Available online at www.most.gov.bd
Volume 06, Issue 01, Pages: 65–72, 2024
DOI: https://doi.org/10.3329/jscitr.v6i1.77377

Journal of Science and Technology Research
Ministry of Science and Technology
Government of the People's Republic of Bangladesh
Bangladesh Secretariat, Dhaka

Seminal Characteristics of Short-term Stored Spermatozoa of Indigenous Aseel Chicken in Bangladesh

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Abstract

The purpose of this study is to look at the seminal characteristics of short-term stored spermatozoa (at 4°C) from indigenous Aseel chickens in Bangladesh. The abdominal massage technique was utilized to collect semen from adult Aseel chickens at 3-day intervals and microscopically analyze the semen quality. The semen was then diluted with egg yolk-citrate (EYC) extender and stored at 4°C. They were then evaluated at various storage times, including Day 0 (0 hours), Day 1 (24 hours), Day 2 (48 hours), Day 3 (72 hours), Day 4 (96 hours), and Day 5 (120 hours). The findings of the study revealed that as storage time progressed, sperm motility, live spermatozoa, and the percentage of normal spermatozoa decreased. Total motility percentage significantly (P<0.05) decreased with the extended storage time in case of all individual cock. Progressive motility percentage was significantly (P<0.05) decreased from Day 0 (57.4±3.21) to Day 5 (0.11±3.21). On the contrary, non-motile sperm percentage significantly increased in Day 5 (99.3±4.85) compared to Day 1 (21.9±4.85). On the other hand, live spermatozoa were significantly (P<0.05) higher (76.68±5.06%) on Day 0 (0 h) and gradually declined day after day and finally it was 0% on day 5 (120 h). Similarly, spermatozoa abnormalities were highest at Day 5 (11.29±1.69% head, 3.33±1.42% mid-piece and 7.26±1.66% tail) and head abnormalities were significantly (P<0.05) affected by the storage time. Therefore, this study concluded that the storage duration had a substantial impact on the quality of Aseel chicken sperm, which may be used for artificial insemination (AI). The outcomes of this study may be valuable for the conservation and enhancement of the indigenous Aseel chicken breed in Bangladesh through the use of AI.

Received: 07.02.2024
Revised: 24.06.2024
Accepted: 30.06.2024

Keywords: Aseel Chicken, Semen Quality, Spermatozoa, Egg Yolk Citrate, Storage.

Introduction

Aseel is a well-known indigenous chicken breed in Bangladesh, raised primarily in the Sarail upazila of Brahmanbaria district. Some urban inhabitants keep them for cockfighting or as a hobby. The Aseel breed is known for its pugnacity, stamina, tenacious fighting abilities, and majestic walk (Jabbar *et al.*, 2015) and has various benefits over other indigenous breeds (Yousaf *et al.*, 2016). The Aseel can be raised in rural locations due to its adaptation to severe circumstances and disease resistance (Jatoi *et al.*, 2014). Meat and eggs from Aseel chickens have aphrodisiac and therapeutic effects because they contain more protein, iron, and amino acids (arginine, threonine, lysine, and valine) than foreign birds (Mohan *et al.*, 2008). However, the Aseel population is diminishing, and bird owners or farmers are interested in purchasing male birds just

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for cockfighting (Sarker *et al.*, 2012). As a result, the Aseel chicken's survival is in jeopardy, and proper conservation measures must be taken. The growing importance of artificial insemination (AI) in poultry reproduction has piqued researchers' interest in creating optimal conditions for short- and long-term semen storage (Van Wambeke, 1967; Lake and Ravie, 1981; Lukaszewicz *et al.*, 2004). The cock ejaculate is typically tiny in volume but extremely concentrated, therefore it is possible to expand it with suitable diluents at particular rates prior to AI and storage (Blesbois, 2007).

The Aseel breed has difficulties such as slow growth, late maturity, broodiness, low persistence, low egg output, and poor fertility and hatchability (Amjad *et al.*, 2012). The low fertility could be attributed to poor semen quality, because there is no baseline data on the semen quality of the Aseel breed (Yousaf *et al.*, 2016). No systematic study of the impact of diluents and preservation time on the quality of Aseel cock semen has been completed. As a result, it is critical to examine Aseel cock semen and determine the influence of storage time on the state of seminal qualities for the advancement of AI in poultry, particularly Aseel chicken.

Materials and Methods

Birds Management

Three (n=3) adult Aseel cocks (Tag numbers 3612, 3616, and 3644) appeared to be in good health and free of obvious anatomical defects. Cocks aged 2.5 to 3 years had body weights of 3.79 kg, 3.06 kg, and 2.78 kg, respectively. The birds were raised in individual cages (0.9×0.76×0.75 m^3). They were treated with Avinex® powder (Renata Limited, Bangladesh) to deworm the digestive and gastrointestinal roundworms. Vaccination was performed against Ranikhet diseases using intramuscular injection of RDV (LRI, Mohakhali, Bangladesh). Each Aseel bird was fed with 200 g commercial cock breeder feed (Nourish Feeds Limited, Bangladesh) in the mash form which containing 2,900 kcal/kg metabolizable energy (ME) and 19% crude protein (CP). Total feeds were supplied in two splits per day with water ad-libitum water supply. In the pre-experimental period, cocks were trained once daily (morning) and cleaning of the cloacal region of each bird was performed prior to semen collection by the abdominal massage technique (Lake, 1957) for four weeks.

Figure 1. Collection of semen from Aseel cock.

Preparation of Diluted Semen

A solution of 2.94% sodium citrate in 100 mL distilled water was made, and 100,000 IU of diluted pronapen solution was added to it. Following that, 1 part egg yolk by volume was combined with 4 parts citrate solution and thoroughly mixed. The semen and diluter were combined at room temperature at a ratio of 1:20 (Chowdhury *et al.*, 2023).

Evaluation of Motility

To evaluate the motility, a drop of semen was placed on a pre-warmed glass slide with a cover slip and examined under a light microscope at high magnification (40×). It was counted as a percentage ranging from zero to one hundred depending on the individual movement of the sperm.

Evaluation of Live Spermatozoa

Two drops of 1% Eosin solution were gently mixed for 15 seconds with a drop of completely mixed sperm sample placed on a clean, warmed glass slide. Then it was mixed with two drops of a 10% aqueous Nigrosin solution. Immediately, a thin smear was created and air-dried. The fixed slides were examined with a phase contrast microscope at 40× magnification. Spermatozoa that were red or dark pink in hue were regarded dead, whilst those that were white or mild pink were thought to be alive.

Evaluation of Morphology

On a clean, dry glass slide, two drops of sodium-citrate buffer solution were deposited, followed by one drop of correctly mixed semen. A smear was produced, which was then air dried before being stained with rose-bengal stain for 3 to 5 minutes. The slide was then cleaned with distilled water to remove any remaining stain, then air dried again. Spermatozoa were examined under a microscope at 40x magnification. Spermatozoa with any of the abnormalities in the head, midsection, or tail were judged abnormal (Islam *et al.*, 2018).

On a clean, dry glass slide two drops of sodium-citrate buffer solution was placed and a small amount (1 drop) of properly mixed semen was added in the buffer solution. A smear was made and after air drying it was stained in rose-bengal stain for 3-5 minutes. Then the slide was rinsed with distilled water for removing additional stain and air dried again. The slide was set on microscope and spermatozoa were observed (40×). Spermatozoa with any of the deformities were in head, mid-piece and tail considered as abnormal (Islam *et al*., 2018).

Statistical Analyses

Data generated from the experiment were compiled, tabulated and analyzed according to the objectives of the study by one-way analysis of variance (ANOVA) and Duncan's multiple range test (DMRT) using SAS software. The difference between values was considered significant when the *P* value was less than 0.05.

Results and Discussion

Effect of Short-term Storage on Aseel Chicken Semen

Effects of storage time on sperm motility of Aseel cock semen collected from different cocks was depicted in Table 1. On Day 0 (0 h), the total sperm motility (%) was 85.36±6.59, 73.66±8.28, and 75.26±9.20 in cock 3616, 3612, and 3644, respectively, with cock 3616 having the highest motility (85.36±6.59%) than the others. Individual cock sperm motility (%) decreased significantly ($P<0.05$) with increasing preservation time.

Sperm motility is a strong indicator of the viability and quality of the sperm sample (Ola *et al.*, 2020). Semen extenders and temperature control play a pivotal role in maintaining cock sperm motility. The current study observed 41.94±4.85% sperm motility at Day 1 (24 h), which is lower than Ciereszko *et al.* (2011) who reported 59.4±8.6% motility after 24 hours of storage with Ovodyl extender in Black grouse semen. Mphaphathi *et al.* (2016) found 55.0±8.0% motility in Venda chicken semen at 5°C after 24 hours of preservation. Shinde *et al.* (2012) from India observed significantly increased motility (87.65±1.00% in Kadaknath chicken and 72.34±3.05% in broiler) after 24 hours of storage. Ola *et al.* (2020) found that Egg Yolk Plasma dilution improved motility in broiler breeder cocks by 69.33±1.70%, 62.00±3.91%, and 55.33±2.52% at 24 h, 48 h, and 72 h, respectively. This low proportion of motility when compared to earlier research could be attributed to differences in breed, age, dilution, and semen processing.

Table 1. Effects of storage time on sperm motility (%) of Aseel cock semen

Cock ID	Storage Time					
	Day 0 (0 h)	Day 1 (24 h)	Day 2 (48 h)	Day 3 (72 h)	Day 4 (96 h)	Day 5 (120 h)
3616 (15)	85.36^{a}±6.59	54.52^{b}±6.59	32.99^{c}±6.59	17.2^{d}±6.59	9.89^{d}±6.59	0.00^{e}±6.59
3612 (15)	73.66^{a}±8.28	40.33^{b}±8.28	29.92^{b}±8.28	9.02^{c}±8.28	6.76^{c}±8.28	1.96^{d}±8.28
3644 (15)	75.26^{a}±9.2	30.97^{b}±9.2	12.94^{b}±9.2	8.14^{b}±9.2	5.49^{b}±9.2	0.00^{b}±9.2
Pooled	78.09^{a}±4.85	41.94^{b}±4.85	28.37^{c}±4.85	11.45^{d}±4.85	7.38^{d}±4.85	0.65^{e}±4.85

Parenthesis with cock ID indicates the number of observations. Values with different superscripts within the same row differed significantly from each other ($P<0.05$).

Effects of Storage Time on Individual Sperm Motility

The effects of storage time on sperm motility of the pooled semen (n=45) samples are presented in Table 2. The mean±SE values of individual motility (%) on Day 0 (0 h), Day 1 (24 h), Day 2 (48 h), Day 3 (72 h), Day 4 (96 h) and Day 5 (120 h) in Egg Yolk Citrate (EYC) extender stored at 4°C were 78.09±4.85, 41.94±4.85, 28.37±4.85, 11.45±4.85, 7.38±4.85 and 0.65±4.85, respectively (Table 2). The progressive motility % fell considerably (P<0.05) from Day 0 (57.4±3.21) to Day 5 (0.11±3.21). In contrast, the percentage of non-motile sperm increased dramatically on Day 5 compared to previous days (Table 2). Cooling spermatozoa to 5˚C causes a phase transition of membrane lipids, resulting in loss of selective permeability and disruption to the phospholipid bilayer in the plasma membrane (Watson, 1981).

Motility is an indicator of spermatozoa viability. In the current study, sperm motility gradually deteriorated in the EYC extender, similar to the findings of Das *et al.* (2016), who reported that the percentage of diluted spermatozoa in native cock semen on Day 0 varied from 60.6±2.4% to 61.7±3.1%, which was much lower values than the current study (78.09±4.85%). Alkan *et al.* (1997) reported a somewhat higher motility (85.83±6.19%) on Day 0. Moreover, Alkan *et al.* (1997) and Das *et al.* (2016) found that diluted spermatozoa had higher motility (%) on Day 1 (76.25±10.34% and 52.9±2.3% to 58.1±3.3%) and Day 2 (43.33±20.78% and 39.2±2.0% to 52.2%). In this study, there was a significant loss in motility of Aseel cock spermatozoa from Day 1 to Day 5 of storage at 4°C. According to Parker and McDaniel (2006), avian sperm motility is affected by the concentration of oxygen and Ca++ ions. The diluent chemicals, storage temperature, and preservation times all have the potential to impact sperm motility. Furthermore, this variance could be attributed to the impacts of varied diluent, method, and storage techniques, as well as age, cock breed, collection interval, and sample ambient temperature.

Effects of Storage Time on Sperm Viability

The effects of storage time on the percentage of living sperm in semen samples are presented in Figure 2. Live sperm concentrations ranged from 76.68 to 0% depending on storage period using an EYC extender at 4°C. The mean±SE values of living spermatozoa at 4°C of storage on Days 0, 1, 2, 3, 4, and 5 were 76.68±5.06%, 52.92±5.06%, 30.20±4.80%, 10.83±5.37%, 3.36±5.37%, and 0%, respectively (Figure 2). On Day 1 (0 h), there was a significantly higher percentage (76.68±5.06%) of viable spermatozoa, which steadily decreased to 0% by Day 5 (120 h). Figure 3 depicts a pictorial representation of live and dead spermatozoa from Aseel cock semen.

Table 2. Effects of storage time on sperm motility of the pooled semen (n=45) at 4°C (Mean±SE)

Parameter	Storage Time					
	Day 0	**Day 1**	**Day 2**	**Day 3**	**Day 4**	**Day 5**
Non-motile (%)	21.9^{c}±4.85	58.05^{b}±4.85	71.63^{b}±4.85	88.54^{a}±4.85	92.6^{a}±4.85	99.3^{a}±4.85
Individual motility (%)	78.09^{a}±4.85	41.94^{b}±4.85	28.37^{c}±4.85	11.45^{d}±4.85	7.38^{d}±4.85	0.65^{e}±4.85
Progressive motility (%)	57.4^{a}±3.21	26.04^{b}±3.21	15.38^{c}±3.21	4.52^{d}±3.21	2.58^{d}±3.21	0.11^{d}±3.21
Oscillatory (%)	17.71^{a}±1.95	15.09^{a}±1.95	12.48^{a}±1.95	6.84^{b}±1.95	4.70^{bc}±1.95	0.54^{c}±1.95
Rotatory (%)	3.04^{a}±0.27	0.72^{b}±0.27	0.51^{b}±0.27	0.09^{b}±0.27	0.09^{b}±0.27	0.00^{b}±1.00

Values with different superscript within the same row differed significantly from each other (P<0.05).

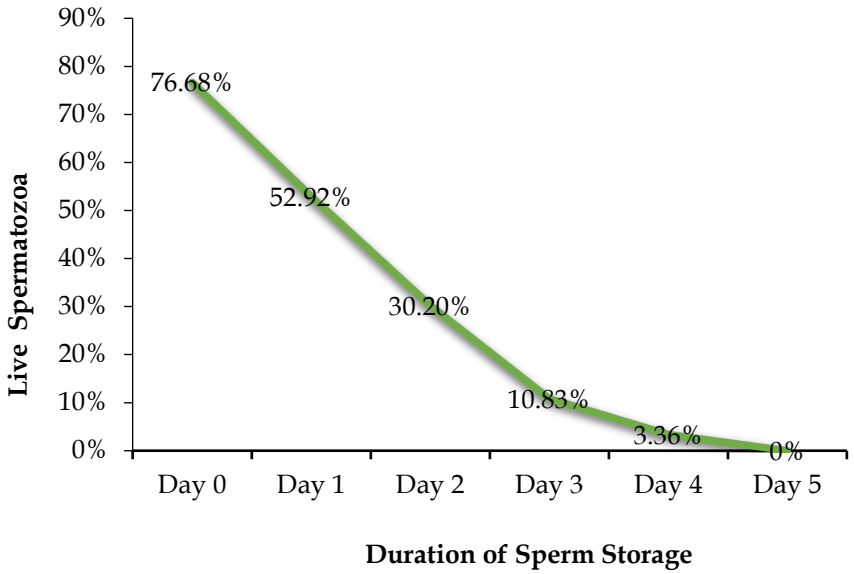

Figure 2. Effects of preservation time on the viability (%) of the spermatozoa.

In the present study, a significantly lower number of live spermatozoa was found on Day 5 (0%), Day 4 (3.35±5.37%), and Day 3 (10.83±5.37%) in comparison to Day 0 (76.68±5.06%), Day 1 (52.92±5.06%) and Day 2 (30.2±4.80%). The number of live spermatozoa was reduced with the increased preservation periods. These observations are also in agreement with the other researchers (Mosenene, 2009; Keskin *et al.*, 1997). Das *et al.* (2016), however, showed live spermatozoa percentage of native cock semen with two different diluents on Day 0, 1 and 2 of storage at 4°C were 69.0±4.5 vs 70.2±4.3; 62.6±4.4 vs 64.9±4.6 and 45.1±3.8 vs 58.0±4.2%, respectively. Shinde *et al.* (2012) observed 87.65±1.00% live spermatozoa in Kadaknath chicken and 77.41±0.92% in broiler. In an experiment with broiler breeder cock semen, Ola *et al.* (2020) found live spermatozoa were 70.00±3.82%, 63.33±7.87% and 55.00±1.81% at 24 h, 48 h and 72 h, respectively using Egg Yolk Plasma diluter.

In this respect, the viability of Aseel cock spermatozoa was found to be less consistent in the current investigation with EYC extender at 4°C. This variance could be attributable to the impact of different diluents, processes, and techniques used for preservation, as well as age, cock breed, collection interval, and sampling ambient temperature.

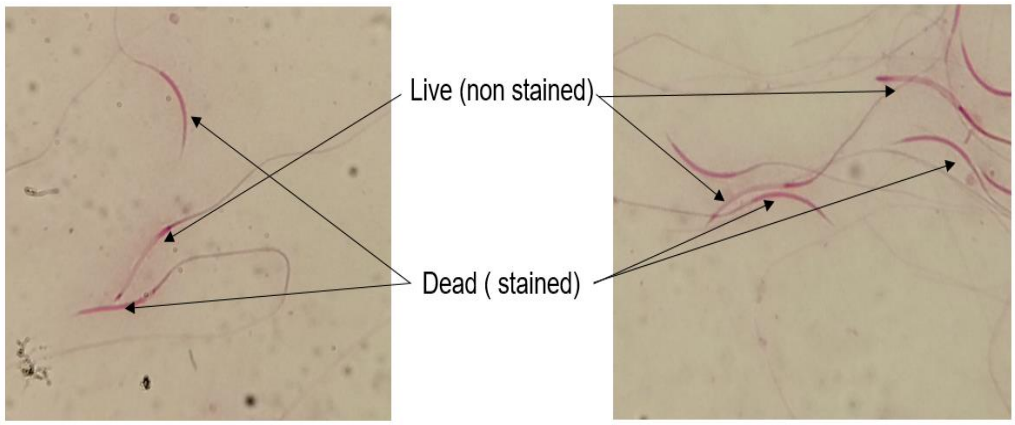

Figure 3. Pictorial representation of live and dead sperm from Aseel cock (100× magnification).

Effect of Storage Time on Sperm Abnormalities

Table 3 shows effect of storage time on the morphological sperm abnormalities. The percentage of morphologically abnormal sperm in the head, midpiece, and tail had varying Mean±SEM values based on storage time (5.57±1.69 to 11.29±1.69%, 1.55±1.42 to 3.33±1.42%, and 6.50±1.66 to 7.26±1.66%).

Table 3. Effects of storage time on the percentage (Mean±SE) of morphological sperm abnormalities

Storage Time	Morphological Abnormalitiers (%)		
	Head	Midpiece	Tail
Day 0	6.94^{ab}±1.69	3.88^{a}±1.42	4.97^{a}±1.66
Day 1	5.57^{b}±1.69	1.55^{a}±1.42	6.5^{a}±1.66
Day 2	6.55^{ab}±1.96	2.01^{a}±1.42	7.22^{a}±1.66
Day 3	7.03^{ab}±1.69	2.86^{a}±1.42	5.31^{a}±1.66
Day 4	6.71^{ab}±1.69	1.6^{a}±1.42	5.89^{a}±1.66
Day 5	11.29^{a}±1.69	3.33^{a}±1.42	7.26^{a}±1.66

Forty five ejaculates were used in this experiment. Values with different superscripts within the same column differed significantly from each other (P<0.05).

On day 5 (120 h), a significantly higher (P<0.05) percentage (11.29±1.69) of head abnormalities were observed. As the storage time progressed, the deformities of the head, midpiece, and tail became more noticeable. Figure 4 illustrates various morphological defects in Aseel cock spermatozoa.

Das *et al.* (2016) reported the head abnormalities of native cocks' sperm stored at 4°C varied from 5.7±5.0 to 9.7±1.3%. Siudzinska and Łukaszewicz (2008) studied semen of four domestic fowl and found 3.00±2.05% swollen head, 1.45±1.42% defective mid-piece and 7.27±4.34% other defect at 24 h in Black Minorca sperm. They also stated that 3.33±3.06% swollen head, 2.70±4.05% mid-piece and 6.42±2.07% other defects at 24 h in White Crested Black Polish sperm.

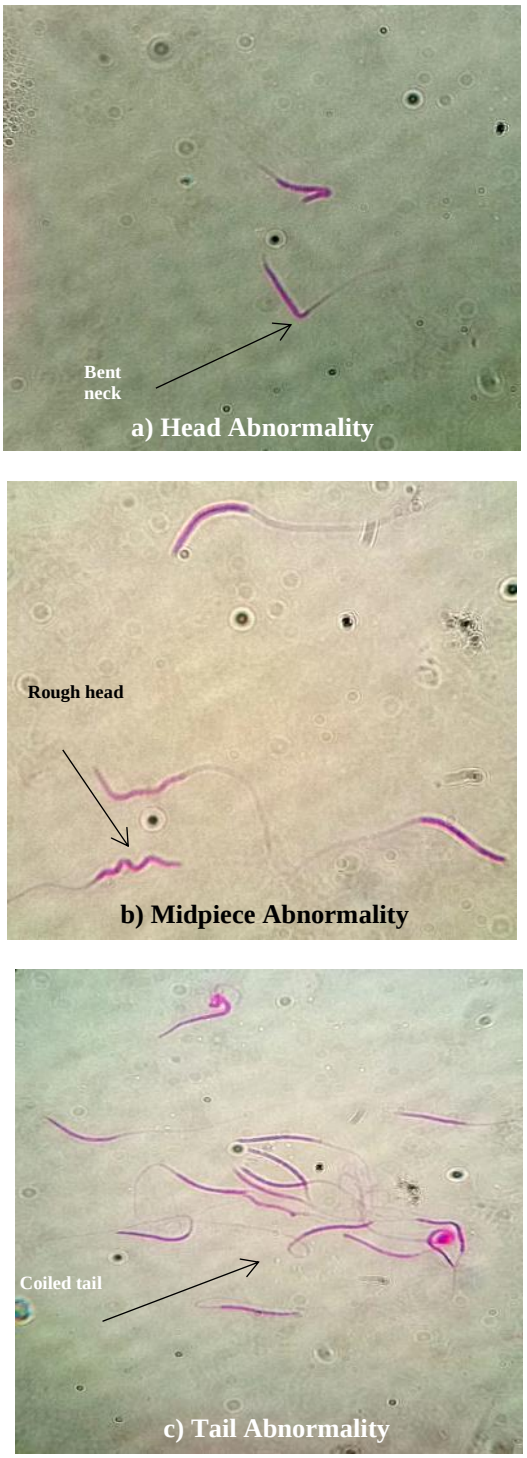

Figure 4. Pictorial representation of morphological abnormalities; a) head, b) midpiece and c) tail of Aseel cock spermatozoa (100× magnification).

Conclusions

Storage time has a considerable impact on the seminal properties of Aseel chicken stored at 4°C. The percentage of non-motile, abnormal, and dead spermatozoa has increased with storage duration. Semen quality retains up to 48 hours of storage and can be used for AI purposes. More research into storage of Aseel semen is needed to investigate the influence of storage on seminal characteristics of the Aseel chicken and to conserve this rich genetic resource.

Acknowledgements

The authors gratefully acknowledge the financial support of the Ministry of Science and Technology, Government of the People's Republic of Bangladesh for financial support (SRG-221054, 2022–2023). The authors also acknowledge the Department of Animal Breeding and Genetics, Bangladesh Agricultural University and Bangladesh Agricultural University Research System for their enormous logistic support. We appreciate the efforts of everyone involved in making this research possible, and we are thankful for the shared vision and dedication to advancing knowledge in this field.

Declaration

There is no conflict of interest regarding publication of this research article.

Authors' Contributions

MDH worked with methodology, investigated and wrote the original draft; MM was involved in investigation, formal analyses and writing of original draft; SD supervised and curated the data; ASA is responsible for conceptualization, funding acquisition, project administration, supervision, data curation as well as writing, reviewing and editing the manuscript.

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