cada dia com uma fêmea distinta. As informações sobre postura, viabilidade embrionária e fertilidade de ovos foram coletadas diariamente durante 14 dias após a cópula. O tempo de permanência dos machos em cópula não alterou a fertilidade dos ovos. Nas fêmeas, a presença e o possível estresse causado pelos machos não alteraram as taxas de postura e viabilidade embrionária dos ovos. A taxa de fertilidade foi superior a 70% apenas de três a cinco dias após a cópula, não obtendo o mesmo desempenho nos demais dias. A partir dessas observações, uma proposta de manejo reprodutivo mais eficiente para grandes populações pode ser implementada com uso de três fêmeas para cada macho, em dias alternados, com coleta de ovos a partir do terceiro dia após a primeira cópula, sendo necessária outra cópula a cada três dias por fêmea, considerando o período de acasalamento de 10 dias consecutivos. Para plantéis menores, sugere-se a proporção de duas fêmeas para cada macho, com um dia de descanso para o macho.

Palavras-chave: Coturnix coturnix; embriodiagnóstico; fertilidade; postura; reprodução.

1. Introduction

In quails, the reduced seminal volume, resulting from the absence of accessory sexual glands, and the production of foam before ejaculation make individual sperm analyses difficult for selecting reproductive males. Sperm storage in sperm storage tubules (SST), which occurs at the uterovaginal junction of the bird's reproductive system, represents, however, an alternative for predicting the reproductive potential of males as it reflects sperm viability in the female's reproductive system, increasing the breeding stock's offtake. Furthermore, it allows the production of fertile eggs for longer, ensuring the perpetuation of the species even without the constant presence of the male[1].

Reproductive management in coturniculture usually involves the insertion and permanence of the male with one or more females during the reproductive period. Its duration depends on the characteristics of the production system and the reproductive potential of the male [2]. In several species of farm animals, high-frequency use of the same breeding male results in reduced sperm quality and volume[3,4,5]. In quails, however, the consequences of this type of management are not yet well established. Furthermore, as they are fast-breeding animals, there is a high possibility of inbreeding, with consequent loss of genetic material, reduced performance, and expression of harmful recessive genes[6].

Therefore, due to the scarcity of information about the ideal time for the male to remain with the female during the mating period, this study aimed to verify posture, fertility, and embryonic viability depending on the usage time of the male for reproduction. It also allowed the evaluation of sperm storage time in the female reproductive system after copulation, and new recommendations were proposed regarding the reproductive management of European quails.

2. Material and methods

The experiment was carried out in the poultry farming sector of the Instituto de Ciências Agrárias da Universidade Federal de Minas Gerais (ICA - UFMG), Brazil, located at latitude 16º 44′ 06″ S, longitude 43º 51′ 42″ W, and average altitude of 648 meters in Montes Claros city in the state of Minas Gerais, with the approval of the Ethics and Animal Use Committee, under protocol no. 45/2022. The experiment design carried out was completely randomized with factorial scheme (2x10), with two genetic groups (ICA1 and ICA2) of males (M) and females (F) combined with ten days of use of the couples for reproduction (Table 1), followed by 14 days of egg collection from each female after copulation.

Table 1. Schematic representation of the experimental design

LINEAGES							
ICA 1				ICA 2			
Male	Female	Copulation (day)	Egg collection (days)	Male	Female	Copulation (day)	Egg collection (days)
1	1	1	2 to 16	10	91	1	2 to 16
	2	2	3 to 17		92	2	3 to 17
	3	3	4 to 18		93	3	4 to 18
	4	4	5 to 19		94	4	5 to 19
	5	5	6 to 20		95	5	6 to 20
	6	6	7 to 21		96	6	7 to 21
	7	7	8 to 22		97	7	8 to 22
	8	8	9 to 23		98	8	9 to 23
	9	9	10 to 24		99	9	10 to 24
	10	10	11 to 25		100	10	11 to 25
...	...	...	...	...	...	...	...
9	81	1	2 to 16	18	171	1	2 to 16
	82	2	3 to 17		172	2	3 to 17
	83	3	4 to 18		173	3	4 to 18
	84	4	5 to 19		174	4	5 to 19
	85	5	6 to 20		175	5	6 to 20
	86	6	7 to 21		176	6	7 to 21
	87	7	8 to 22		177	7	8 to 22
	88	8	9 to 23		178	8	9 to 23
	89	9	10 to 24		179	9	10 to 24
	90	10	11 to 25		180	10	11 to 25

Note: *Male 1 was placed with female "a" of the same lineage during day 1 of mating. On the following day, the same male was placed with female "b" and so on, until reaching female "j" on day 10 of mating. Eggs were collected the next day after copulation for 14 days.*

Nine male European quails from each genetic group (ICA1 and ICA2) were used, totaling 18 males aged 75 to 90 days, sexually mature. For each male, ten females of the same age and lineage (n=180) were used, totaling 178 animals. The males were changed daily from cages to mate with a new female kept with the male for 24 hours. The animals remained in a closed shed with fans, in single-level galvanized wire cages, housed individually, with food and water provided *ad libitum*. The average minimum and maximum daily temperatures during the experiment were 20.5ºC and 32.9°C, respectively, and humidity was 50%.

After one day of copulation for each female, the eggs were collected in the late afternoon. They were identified during the subsequent 14 days, allowing us to investigate the influence of sperm storage time within the SST on egg-laying and fertility. After identification, the eggs were maintained in a commercial incubator at 37.8°C and 60% humidity, with continuous ventilation and automatic turning every hour for 14 days.

The eggs remained at 4°C for 48 hours to interrupt development to ensure the death of the embryos after 14 days of incubation. After this procedure, the eggs were broken and subjected to embryodiagnosis. The classification used to evaluate embryos during the stages of embryonic development may be observed in Figure 1.

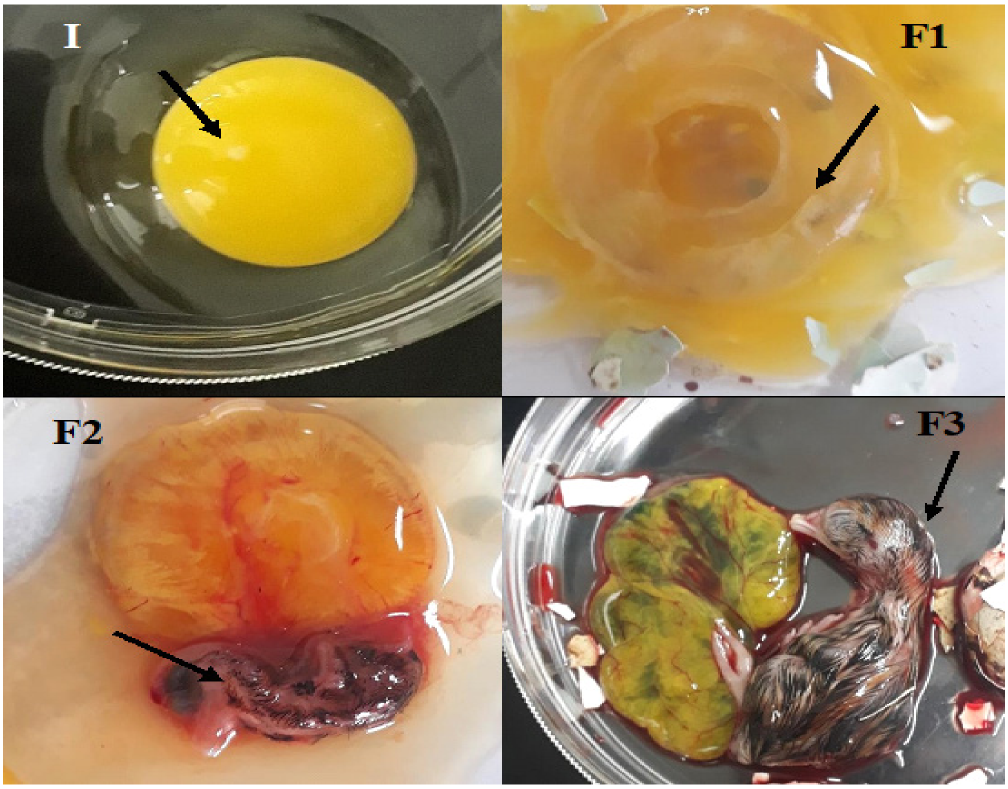

Figure 1. Classification of embryonic development phases. I = Infertile; F1 = Phase of appearance of the embryonic disc, formation of blood vessels, and development of eyes and beaks; F2 = Embryo formation and growth phase, appearance of feathers; F3 = fully formed embryo stage, ready for hatching. Photos: Authors, 2022.

The number of eggs laid over 14 days of collection (E/D), the proportion of eggs laid that were viable (V/E), the proportion of viable eggs that were fertile (F/V), and the proportion of fertile eggs that reached phase 3 of embryonic development (E/F) were measured to verify reproductive performance per female. For all response variables, E/D, V/E, F/V, and E/F, analyses of variance were performed to verify the occurrence of interactions between the factors of genetic group (1 and 2), time of use of males (1 to 10 days), and number of days after female copulation (0 to 14 days). Considering non-significant (ns) interactions, joint regression analyses of variance were performed to verify the linear and quadratic effects of the number of days of use in males and the storage time of sperm in females, according to the model: $z_{ijk} = \alpha 0 + \beta_1 x + \beta_2 x^2 + \gamma_1 y + \gamma_2 y^2 + eijk$.

Where: $zijk$ = observed value for E/D, V/E, F/V, or E/F; α_0 = model intercept; x = number of days in service of the male; β_1 = linear effect of male usage time in days; β_2 = quadratic effect of the males' usage time in days; y = number of days between female copulation and egg laying; γ_1 = linear effect of sperm storage time in females; γ_2 = quadratic effect of sperm storage in females; e = experimental error associated with the observation. All statistical analyses were performed using SAS software. For the characteristics that presented a quadratic effect, the derivative of the model estimated was performed as a function of the effect. The derivative equal to zero was set to obtain the maximum point.

3. Results and discussion

The absence of a linear or quadratic effect on the time males were used for reproduction for all characteristics evaluated indicates that the use of males for up to ten consecutive days did not interfere with fertility and embryonic quality (Table 2). Quail spermatogenesis lasts 8 to 12 days, and 92.5 x 10^6 spermatozoa are produced per gram of testicles per day on average (7) so that an ejaculate contains an average concentration of 12x10^9 sperm per mL (8). This condition differs from that observed in most domestic production mammals, whose spermatogenesis lasts between 40-60 days on average (9, 10, 11). This intense sperm renewal in quails makes it possible to use the male for mating for several consecutive days without reducing fertility, as confirmed in this research.

Table 2. Model statistics for the variables: eggs per female day (E/D), eggs laid that were viable (V/E), viable eggs that were fertilized (F/V), and fertile eggs that reached phase 3 of development (E/F).

Variables	Statistics	α_0	β_1	β_2	γ_1	γ_2
E/D	Parameter	0.4975	---	---	---	---
	p-value	<0.0001	ns	ns	ns	ns
V/E	Parameter	0.8940	---	---	---	---
	p-value	<0.0001	ns	ns	ns	ns
F/V	Parameter	0.5948	---	---	0.0568	-0.0069
	p-value	<0.0001	ns	ns	0.0006	<0.0001
E/F	Parameter	0.6093	---	---	-0.0500	---
	p-value	<0.0001	ns	ns	<0.0001	ns

α_0 intercept of the equation; β_1 and β_2 linear and quadratic coefficients for the number of days of males in reproductive use; γ_1 and γ_2 linear and quadratic coefficients for the number of days after mating with the female; ns non-significant.

A reduction in fertility and egg laying was found resulting from stress when female quails were kept in cages with the male for 16 weeks in a ratio of 1M:2F, which demonstrates that the length of time the male spends with the females and the ratio M:F can negatively interfere with the productivity of the breeding stock [12]. The stressful effect of the presence of the male may be reduced when males and females are in individual cages close to each other throughout the breeding stock period [13]. However, the maintenance of egg laying and viability (p-value <0.0001, Table 2) in this experiment indicated that the presence of the male and collection of eggs for 14 days after copulation did not sufficiently stress the female to the point of changing the laying or quality of eggs when kept in the 1M:1F ratio for 24 hours. Indirectly, there was no exhaustion in the male, possibly causing a reduction in libido and reproductive potential.

For the characteristic of fertile viable eggs (F/V), quadratic behavior occurred depending on the number of days after copulation, reflecting the sperm storage time in the SST. The F/V ratio was higher than 70% between the third and fifth day after mating. Until the eighth day after copulation, the percentages exceeded at least 60%, followed by a significant reduction from the ninth day onwards and reaching the lowest estimated value of 3.8% on the fourteenth day (Figure 2). That indicates that waiting, at least the third day after the first copulation, to collect the egg can bring advantages in fertility with a direct impact on incubation performance.

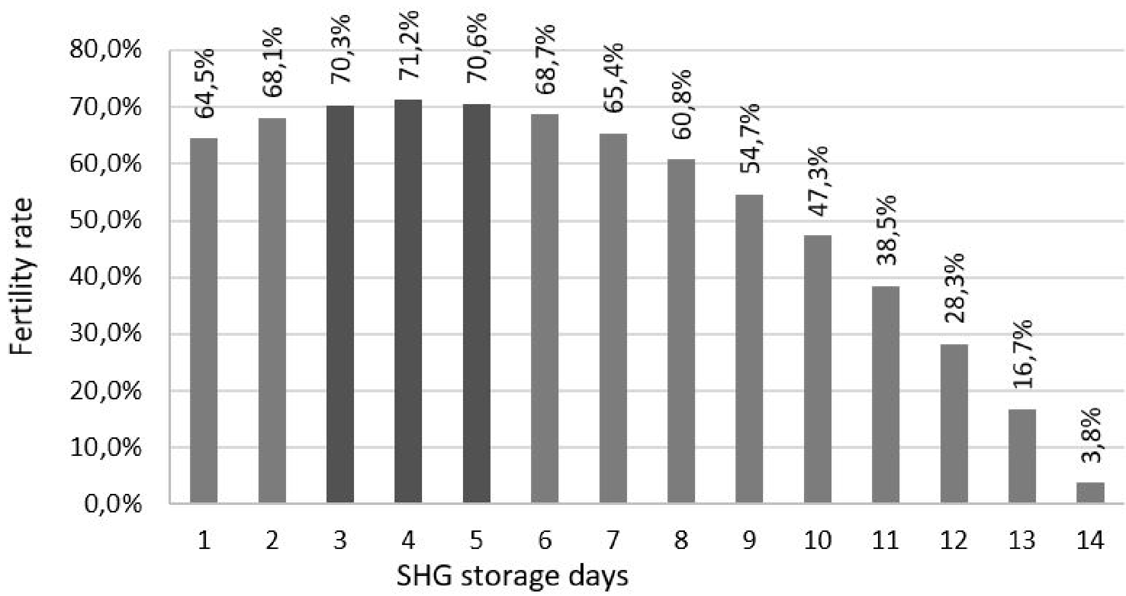

Figure 2. Egg fertility as a function of sperm storage days in female host glands.

The obtaining of fertile eggs until the fourteenth day after mating, despite the reduction in fertility after the fifth day of laying, is probably due to infundibular tubules (ITs) in quails. These, in addition to SST, can store a small amount of sperm on the way up to the oviduct. The presence of fertile sperm in both storage tubules (SST and ITs) of quails for 14 days was also reported by other authors [1]. For fertile eggs that reached phase 3 (E/F), the model chosen includes intercept and linear effect for the number of days after copulation. Thus, each day after copulation, the chance of a fertilized egg developing to stage 3 is reduced by 5%. During the storage of sperm in the SST and ITs, there is a reduction in sperm quality due to the production of free radicals, which can impact subsequent embryonic development [14]. Evidence shows that, upon entering the SST, sperm become immobile due to the local acidic pH. This immobility reduces the production of free oxygen radicals before ascending to the infundibulum, the site of fertilization [15, 16]. In addition to sperm characteristics, factors such as egg storage conditions, incubation temperature and humidity, and the female's age can also affect embryonic mortality [17].

Based on the results of this study, it was possible to propose different reproductive management for European quails to optimize the use of males and obtain a higher fertility rate, as outlined in Table 3. One represents the traditional management scheme (A), and two alternative ones (B and C). For all schemes, the effective size [4MF/(M+F)] and the inbreeding increase rate (0.5/N) were calculated, considering two hypothetical populations, 96 and 48 females, respectively. M represents the number of males, F is the number of females, and N is the effective size.

Table 3. Schemes A, B, and C of quail mating system.

Male schemes	Male	Female	Copulation days	Start of egg collection
A (1M:2F)	1	A	1st, 3rd, 5th, ...	2nd day
		B	2nd, 4th, 6th, ...	3rd day
B (1M:2F)	1	A	1st, 4th, 7th, ...	4th day
		B	2nd, 5th, 8th, ...	5th day
		Rest	3rd, 6th, 9th, ...	Rest
C (1M:3F)	1	A	1st, 4th, 7th, ...	4th day
		B	2nd, 5th, 8th, ...	5th day
		C	3rd, 6th, 9th, ...	6th day

In scheme A (Table 3), usually used in breeding stocks, considered traditional management, the male is kept inside the cage with the same female for the entire day and, on the following day, it is placed with another female for 24 hours, alternating between these two females throughout the reproductive period. The fertilized eggs are collected the day after copulation.

Scheme B (Table 3) considers a day of rest for the male. It remains with a female for 24 hours, the next day with another female, and on the third day, the male is kept alone to rest. This recommendation is valid for smaller breeding stocks, as the resting day allows greater fertility control. Fertilized eggs can be collected from the third day after mating due to fertility exceeding 70%, according to the results presented (Figure 2).

In scheme C (Table 3), three females are used per male during the mating period, alternating between the three females, as the male can spend ten consecutive days in copulation without interfering with fertility. Egg collection in this scheme can also be carried out three days after copulation.

Using three females is more appropriate in higher breeding stocks, as it allows for better reproductive efficiency and enables a higher selection pressure in males without causing inbreeding problems and a rapid reduction in genetic variability due to the higher number of individuals. From a reproductive point of view, it does not reduce the fertility rate of females since after three days of copulation with the first female, the male returns to copulate with her, maintaining the storage of sperm in the SST. Among the schemes presented, C presents higher efficiency and offtake rate for the animals, as the male quail can mate with more females and maintain a high fertility rate. However, to adopt this method, the effective size of the breeding stock must be considered to avoid reducing the number of families and population variability and, as a consequence, inbreeding.

Considering a hypothetical breeding stock with 96 females, using schemes A and B, 48 males would be needed for reproduction, generating an effective size of 128 animals and an increase in inbreeding of 0.39%. With scheme C, 32 males would be needed, generating an effective size of 96 animals, and the inbreeding increase rate would be 0.52%.

For a smaller hypothetical population, with only 48 breeding females, schemes A and B would require 24 males, with an effective size of 64 animals, with a 0.78% increase in inbreeding. In scheme C, 16 males would be required, with an effective size of 48 animals, with an estimated increase in inbreeding of 1.04%, generating a faster reduction in variability and a higher occurrence of congenital abnormalities and reproductive failures in future generations. Therefore, scheme A, considered the traditional one, can be replaced by C in larger populations, as it optimizes the use of males. Scheme B would be more suitable for small breeding stocks to reduce inbreeding and provide animals with rest time without harm to hatching. It is relevant, thus, to adapt reproductive management according to the size of the breeding stock and the risk of inbreeding to be accepted.

4. Conclusion

The male quail can remain in reproduction for ten consecutive days without changing sperm viability, laying, and egg fertility, while females have fertility greater than 70% from the third to the fifth day post-coital. This condition allows reproductive management using up to three females for each male, on alternate days, with eggs collected from the third day after the first mating, requiring another mating every three days per female.

Statement of conflict of interest

There are no conflicts of interest to declare.

Data availability statement

The data will be provided upon request.

Author contributions

Conceptualization: L. F. Crocomo, F. Ferreira and F. G. Silva. Data curation: N. P. M. O. Barbosa, D. D. Pereira and J. V. S. Prates. Formal analysis: F. G. Silva. Methodology: L. F. Crocomo, F. Ferreira and F. G. Silva. Investigation: N. P. M. O. Barbosa, D. D. Pereira and J. V. S. Prates. Project Administration: L. F. Crocomo, F. Ferreira and F. G. Silva. Supervision: L. F. Crocomo, F. Ferreira and F. G. Silva. Resources: L. F. Crocomo, F. Ferreira and F. G. Silva. Visualization: N. P. M. O. Barbosa, D. D. Pereira and J. V. S. Prates. Writing (original draft, review and editing): L. F. Crocomo, F. Ferreira, F. G. Silva, N. P. M. O. Barbosa, D. D. Pereira, J. V. S. Prates and A. L. M. Athayde

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