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Age-related declines in ejaculate quality and sperm kinematics vary among strains of Japanese Quail (*Coturnix japonica*)

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Abstract

For successful breeding programs, it is important to quantify the useful period of a male's reproductive life and it is often done simply by measurement of semen quality. This information is lacking for Japanese quail so we tested whether there is a decline in ejaculate quality and sperm kinematics with age, and whether the decline varies among strains. Nine males (n = 9) from each of 5 strains (A, B, C, D, E) were subjected to 4 semen collections at 4 ages (8, 16, 26, 36 weeks). Ejaculate volume, sperm concentration and total sperm per ejaculate were measured, and sperm kinematics were analysed using a Sperm Class Analyser (SCA[®]). Ejaculate volume peaked at Week 26 in all strains, and thereafter declined significantly only in Strain A. Peak values for sperm concentration and total sperm per ejaculate were observed at Week 16 for most strains, after which sperm concentration declined significantly by Week 26 in strains A, D and E, whereas total sperm per ejaculate declined significantly only for Strains A and E at Week 36. There were declines in percent motile sperm, progressive motile sperm, and rapid sperm, and in curvilinear velocity, straight line velocity and average velocity path, by Week 16 for most strains. Linearity declined by Week 26 in some strains, and all strains showed a significant decline in beat cross frequency by that age. The declines in lateral head displacement were also significant at Week 26 for Strains A and E. In conclusion, the ability of CASA to detect age-related changes in sperm kinematics makes it a valuable tool for identifying the best male and thus improving quail flock fertility. It is essential that breeders understand that age affects both sperm production and sperm kinematics, and that the changes vary with strain.

Key words: Quail reproduction, age, fertility, strain, sperm motility, CASA

Running title: Sperm kinematics in aging quail

1.0 Introduction

Successful fertilization depends on the production of good quality gametes from both males and females, as well as the success of the physical act of mating, the transportation of sperm to the egg fertilization site, and the union of sperm and ova. Generally, from the start until the peak of production, both sexes produce good quality gametes. Thereafter, quality deteriorates. This decline with age needs to be understood if we are to offer gender-specific strategies for delaying the process or for improving breeding programs by selection of individuals with desirable characteristics.

In Japanese quail, egg fertility is highest between the ages of 10 and 20 weeks, with a peak at 14-16 weeks (Woodard & Abplanalp, 1967; Narahari et al., 1988; Farooq et al., 2012). Flock fertility thus declines after the peak, yet the available data suggests there are no changes in male reproductive behaviour, testosterone secretion, or testis weight until they are 70 weeks old (Ottinger et al., 1983). Obviously, both sexes contribute to successful egg fertilization, and therefore to any decline in fertility, but there has been little exploration of the role of the male. Importantly, a male can serve multiple females over a long period, and thus contribute to the fertility of several hundred eggs, so male fertility is clearly important, yet there has been little exploration of how it is affected by advancing age.

The most commonly used methods of assessing male fertility are semen volume and colour, and sperm concentration, motility, viability, and morphology, and age-related decline is also assessed by physical ability to copulate and the genetic quality of the gametes (Sexton et al., 1989; Cordero & Miller, 1992; Donoghue & Wishart, 2000; Sharpe et al., 2003; Pizzari et al., 2008). In Japanese quail, some studies have revealed lower sperm production, higher proportions of testicular abnormalities, and reductions in sexual activity by age 1 year (Eroschenko et al., 1977; Ottinger, 1983), but little is known of changes in traditional sperm quality traits, let alone sperm kinematics.

Kinematic measures of sperm motility are widely considered important for assessing ejaculates and fertility in a wide variety of species (Amann, 1989; Bakst & Cecil, 1992; Farrell et al., 1993; Moore & Akhondi, 1996; Holt et al., 1997; Klimowicz et al., 2008; Long et al., 2010). The technology has also been used to evaluate potential fertility in a variety of poultry species, including the broiler chicken and turkey, as well as quail (King et al., 2000; Long et al., 2010, Farooq et al., 2017, 2018). Moreover, changes in sperm motility parameters with advancing age have been documented in humans (Sloter et al., 2006; Johnson et al., 2015). This research history makes it clear that an investigation of this type will provide vital

information for managing the contribution of the flock fertility in quail. Should age-related issues arise, a solution might be found in variation among strains that could be a factor in selection programs. For quail, age-related decline in male fertility has not been applied to commercial operations, a potentially serious problem when industry populations carry genetic loads that are sensitive to inbreeding (Woodard et al., 1973; Minvielle, 1998, 2004; Cheng, 2002).

In the present study, therefore, we tested whether ejaculate quality and sperm kinematics decline with age in Japanese quail, and whether the effects of age vary among strains.

2.0 Materials and methods

2.1 Housing and management

Japanese quail (9 males for each of 5 strains; $n = 45$) were randomly selected from five meat-type breeder strains (labelled A, B, C, D, E), all with similar body weight at age 4 weeks. The birds had been hatched and reared under commercial conditions (Game Farm Pty Ltd; Galston NSW, Australia) until age 6 weeks, after which they were transferred to the avian laboratory at The University of Western Australia (32°13'S; 115° 38'E). They were housed individually in quail cages (19 cm wide x 40 cm deep x 23 cm high; Cimuka Quail Cages, Turkey) in an environment maintained at 22-26°C with adequate ventilation and a 14:10 light:dark cycle. Feed and water were supplied *ad libitum*. The feed was a quail breeder diet containing 20% CP and 11.5 MJ/kg ME. Cages were enriched by the provision of a sand bath in each pen, with the sand renewed daily. Before the start of measurements, the males were conditioned to being handled and to semen collection using a teaser female (Farooq et al., 2014). All procedures were carried out in accordance with Protocol RA/3/100/941 approved by the Animal Ethics Committee, The University of Western Australia.

2.2 Experimental design

For every male, 4 ejaculates were collected at ages 8, 16, 26 and 36 weeks (total 720 ejaculates). The range of ages was selected to cover the normal production period in commercial quail breeding, in which males are generally culled at age 40 weeks. Ejaculate volume, sperm concentration, and total sperm in the ejaculate were recorded, and then duplicate semen samples were assessed for sperm kinematics, as described below.

2.3 Semen collection and preparation

Immediately collection, ejaculate volume was measured using a micro-pipette (0.1-0.2 µl accuracy) and the semen was diluted 1:2 with NaCl-TES collection buffer (50 mM NaCl and 20 mM *N*-tris-[hydroxymethyl]-methyl-2-aminoethane-sulphonic acid, adjusted to pH 7.40 at 25 °C (Holm & Wishart, 1998) and stored for 10 min at 30 °C. To measure sperm concentration, 1.5 µl neat semen was diluted with 598.5 µl formalin buffer (2% formalin in PBS buffer) in a 1 ml vial, mixed well and transferred to a polystyrene cuvette. Absorbance at 560 nm in a spectrophotometer (Metertech UV/VIS SP8001, Taipei, Taiwan) was compared with a standard curve established in our laboratory for measuring sperm concentration in the emu and in Japanese quail (Sood et al., 2012; Farooq et al., 2017).

2.4 Sperm-class-analyser-computer assisted semen analysis (SCA[®]-CASA)

Semen samples were diluted in a medium comprising motility buffer (150 mM NaCl and 20 mM *N*-tris-[hydroxymethyl]-methyl-2-aminoethane-sulphonic acid, 35 mM glucose and 0.1 % bovine serum albumin adjusted to pH 8.00 at 25 °C) and 10% proctodeal gland foam extract (Farooq et al., 2017). The foam extract had been prepared as described by Farooq et al., (2014). For each male age, fresh foam was collected and extracted.

The CASA equipment has been described previously (Farooq et al., 2017). In brief: we used the Motility Module of the Sperm Class Analyzer (SCA[®]) version 5.2 (Microptic S.L., Barcelona, Spain). Videos were captured using a Basler A602fc digital camera (Microptic S.L.) mounted on an Olympus BX53 microscope (Olympus Optical Co., Tokyo, Japan) equipped with phase-contrast optics and a motorized heated stage (Prior Optiscan II, Prior Scientific Intl.). The capture properties of the SCA[®] system were: 25 images per sec; frame rate 60Hz; optics = Ph- (negative phase contrast); minimum particle size 10 µm, maximum particle size 130 µm; progressivity at 70% STR; green light filter; standard count four-chamber Leja 10 slides (Leja products BV, Nieuw-Vennep, Netherlands).

For motility assessment, 2-3 µl diluted semen was loaded on to a pre-warmed slide, placed on a heated stage at 39 °C, and allowed 45 sec to equilibrate before recordings were made. Ten to 15 fields were captured until a total of 200 motile spermatozoa were collected (WHO, 1999). Fields were captured randomly to eliminate bias toward higher sperm concentration or motility, but fields that included debris or clumps of sperm were excluded. All captured sperm tracks were verified visually to delete incorrectly recorded tracks due, for example, to colliding spermatozoa.

The kinematic measures were: percent motile sperm (%MOT); percent progressive sperm (%PROG); percent rapid sperm (%Rapid); percent medium sperm (%medium); curvilinear velocity (VCL); straight line velocity (VSL); velocity average path (VAP); linearity (LIN); beat cross frequency (BCF); straightness (STR); and lateral head displacement (ALH). For allocation of sperm into slow, medium and rapid categories, the following SCA[®] “VCL intervals” were selected: slow sperm $<45\mu\text{m}/\text{sec}$ < medium sperm $<160\mu\text{m}/\text{sec}$ < rapid sperm.

2.5 Statistical analysis

The motility variables approximated a normal distribution and normal values were used for ejaculate volume, sperm concentration and total sperm per ejaculate. Mixed model procedure of PASW 18.0 (Release Version 18.0.0 (SPSS, Inc., 2009, Chicago, IL www.spss.com) was used to analyze the data. The factors were age and strain, with the interaction term included, and male ID was used as a random effect. Means were compared using least significant differences (LSD). The linear regression analysis procedure of PASW 18.0 (Release Version 18.0.0 (SPSS, Inc., 2009, Chicago, IL www.spss.com) was used to investigate the age-related changes in each measure. All significant values are reported at $P < 0.05$ level unless specified otherwise.

3.0 Results

3.1 Effects of age and strain on ejaculate volume and sperm production

There was significant variation among strains and among ages for all three measures of sperm production. There was a significant interaction between the effects of strain and age for ejaculate volume ($F = 3.6$; $df = 12$; $p < 0.01$) and total sperm per ejaculate ($F = 2.9$; $df = 12$; $p < 0.01$) but not for sperm concentration ($F = 0.67$; $df = 12$; $p < 0.78$). As can be seen in Figure 1, ejaculate volume increased with age, with a significant change in Strains A, B and E between Weeks 8 and 16, that was not significant until Week 26 in Strains C and D. For all strains, ejaculate volume peaked at Week 26 after which a decline was detectable only in Strain A. In all strains, sperm concentration peaked at Week 16 and, in strains A, D and E it decreased significantly by Week 26. For Strain B, the decline from peak value was not significant until Week 36 and, in Strain C, there was no significant change after the peak at Week 16. Values for total sperm per ejaculate peaked at Week 16 in Strains A, B and E, but

not until Week 36 in Strains C and D. Significant declines from peak values at Week 36 were observed only in Strains A and E.

Figure 1. Changes with age in ejaculate volume, sperm concentration and total sperm per ejaculate for five strains of Japanese quail (A ○; B ●; C □; D ■; E ◆). For each age 36 ejaculates (9 male x 4 ejaculates) per strain were analyzed.

Between Weeks 8 and 26, Strain A showed the greatest weekly rate of increase in ejaculate volume ($+ 0.40 \mu\text{L}$ per week; $p < 0.01$), sperm concentration ($+ 0.01 \times 10^6/\mu\text{L}$ per

week; $p < 0.09$ and total sperm per ejaculate ($+ 0.72 \times 10^6/\mu\text{l}$ per week; $p < 0.01$) while Strain D showed the smallest values (Table 1).

3.2 Effect of age and strain on %MOT, %PROG, %Rapid and %Medium sperm

The effect of the interaction between age and strain was significant for %MOT ($F = 3.29$; $df=12$; $p < 0.01$) but not for %PROG ($F = 1.15$; $df = 12$; $p < 0.31$), %Rapid ($F = 1.61$; $df = 12$; $p < 0.08$) and %Medium ($F = 0.75$; $df = 12$; $p < 0.70$). The data are presented in Figure 2. In Strains B, D and E, %MOT declined significantly by Week 16 and then declined further by Week 26. In Strain C, the decline was not significant until Week 26. In Strain A, there was no significant change in %MOT throughout the study but, in all other strains, it increased between Week 26 and Week 36.

In all strains, %PROG declined significantly between Weeks 8 and 16 (Figure 2), and a further decline was observed at Week 26 in Strains B, C and E. Between Weeks 26 and 36, there was a detectable increase in %PROG in all strains.

Figure 2. Changes with age in percentages of motile, progressive, rapid and medium spermatozoa for five strains of Japanese quail (A ○; B ●; C □; D ■; E ◆). At each age, 36 ejaculates per strain were analyzed.

There was a significant decline in %Rapid by Week16 in Strains A, B and D (Figure 2), and by Week 26 this decline was significant for all strains, compared to the values for Weeks 8 and 16. In four strains (A, B, C, D) there was a significant increase in %Rapid between Weeks 26 and 36. For % Medium, there was a significant decrease at Week 16 in all strains, followed by significant increases at Weeks 26 and 36.

From Weeks 8 to 26, the rates of decline in %MOT, %PROG and %Rapid differed among strains, with the greatest rate observed in Strain E and the smallest in Strain A (Table 1).

3.3 Effects of age and strain on sperm kinematics

There were significant interactions between age and strain for VCL ($F=2.36$, $df = 12$; $p < 0.01$), VSL ($F = 2.52$; $df = 12$; $p < 0.01$), VAP ($F = 2.47$; $df = 12$; $p < 0.01$), LIN ($F = 3.26$; $df = 12$; $p < 0.01$), STR ($F = 3.38$; $df = 12$; $p < 0.01$) and BCF ($F=1.77$; $df = 12$; $p < 0.05$) but not for ALH ($F = 1.21$; $df = 12$; $p < 0.26$). There were significant declines in VCL, VSL and VAP at Week 16 in all strains (Figure 3). Velocity parameters improved in Strain A between Weeks 16 and 36. In Strain B, VCL improved between Weeks 16 and 36 but no there were detectable changes in VSL or VAP. There were no detectable changes in Strain C between Weeks 16 and 36. In Strain E, all velocity parameters continued to decline until Week 36, in contrast with Strain D in which there were significant improvements after Week16.

From Weeks 8 to 26, the rate of decline in VCL, VAP and VSL differed among strains, with the greatest rate observed in Strain E; VCL and VSL declined in Strain B and VAP declined in Strain D (Table 1).

The decline in LIN was significant at Week 26 in Strains A, D and E, but did not become significant until Week 36 in Strain B (Figure 3). No decline in LIN was detectable in Strain C throughout the study period. The values for BCF declined by Week 26 in all strains, after which there was a return to high values in Strains A, C and D.

The decline in ALH was significant at Week 26 only in Strains A and E; at Week 36, there was a significant improvement in ALH in Strains A and B (Figure 3).

From Weeks 8 to 26, rate of decline in BCF, LIN and ALH differed among strains (Table 1). For BCF, the greatest decline was observed in Strain D and the smallest in Strain B; for LIN, the greatest decline was observed in Strain E and the smallest in Strain C; for ALH, the greatest decline was observed in Strain E and the smallest in Strain B (Table 1).

Table 1. Estimated changes in ejaculate and sperm kinematics parameters in Japanese quail as age increases from 8 to 26 weeks. At each age, 36 ejaculates per strain were analyzed.

Parameters	Strain				
	A	B	C	D	E
Ejaculate volume (μl)	+0.40; $p < 0.01$ [95% CI, 0.50 -0.30]	+0.240; $p < 0.01$ [95% CI, 0.34, 0.14]	+0.132; $p < 0.01$ [95% CI, 0.20, 0.06]	+0.12; $p < 0.06$ [95% CI, 0.25, 0.007]	+0.18; $p < 0.01$ [95% CI, 0.29, 0.072]
Sperm conc. (x 10⁶/μl)	+0.01; $p < 0.09$ [95% CI, 0.02, -0.002]	+0.007; $p < 0.30$ [95% CI, 0.02 -0.006]	+0.01; $p < 0.06$ [95% CI, 0.02, -0.001]	-0.006; $p < 0.23$ [95% CI, 0.004, -0.017]	+0.003; $p < 0.68$ [95% CI, 0.02, -0.013]
Total sperm/Ejaculate (x 10⁶/μl)	+0.72; $p < 0.01$ [95% CI, 0.97, 0.47]	+0.42; $p < 0.01$ [95% CI, 0.67, 0.16]	+0.27; $p < 0.01$ [95% CI, 0.42, 0.13]	+0.07; $p < 0.46$ [95% CI, 0.27, -0.12]	+0.33; $p < 0.05$ [95% CI, 0.67, -0.006]
%MOT	0.44; $p < 0.09$ [95% CI, -0.80, 0.02]	-0.64; $p < 0.01$ [95% CI, -0.25, -1.03]	-0.48; $p < 0.05$ [95% CI, -0.75, -0.88]	-0.61; $p < 0.01$ [95% CI, -0.17, -1.05]	-0.91; $p < 0.01$ [95% CI, -0.45, -1.36]
%PROG	-0.47; $p < 0.01$ [95% CI, -0.13, -0.81]	-0.49; $p < 0.01$ [95% CI, -0.14, -0.83]	-0.26; $p < 0.14$ [95% CI, 0.08, -0.61]	-0.42; $p < 0.05$ [95% CI, -0.03, -0.81]	-0.81; $p < 0.01$ [95% CI, -0.48, -1.15]
%Rapid	-0.44; $p < 0.01$ [95% CI, -0.18, -0.69]	-0.50; $p < 0.01$ [95% CI, -0.19, -0.81]	-0.44; $p < 0.01$ [95% CI, -0.13, -0.75]	-0.51; $p < 0.01$ [95% CI, -0.21, -0.80]	-0.65; $p < 0.01$ [95% CI, -0.36, -0.94]
VCL (μm/s)	-0.46; $p < 0.11$ [95% CI, 0.11, -1.03]	-0.45; $p < 0.10$ [95% CI, 0.10, -1.0]	-0.78; $p < 0.01$ [95% CI, -0.24, -1.3]	-0.50; $p < 0.07$ [95% CI, 0.04, -1.05]	-1.1; $p < 0.01$ [95% CI, -0.60, -1.69]
VAP (μm/s)	-0.60; $p < 0.05$ [95% CI, -0.06, -1.13]	-0.46; $p < 0.09$ [95% CI, 0.08, -1.0]	-0.60; $p < 0.05$ [95% CI, -0.08, -1.13]	-0.53; $p < 0.01$ [95% CI, 0.18, -1.09]	-1.23; $p < 0.01$ [95% CI, -0.70, -1.77]
VSL (μm/s)	-0.62; $p < 0.05$ [95% CI, -0.10, -1.14]	-0.46; $p < 0.08$ [95% CI, 0.06, -1.0]	-0.47; $p < 0.07$ [95% CI, 0.04, -1.00]	-0.50; $p < 0.07$ [95% CI, 0.43, -1.06]	-1.23; $p < 0.01$ [95% CI, -0.70, -1.77]
BCF (Hz)	-0.32; $p < 0.05$ [95% CI, -0.001, -0.062]	-0.02; $p < 0.05$ [95% CI, -0.01, -0.05]	-0.04; $p < 0.01$ [95% CI, -0.18, -0.07]	-0.008 $p < 0.058$ [95% CI, 0.02, - 0.036]	-0.03; $p < 0.05$ [95% CI, -0.005, -0.07]
LIN %	-0.31; $p < 0.01$ [95% CI, -0.093, -0.52]	-0.10; $p < 0.23$ [95% CI, 0.07, -0.28]	+0.04; $p < 0.64$ [95% CI, 0.24, -0.15]	-0.11; $p < 0.30$ [95% CI, 0.10, - 0.34]	-0.47; $p < 0.01$ [95% CI, -0.22, -0.72]
ALH (μm)	-0.01; $p < 0.05$ [95% CI, -0.003, -0.03]	-0.009; $p < 0.17$ [95% CI, 0.004, -0.02]	-0.01; $p < 0.05$ [95% CI, -0.001, -0.02]	-0.006; $p < 0.01$ [95% CI, 0.006, -0.01]	-0.02; $p < 0.01$ [95% CI, -0.10, -0.03]

Figure 3. Changes with age in curvilinear velocity (VCL), straight line velocity (VSL), average path velocity (VAP), linearity (LIN), beat cross frequency (BCF), and amplitude of lateral head movement (ALH) for spermatozoa from five strains of Japanese quail (A ○; B ●; C □; D ■; E ◆). At each age, 36 ejaculates per strain were analyzed.

4.0 Discussion

In general, there are declines with age in most measures of ejaculate quality and sperm kinematics in Japanese quail. Peak values in sperm production were observed around Week

16 and then fell as birds reached 36 weeks of age, the limit of the present study. However, measures of sperm motility and kinematics seemed to be much more susceptible to aging, with most measures lower at Week 16 than Week 8, often followed by further declines until Week 26. These observations suggest that sperm kinematics are a better predictor of decline in male reproductive capacity than the classical measures of ejaculate quality. Importantly, the age-related declines in both ejaculate quality and sperm kinematics clearly vary with strain, suggesting that genetic variation can be used to overcome fertility limitations through selection and breeding.

Although sperm concentration declined after Week 16, and ejaculate volume after Week 26, overall sperm numbers per ejaculate were not much lower in 36-week-old males than in younger males, suggesting that older males will be able to supply sufficient sperm for egg fertilization, at least until age 36 weeks. Commercial breeding quail are generally culled for age around 40 weeks, so there should be no concerns about males limiting flock fertility. Certainly, male quail seem to be able to maintain their testis weight, plasma testosterone concentrations and sexual behavior until far greater ages, with reports available for birds that are 70-240 weeks old (Cherkin & Eckardt, 1977; Ottinger et al., 1983). However, our CASA data analysis provides clear quantitative evidence that older males produce fewer motile sperm and that these sperm have poorer forward movement. The CASA parameters of sperm movement (VCL, VSL) have previously been reported to be positively correlated with sperm mobility and egg fertility in turkeys as well as quail (King et al., 2000; Hirano et al., 2001; Farooq et al., 2018). In humans also, age-related declines in %MOT, %Rapid and %PROG and other sperm kinematics are linked to lower egg fertilization rate (Abramsson, 1988; Slotter et al., 2006). We therefore conclude that CASA measures of sperm kinematics offer value in managing the fertility of male Japanese quail.

Interestingly, for some of the strains in the present study, there was no detectable change with age in STR and ALH. In humans, better ALH has been linked with the ability of sperm to penetrate the cervical mucus and fuse with the oocyte (Barlow et al., 1991). There are major differences between birds and mammals in the biology of fertilization but, assuming poor ALH imposes similar constraints on quail sperm, it is feasible that some strains better maintain the ability of their sperm to fuse with ova as they age.

In most strains, we observed some improvement in the number of motile sperm and in sperm kinematics between Week 26 and Week 36 (Figure 3), suggesting that declines in semen quality can be reversed. A mechanism for such recovery is not evident, but we did observe some feather loss and re-growth in this period. Molting might 'recondition' male quail and increase sperm quality, perhaps by evoking changes in the morphology and

physiology of the reproductive organs (Faitarone et al., 2008; Arora & Vatsalya, 2011). In turkeys also, molting has been associated with increased semen output (Woodard et al., 1975). Further work with quail is needed to test this possibility over the range of ages we have studied.

The variation among quail strains in age-related declines in sperm kinematics and other aspects of reproductive performance might be explained in several ways: i) as a side-effect of intensive selection of quail for growth rate, with subsequent effects on metabolic endocrine systems (Ottinger, 2001), many of which are known to affect testis function in mammals (Guan & Martin, 2017); ii) variation in the age at which quail present with testicular pathology, such as Sertoli cell tumors, diseases that would decrease sperm production (Gorham and Ottinger, 1968); iii) decline with age in the activity of testosterone dependent aromatase and arginine vasotocin (Panzica et al., 1996), leading to declines in intra-testicular and circulating concentrations of testosterone, a critical factor in spermatogenesis, seminiferous tubule integrity, the production of seminiferous fluid; iv) variation in the seasonality of reproductive activity (Thurston & Korn, 2000); v) variation in the secretion of Ca^{+2} and glutamate by the accessory reproductive glands (Froman & Feltmann, 2005); vi) variation in morphology and function of sperm mitochondria, factors known to depend on genetics in the chicken (Froman & Kirby, 2005); vii) variation in the size and function of the cloacal glands that produce the foam that is important for sperm motility in quail (Farooq et al., 2014).

In general, breeder quail are genetically selected primarily for traits such as growth rate or feed efficiency. Reproductive performance is not always assessed and, if it is, it is measured by judging physical traits, such as cloacal gland area, that are correlated with testosterone production and testis size. However, a male with a large cloacal gland and high testosterone levels could produce large volumes of semen with good sperm concentrations, but with poor sperm quality and therefore poor overall fertility. To avoid this situation, quail breeders should incorporate evaluation of sperm kinematics in their genetic selection plans. Clearly, the ability of CASA to detect age-related changes in sperm kinematics makes it a valuable tool for identifying good males, or least eliminating the worst ones, and thus improving flock fertility.

In conclusion, with advancing age, there are reductions in sperm production and sperm kinematics in Japanese quail, but the timing of peak values and the rates of decline differ among strains.

Conflict of Interest

None of the authors have any conflict of interest to declare.

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