


Ultrasonographic features of the regressing yolk sac of normal captive Brown kiwi chicks (*Apteryx mantelli*) vary with age

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Abstract

Brown kiwi (*Apteryx mantelli*) are a culturally significant ratite species endemic to New Zealand. Chicks can develop diseases of the yolk sac which can be fatal if not promptly recognized and treated. Ultrasound examination of the yolk sac is an important component in the evaluation of chicks with suspected yolk sac disease. However, there are currently no reports of the normal ultrasonographic appearance of the regressing yolk sac in this species. The objective of this prospective reference interval study was to describe the normal ultrasonographic appearance of the yolk sac in brown kiwi chicks. Focused coelomic sonographic examinations were performed on 29 healthy chicks from >1 day of age until the yolk sac was completely absorbed or was <1 mL in volume. 41.6% (57/137) of yolks were round/ovoid in shape and 58.6% (80/137) had lobulated margins with invaginations. All yolks (137/137) were hyperechoic relative to adjacent coelomic structures. The heterogeneity of the echogenicity of the yolk was significantly associated with age (increased heterogeneity in younger birds) and was homogeneous, mildly heterogeneous, and moderately heterogeneous in 33% (46/138), 46% (63/138), and 21% (29/138) of all examinations respectively. Intrasac anechoic pockets were seen in 14% (20/139) of yolk sacs. The rate of yolk sac resorption slowed with age. In all examinations identifying a completely absorbed yolk sac or a yolk sac <1 mL in volume, chicks were less than 21 days old. The results of this study provide a reference of normal for the sonographic appearance of the yolk sac in captive brown kiwi chicks.

KEYWORDS

anatomy, avian, coelom, regression, ultrasound

1 | INTRODUCTION

Brown kiwi chicks (*Apteryx mantelli*), a culturally significant ratite species endemic to New Zealand, are listed as vulnerable by the IUCN Red List.^{1,2} A national conservation program (“Operation Nest Egg”) strives to increase the survival of kiwi chicks to adulthood. Eggs or

young chicks are collected from the wild and chicks are reared in captivity. Chicks are released back into the wild when they reach approximately 1000 g in bodyweight and are significantly less likely to be predated.³ In one study, it has been documented that 4.3% of kiwi chicks raised in captivity presented with retained or infected yolk sacs.⁴ The avian yolk sac (*saccus vitellinus*) is an intra-coelomic structure containing residual yolk internalized into the coelom prior to hatching (Figure 1). Posthatching, the yolk sac contents are absorbed via the

Abbreviations: CI, confidence intervals; IQR, interquartile range; OR, odds ratios.

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FIGURE 1 Postmortem photograph of a brown kiwi (*Apteryx mantelli*) chick that died during hatch. The yolk sac (white asterisks) has been removed from the coelom. [Color figure can be viewed at wileyonlinelibrary.com]

vitelline stalk and provide nutritional support to the chick during the neonatal period.⁵ Diseases of the yolk sac are a cause of morbidity and mortality in brown kiwi chicks. Yolk sac retention is defined as abnormal resorption of the yolk sac, resulting in a yolk of disproportionate size for the age of the chick,⁶ whereas yolk sacculitis involves infection of the yolk sac.⁷ Both yolk sac retention and yolk sacculitis can result in significant morbidity or death of brown kiwi chicks, and for an optimal outcome, require early recognition, medical therapy, and often surgical removal of the yolk sac (deutectomy).⁵

Clinical findings supportive of yolk sac disease are identified through clinical history, physical examination findings, hematology, radiography, and coelomic ultrasound.⁸ Clinical signs of kiwi chicks with yolk sac retention or yolk sacculitis are nonspecific and include abnormal weight trends, reduced appetite, and reduced activity levels. On physical examination chicks may have a distended coelom and radiographs may show increased soft tissue opacity in the caudoventral coelom, though these findings may be less obvious with smaller retained yolk sacs.⁵ Additionally, at our institution, focused ultrasound examination of the yolk sac is used as a noninvasive and simple means for assessment of the yolk sac when disease is suspected, making it an excellent tool for repeated assessments if clinically indicated. However, currently, there is only a single case report describing the radiographic and CT appearance of a retained yolk sac in brown kiwi,⁹ and there are no published descriptions of the normal sonographic appearance of the yolk sac during regression. Additionally, the normal rate of yolk sac regression and normal age of complete regression of the yolk sac in brown kiwi chicks is unknown. This lack of information puts limitations on the interpretation and clinical relevance of sonographic findings relating to the yolk sac for this species and subsequently limits the utility of ultrasound for identifying surgical candidates. Normal yolk sac resorption and diseases of the yolk sac have been studied in other avian species.^{6,10,11,12,13,14} Currently there are varying reports of the normal length of time for yolk sac utilization, ranging from 4 to 15 days,

with precocial species such as the ostrich (*Struthio camelus*) being at the higher end of this range.^{6,12,15,16} Of all birds, kiwi lay the biggest eggs relative to their size (ca. 400% above allometrically expected value), and the yolk content of the egg is very high (yolk content of up to 62% of fresh egg mass, with expected 40%–50%).⁴ It is currently unknown whether these unique features of brown kiwi are associated with clinically relevant species-specific variation in yolk sac morphometrics, which also puts limitations on our assessment of chicks with possible yolk sac disease. Documenting the normal sonographic appearance and morphometrics of the yolk sac is the first step in increasing the utility of ultrasound for the assessment of suspected yolk sac disease in kiwi chicks and ultimately contributing to conservation management of this species.

The objectives of this study were (a) to describe the sonographic appearance of the yolk sac throughout regression in a group of clinically healthy brown kiwi chicks hatched and raised in captivity and (b) to describe the rate of regression and age of complete regression of the yolk sac in these chicks.

2 | MATERIALS AND METHODS

2.1 | Selection and description of subjects

A prospective reference interval study design was used. The study was approved by and conducted in accordance with an Institutional Animal Care and Use Committee (Massey University Animal Ethics Committee [MUAEC protocol number: 21/44]) and the New Zealand Department of Conservation (authorization number: 96030-RES).

The source population consisted of all chicks hatched during the study period (October 31, 2021–December 2, 2021) through Operation Nest Egg at the National Kiwi Hatchery, Rotorua, New Zealand and the Crombie Lockwood Kiwi Burrow, Taupo, New Zealand. The eligible population consisted of healthy chicks from the source population. Recruitment stopped after the first 30 eligible chicks were included. A sample size of 30 chicks was chosen based on a previous study involving ultrasound measurement of yolk sac size.¹⁷ Birds were excluded if there were any clinical concerns by experienced hatchery staff regarding weight trend, food consumption, coelomic palpation findings, or activity levels. Final decisions for inclusion were made by a 2nd-year radiology resident (E.S.) in consultation with K.M. (associate professor in Wildlife Health, BVSc, PhD), experienced wildlife veterinarian. As per normal husbandry protocols at the two hatcheries, chicks were housed individually or in pairs in brooders and were fed a formulated captive kiwi diet comprising of ox heart, ox mince, carrot, peas, corn, wheat germ, cat biscuits, currants, banana, apple, pear, CaCO₃, soy oil, and vitamin mix.

2.2 | Data recording and analysis

All data collection and sonographic examinations were performed by a 2nd-year radiology resident (E.S.) with 2 years of clinical experience

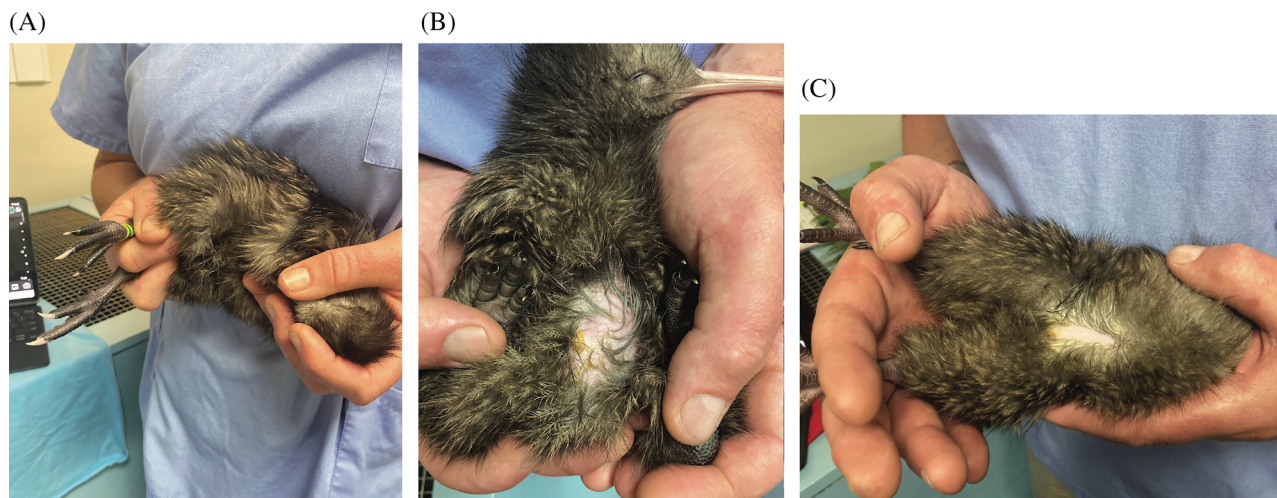


FIGURE 2 A, Brown kiwi chick restrained in left lateral recumbency for sonographic examination of the yolk sac. B, Brown kiwi chick restrained in dorsal recumbency for sonographic examination of the yolk sac. C, Brown kiwi chick restrained in left lateral recumbency with feathers parted to show midline featherless tract. [Color figure can be viewed at [wileyonlinelibrary.com](https://onlinelibrary.wiley.com/doi/10.1111/vru.13280)]

in sonographic evaluation of brown kiwi yolk sacs with prior consultation with M.O. (ECVDI boarded radiologist) regarding image collection and analysis. The first sonographic examination was performed from as early as 1 day of age (>24 h since hatch) and measurements were repeated up to every other day. The data collection endpoint for each chick was either “could not identify the yolk” or “yolk was very small (calculated approximate ellipsoid volume <1 mL)” or “chick left premises”.

A 3–10 MHz electronic convex hand-held Clarius Ultrasound Scanner (C7Vet HD3, Clarius Mobile Health Corp.) was used for the sonographic examination of the yolk sac. Clarius Ultrasound App (Clarius Mobile Health Corp.) on an Android tablet (Samsung Tab S2, Samsung Electronics) was used for image viewing during the examination. The sonographer was aware of the chick identification and age of the chick at the time of data recording. The sonographer was not aware of the previous examination findings for a chick at each examination.

As per the routine husbandry protocol at the hatchery, chicks were weighed and underwent a general physical examination, followed by a focused coelomic ultrasound examination. Chicks were restrained for the examinations by experienced hatchery staff. If the chick was determined by the handler to be showing behavior concerning an unacceptable level of stress during the examination, the procedure was discontinued. Specific stress-associated behaviors monitored for included vocalizing, increased respiratory effort, bubbling of mucous from the nostrils, and/or freezing behavior (described as cessation of struggling/becoming still and closing eyes). For the sonographic examination, chicks were held with two hands suspended in left lateral recumbency (Figure 2A). If the yolk was not identified with lateral positioning within approximately 15 s of scanning, the chick was held in dorsal recumbency with two hands (Figure 2B). A swab moistened with warm water was used to part the feathers and reveal the midline featherless tract (Figure 2C). A small amount of sterile lubricant jelly (SURGI-gel® PLUS, Contiform International Pty Ltd.) was applied to

the skin to act as a contact medium, taking care to avoid the umbilicus to limit the risk of possible iatrogenic infection. If the bird was in dorsal recumbency, the feathers to the left of the umbilicus were parted and sterile jelly applied to the skin. The probe was gently positioned on the ventrum in a sagittal plane on midline immediately cranial to the umbilicus or left parasagittal to the umbilicus, for the left lateral and dorsal holds, respectively.

The yolk sac was identified with B-Mode examination, and the depth and gain were adjusted to optimize the image. A single focal point was positioned at the level of the yolk sac. The ultrasound beam was then fanned through the yolk sac to assess the content, margins, and periyolk sac tissues. Three sagittal and three transverse still B-Mode images were acquired for later measurement of size in mm. Power Doppler examination of the yolk sac and periyolk sac tissues was then performed. Upon completion of the examination, the gel was cleaned from the skin and feathers with a moist gauze swab and then dried with a dry swab.

Digital Imaging and Communications in Medicine (DICOM) files were exported for review on an imaging software station (Horos) on a computer (MacBook Air, macOS Monterey Version 12.3, Apple, One Apple Park Way).

Images were reviewed by the sonographer (E.S.) and image interpretation was based on independent opinion. The height, width, and length of the yolk sac were measured in each available image. If portions of the margin of the yolk were not visible, the position of the margin was estimated based on the visible portions of the yolk in that image. The subjective echogenicity of the yolk content relative to surrounding tissues was recorded as hypochoic, isochoic, or hyperchoic. The subjective homogeneity of the echogenicity of the yolk sac content was recorded as homogeneous, mildly heterogeneous, or moderately heterogeneous. The shape of the yolk sac was recorded as round/ovoid or invaginated and/or lobulated margins. The approximate volume of the yolk sac was calculated assuming an ellipsoid shape (volume = $4/3 \times P \times \text{height}/2 \times \text{width}/2 \times \text{length}/2$). The presence

of any coelomic-free fluid seen at the margins of the yolk sac was recorded. Any other identified features of the yolk sac were described where possible. Other data collected included final egg weight prior to hatch, chick hatch weight, chick weight on each day of ultrasound examination, and lowest chick weight throughout the examination period.

2.3 | Statistics

Data analysis was performed by a veterinary epidemiologist (E.V.) using open-source statistical analysis software (R version 4.2.1, 2022, The R Foundation for Statistical Computing). The distribution of continuous variables was checked visually using histograms. For symmetric distributions, the variables were summarized using the mean and standard deviation, and the median and interquartile range (IQR) was used for non-symmetric distributions. Confidence intervals for proportions were calculated using the exact binomial method. To account for repeated observations on the same chicks, associations between age and binary outcomes (presence of free fluid at the yolk sac margins, presence of intrasac anechoic pockets, margin shape, Doppler flow, positioning of the transducer) were tested using mixed-effect logistic regressions with a random intercept for chicks. Regression coefficients were exponentiated to obtain odds ratios (OR), and the boundaries of the 95% confidence intervals (CI) of the coefficients were exponentiated to obtain CIs for ORs. Similarly, associations between age and categorical outcomes taking three or more values (echogenicity) were tested using multinomial regression assuming nonproportional odds (i.e., we assume that the effects of age on the yolk sac being mildly heterogenous compared with homogenous and being moderately heterogenous compared with homogenous are different). The time to loss of yolk sac was explored using a Kaplan–Meier graph. To explore the relationship between the approximate yolk sac volume and age, a mixed-effect linear regression was built using a random intercept for chick, volume as the outcome, and a 3-degree polynomial for age. Once the polynomial for age was fitted, birth weight and chick weight were not significantly associated with yolk sac volume and so were not retained in the model. Predicted volume with a 95% confidence interval as well as prediction intervals were calculated for a chick age ranging from 0 to 30 days. For all tests, the *P*-value significance threshold was set at 0.05.

3 | RESULTS

A total of 151 ultrasound examinations were performed on 29 brown kiwi chicks (one was excluded due to an abnormal weight graph), with a median total of 5 (IQR, 5–6) scans performed on each individual chick. The mean egg weight prior to hatch was 402.09 g (SD, 27.66 g; 95% CI, 391.9–412.3). The mean chick weight at hatch was 339.5 g (SD, 27.4 g; 95% CI, 329.5–349.5). The mean age of lowest chick weight was 9.6 days (SD, 2.1 days; 95% CI, 8.8–10.4). Median age of the first coelomic ultrasound examination, mean age of the final coelomic ultrasound examination, total number of coelomic ultrasound examinations per

TABLE 1 Population results for captive brown kiwi (*Apteryx mantelli*) chicks ($n = 29$) undergoing ultrasonographic evaluation of the regressing yolk sac including median age of the first coelomic ultrasound examination, mean age of the final coelomic ultrasound examination, total number of coelomic ultrasound examinations per chick, reason for final coelomic ultrasound examination, and total number of coelomic ultrasound examinations per age group.

Variable	Value
Median age of the first coelomic examination	2 days (IR 2–4 days)
Mean age of final coelomic examination	20.4 days (SD 2.3 days)
Number of examinations per chick	
4	4/29 (14%)
5	15/29 (52%)
6	10/29 (34%)
Reason for final examination	
Yolk sac < 10 mm ³	11/29 (37.9%)
Yolk sac was not identified	12/29 (41.4%)
Chick left the hatchery	6/29 (20.7%)
Number of examinations per age group	
1–5 days	36/151 (23.8%)
6–9 days	30/151 (19.9%)
10–15 days	43/151 (28.5%)
16+ days	42/151 (27.8%)

^aTwenty-nine brown kiwi chicks undergoing a total of 151 focused coelomic ultrasound examinations.

chick, reason for final coelomic ultrasound examination, and total number of coelomic ultrasound examinations per age group are described in Table 1. Six chicks (6/29 20.7%) left the site (“left the premises”) before a very small (<1 mL approximate ellipsoid volume) or absent yolk sac was documented.

The frequency of categorical variables, including the presence of coelomic fluid at the margin of the yolk, the echogenicity (i.e., hypoechoic, isoechoic, hyperechoic) and homogeneity (i.e., homogeneous, mildly heterogeneous, moderately heterogeneous) of the yolk, presence of intrasac anechoic pockets, the shape (i.e., rounded/ ovoid or invaginated/lobulated), presence of flow within vessels with Power Doppler examination, transducer position and count of other sonographic features are shown in Table 2 and examples of these variables are demonstrated in Figure 3(A–E).

There were several sonographic observations associated with the yolk sac, including the presence of free fluid at the margins. Overall, this was seen in 20% of examinations (29/139, 95% CI), with an incidence of 33% (12/36) of all examinations undertaken between 1 and 5 days of age and dropping to 16.5% in exams performed on birds >5 days of age. After accounting for repeated measures in chicks, chicks aged 1–5 days were significantly more likely to have free fluid at the yolk sac margins than chicks aged 6 days or more (OR = 2.6; 95% CI, 1.05–6.30; $P = 0.0381$).

A homogeneous echogenicity of the yolk sac was seen more frequently in older chicks. In all chicks ($n = 35$) aged 1–5 days, the

TABLE 2 Ultrasonographic findings of sequential examinations of the regressing yolk sac of captive brown kiwi (*Apteryx mantelli*) chicks ($n = 29$).

Variable	Age (days)				All
	1-5	6-9	10-15	16+	
Size					
Height (IQR; mm)	45.3 (38.8–48.3)	35.7 (31.2–39.3)	22.7 (18.7–26.2)	9.9 (0–16.6)	
Length (IQR; mm)	50.2 (47.1–54.1)	40.8 (36.0–48.1)	26.3 (21.2–30.2)	13.8 (0–18.5)	
Width (IQR; mm)	38.7 (34.6–43.4)	31.0 (28.0–35.4)	24.0 (19.9–26.8)	12.1 (0–16.9)	
Volume (IQR; mL)	48.1 (36.5–58.7)	21.5 (19.4–33.9)	7.0 (3.7–11.0)	0.9 (0–2.8)	
Surrounding free fluid (%; 95% CI)					
Not seen	24 (66.6%, 49.0–81.4)	25 (83.3%, 65.3–94.4)	36 (83.7%, 69.3–93.2)	25 (83.3%, 65.3–94.4)	110 (79.1%, 71.4–85.6)
Seen	12 (33.4%, 18.6–51.0)	5 (16.7%, 5.6–34.7)	7 (16.3%, 6.8–30.7)	5 (16.7%, 5.–34.7)	29 (20.9%, 14.4–28.6)
Total	36 (100.0%)	30 (100.0%)	43 (100.0%)	30 (100.0%)	139 (100.0%)
Echogenicity (1) (%; 95% CI)					
Homogeneous	0 (0.0%, 0–9.7)	1 (3.3%, 0.1–17.2)	25 (58.1%, 42.1–73.0)	20 (69.0%, 49.2–84.7)	46 (33.3%, 25.5–41.9)
Mildly heterogeneous	16 (44.4%, 27.9–61.9)	21 (70.0%, 50.6–85.3)	17 (39.5%, 25.0–55.6)	9 (31.0%, 15.3–50.8)	63 (45.7%, 37.2–54.3)
Moderately heterogeneous	20 (55.6%, 38.1–72.1)	8 (26.7%, 12.3–45.9)	1 (2.3%, 0.1–12.3)	0 (0.0%, 0–11.9)	29 (21.0%, 14.5–28.8)
Total	36 (100.0%)	30 (100.0%)	43 (100.0%)	29 (100.0%)	138 (100.0%)
Echogenicity (2) (%; 95% CI)					
Hyperechoic	36 (100.0%, 90.3–100)	30 (100.0%, 88.4–100)	43 (100.0%, 91.8–100)	29 (100.0%, 88.1–100)	138 (100.0%, 97.4–100)
Isoechoic	0 (0.0%, 0–9.7)	0 (0.0%, 0–11.6)	0 (0.0%, 0–8.2)	0 (0.0%, 0–11.9)	0 (0.0%, 0–2.6)
Hypoechoic	0 (0.0%, 0–9.7)	0 (0.0%, 0–11.6)	0 (0.0%, 0–8.2)	0 (0.0%, 0–11.9)	0 (0.0%, 0–2.6)
Total	36 (100.0%)	30 (100.0%)	43 (100.0%)	29 (100.0%)	138 (100.0%)
Anechoic pockets (%; 95% CI)					
Not seen	28 (77.8%, 60.8–89.9)	28 (93.3%, 77.9–99.2)	37 (86.0%, 72.1–94.7)	26 (86.7%, 69.3–96.2)	119 (85.6%, 78.7–91.0)
Present	8 (22.2%, 10.1–39.2)	2 (6.7%, 0.8–22.1)	6 (14.0%, 5.3–27.9)	4 (13.3%, 3.8–30.7)	20 (14.4%, 9.0–21.3)
Total	36 (100.0%)	30 (100.0%)	43 (100.0%)	30 (100.0%)	139 (100.0%)
Shape (%; 95% CI)					
Invaginations or lobulated margins	21 (58.5%, 40.8–74.5)	12 (41.4%, 23.5–61.1)	11 (25.6%, 13.5–41.2)	13 (44.8%, 26.4–64.3)	57 (41.6%, 33.3–50.3)
Round or ovoid	15 (41.7%, 25.5–59.2)	17 (58.6%, 38.9–76.5)	32 (74.4%, 58.8–86.5)	16 (55.2%, 35.7–73.6)	80 (58.6%, 49.7–66.7)
Total	36 (100.0%)	29 (100.0%)	43 (100.0%)	29 (100.0%)	137 (100.0%)
Doppler (%; 95% CI)					
None	3 (8.3%, 1.8–22.5)	5 (16.7%, 5.6–34.7)	1 (2.3%, 0.1–12.3)	4 (13.3%, 3.8–30.7)	13 (9.4%, 5.1–15.5)
Within	12 (33.3%, 18.6–51.0)	15 (50.0%, 31.3–68.7)	5 (11.6%, 3.9–25.1)	0 (0.0%, 0–11.6)	32 (23.0%, 16.3–30.9)
Within and at margins	9 (25.0%, 12.1–42.2)	2 (6.7%, 0.1–22.1)	8 (18.6%, 8.4–33.4)	1 (3.3%, 0.1–17.2)	20 (14.4%, 9.0–21.3)
Only at margins	6 (16.7%, 6.4–32.8)	8 (26.7%, 12.3–45.9)	23 (53.5%, 37.7–68.8)	15 (50.0%, 31.3–68.7)	52 (37.4%, 29.4–46.0)
Not performed	6 (16.7%, 6.4–32.8)	0 (0.0%, 0–11.6)	6 (14.0%, 5.3–27.9)	10 (33.3%, 17.3–52.8)	22 (15.8%, 10.2–23.0)
Total	36 (100.0%)	30 (100.0%)	43 (100.0%)	30 (100.0%)	139 (100.0%)

(Continues)

TABLE 2 (Continued)

Variable	Age (days)				
	1–5	6–9	10–15	16+	All
Scan position (%; 95% CI)					
Cranial to umbilicus	36 (100.0%, 90.3–100)	30 (100.0%, 88.4–100)	32 (74.4%, 58.8–86.5)	6 (14.3%, 5.4–28.5)	104 (68.9%, 60.8–76.2)
Caudolateral to umbilicus	0 (0.0%, 0–9.7)	0 (0.0%, 0–11.6)	11 (25.6%, 13.5–41.2)	36 (85.7%, 71.5–94.6)	47 (31.1%, 23.8–39.2)
Total	36 (100.0%)	30 (100.0%)	43 (100.0%)	42 (100.0%)	151 (100.0%)
Count of all other sonographic features (%; 95% CI)					
None	9 (25.05%, 12.1–42.2)	11 (36.7%, 19.9–56.1)	34 (79.1%, 64.0–90.0)	26 (86.7%, 69.3–96.2)	80 (56.7%, 48.9–65.9)
One feature	20 (55.6%, 38.1–72.1)	15 (50.0%, 31.3–68.7)	9 (20.9%, 10.0–36.0)	4 (13.3%, 3.8–30.7)	48 (34.5%, 26.7–43.1)
Two features	3 (8.3%, 1.8–22.5)	4 (13.3%, 3.8–30.7)	0 (0.0%, 0–8.2)	0 (0.0%, 0–11.6)	7 (5.0%, 2.0–10.1)
Three features	4 (11.1%, 3.1–26.1)	0 (0.0%, 0–11.6)	0 (0.0%, 0–8.2)	0 (0.0%, 0–11.6)	4 (2.9%, 0.8–7.2)
Total	36 (100.0%)	30 (100.0%)	43 (100.0%)	30 (100.0%)	139 (100.0%)
Count of specific other sonographic features ^a					
A					3 (2.2%)
B					4 (2.9%)
C					13 (9.4%)
D					16 (11.5%)
E					8 (5.8%)
F					5 (3.6%)
G					1 (0.7%)
H					2 (1.4%)
I					1 (0.7%)
J					4 (2.9%)
K					11 (7.9%)
L					1 (0.7%)
M					1 (0.7%)

^aOther ultrasonographic features of the regressing yolk sac of brown kiwi chicks obtained with a 3–10 MHz convex hand-held Clarius ultrasound scanner (C7Vet HD3, Clarius Mobile Health Corp.). A, Large swirl, characterized by well-defined hyperechoic curvilinear echogenicity and adjacent ill-defined hypoechoic region. B, Ill-defined hyperechoic curvilinear echogenicity. C, Ill-defined hypoechoic curvilinear echogenicity. D, Well-defined hyperechoic curvilinear echogenicity. E, Ill-defined hypoechoic region. F, Ill-defined hyperechoic region; G, Ill-defined regions of differing echogenicity. H, Small irregularly shaped hyperechoic region. I, Well-defined hypoechoic region; J, Septation with well-defined hyperechoic curvilinear echogenicity; K, Scattered hyperechoic foci. L, Mildly hypoechoic region with smooth curved well-defined cranioventral margins. M, Well-defined hypoechoic curvilinear echogenicity.

echogenicity of the yolk was described as mildly heterogeneous or moderately heterogeneous. In contrast, in chicks aged 10–15 days and 16+ days, the yolk was described as homogeneous in echogenicity in 25 of 43 (58.1%) and 20 of 29 (69.0%) chicks, respectively. There was a statistically significant association between heterogeneity of the yolk echogenicity with age (OR age for mildly heterogeneous vs. homogenous = 0.72, $P < 0.001$; OR age for moderately heterogeneous vs. homogenous = 0.54, $P < 0.001$), with younger chicks having more heterogeneous yolks (mildly and moderately heterogeneous echogenicity; Figure 4).

All yolks were described as hyperechoic relative to adjacent coelomic structures. Intrasac anechoic pockets were seen in the yolk sac of brown kiwi chicks in each of the age groups, with an overall incidence of 20 of 139 (14.4%). There was no statistically significant association between intrasac anechoic pockets and age.

Yolk sacs with either round/ovoid or lobulated/invaginated margins were seen in kiwi chicks in each of the age groups, with round/ovoid shaped yolk sacs more commonly seen in chicks 10–15 days compared with any other age (OR compared with 1–5: 4.07; 95% CI, 1.60–10.89; P value, 0.004). Evidence of flow within vessels within the yolk sac

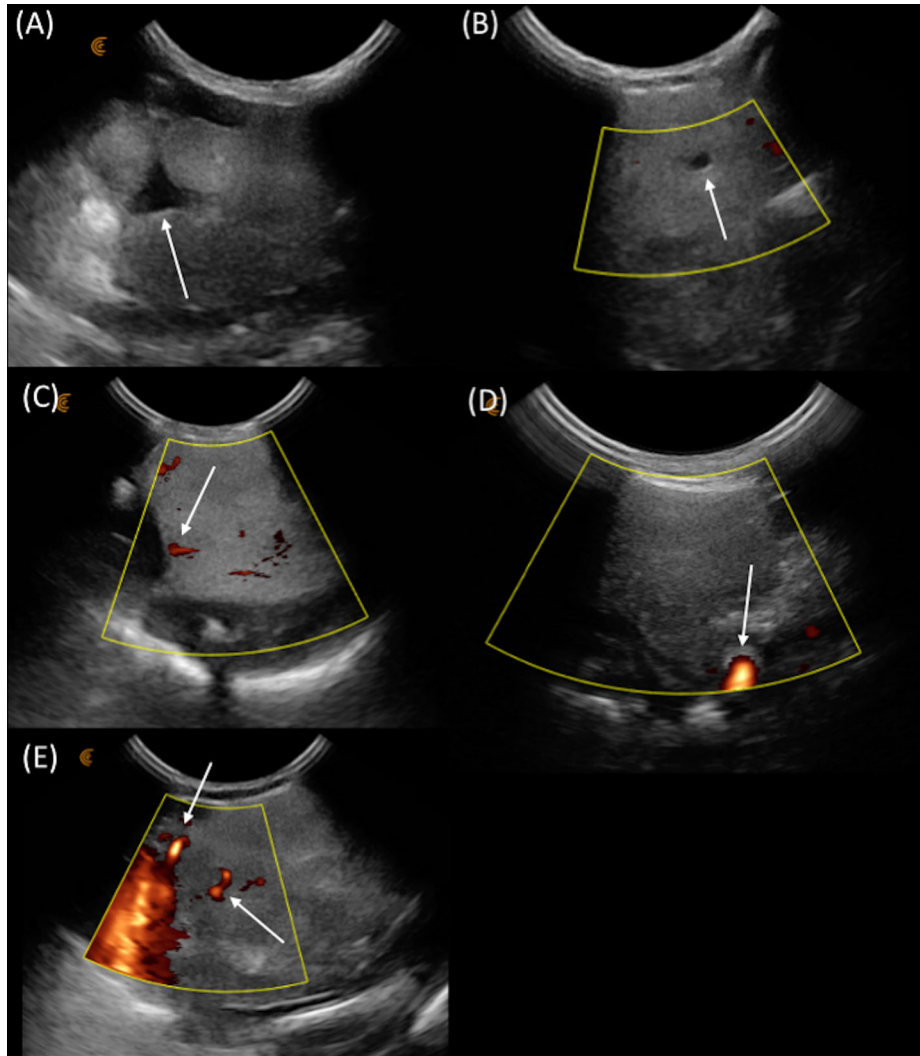


FIGURE 3 Examples of ultrasonographic features of the regressing yolk sac of brown kiwi chicks obtained with a 3–10 MHz convex hand-held Clarius ultrasound scanner (C7Vet HD3, Clarius Mobile Health Corp.). White arrows point to the identified feature in each image. A, Anechoic free coelomic fluid at the margins of the yolk sac. B, Anechoic pockets within the yolk sac. Power Doppler examination findings including evidence of flow within vessels within the yolk sac (C), at the margins of the yolk sac (D), and within and also at the margins of the yolk sac (E). [Color figure can be viewed at [wileyonlinelibrary.com](https://onlinelibrary.wiley.com)]

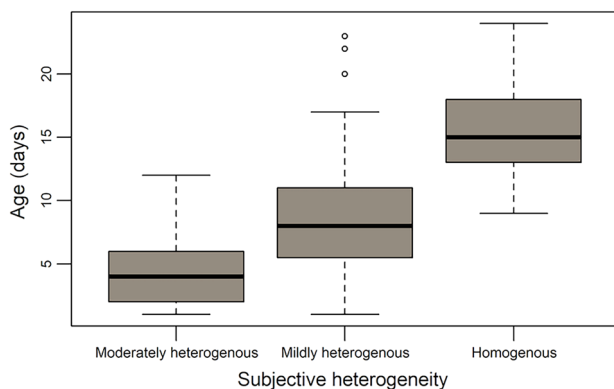


FIGURE 4 Subjective assessment of the ultrasonographic heterogeneity of the echogenicity of the regressing yolk sac of captive brown kiwi chicks ($n = 29$). [Color figure can be viewed at [wileyonlinelibrary.com](https://onlinelibrary.wiley.com)]

during Power Doppler examination was seen in 12 of 36 (33%) and 15 of 30 (50%) of examinations of chicks 1–5 and 6–9 days of age, respectively, although this difference was not statistically significant. In 15 of 30 (50%) of Power Doppler examinations of chicks aged 16+ days, evidence of flow within vessels was only seen at the margin of the yolk sac. Positioning of the transducer caudolateral to the umbilicus was required to identify the yolk sac in 11 of 43 (25.6%) and 36 of 42 (85.7%) of examinations in chicks aged 10–15 days and 16+ days, respectively (OR = 26.7; 95% CI, 8.17–338.06; $P = 0.001$).

Thirteen “other sonographic features” of the regressing yolk sac were seen and described (Figure 5). These included the following: a large swirl characterized by well-defined hyperechoic curvilinear echogenicity and adjacent ill-defined hypoechoic region; ill-defined hyperechoic curvilinear echogenicity; ill-defined hypoechoic curvilinear echogenicity; well-defined hyperechoic curvilinear echogenicity; ill-defined hypoechoic region; ill-defined hyperechoic region;

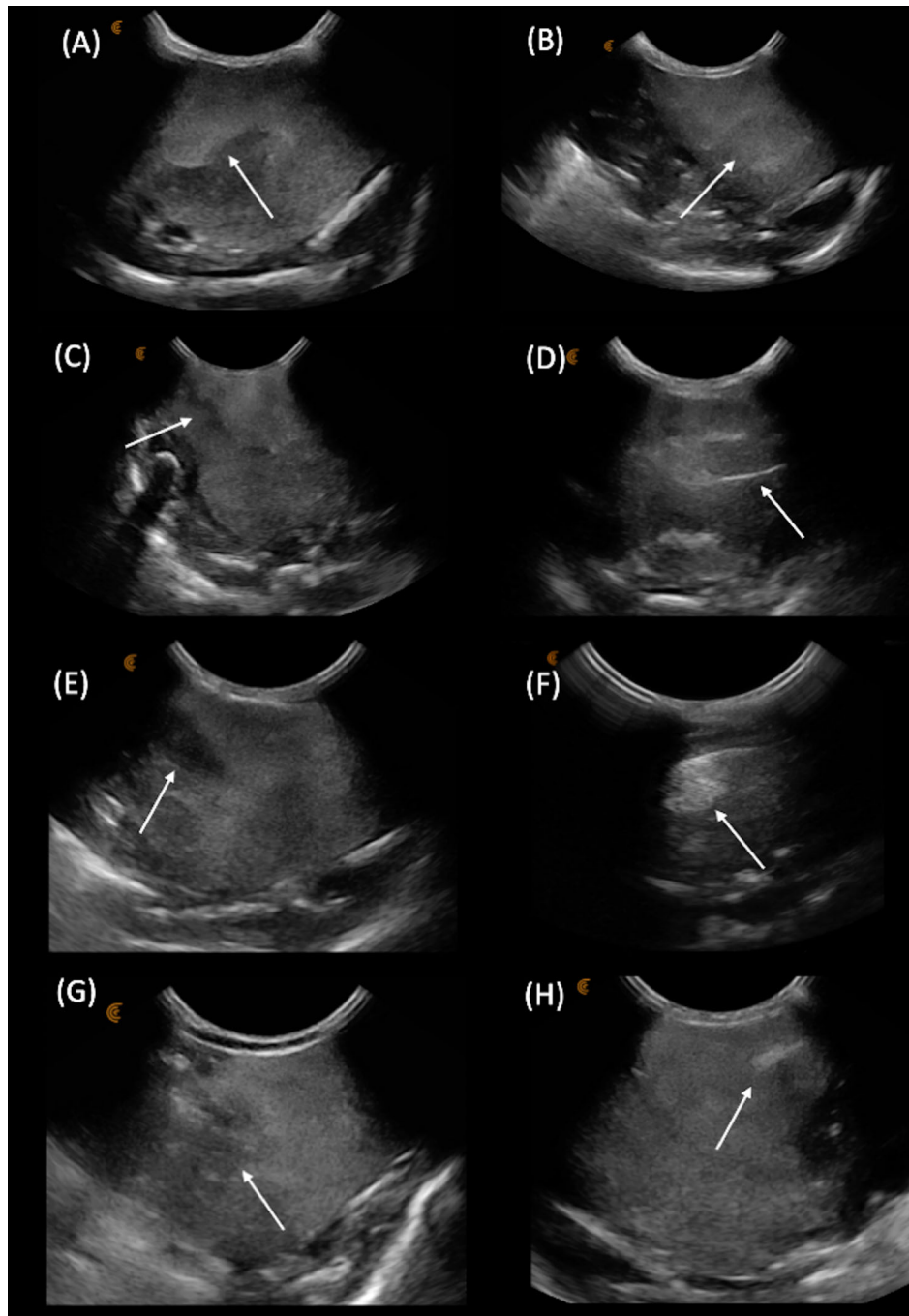


FIGURE 5 Examples of ultrasonographic features of the regressing yolk sac of brown kiwi chicks obtained with a 3–10 MHz convex hand-held Clarius Ultrasound Scanner (C7Vet HD3, Clarius Mobile Health Corp.). White arrows point to identified feature in each image. A, Large swirl, characterized by well-defined hyperechoic curvilinear echogenicity and adjacent ill-defined hypoechoic region. B, Ill-defined hyperechoic curvilinear echogenicity. C, Ill-defined hypoechoic curvilinear echogenicity. D, Well-defined hyperechoic curvilinear echogenicity. E, Ill-defined hypoechoic region. F, Ill-defined hyperechoic region. G, Ill-defined regions of differing echogenicity. H, Small irregularly shaped hyperechogenicity. I, Well-defined hypoechoic region. J, Septation with well-defined hyperechoic curvilinear echogenicity. K, Scattered hyperechoic foci. L, Mildly hypoechoic region with smooth curved well-defined cranioventral margins. M, Well-defined hypoechoic curvilinear echogenicity. [Color figure can be viewed at wileyonlinelibrary.com]

ill-defined regions of differing echogenicity; small irregularly shaped hyperechogenicity; well-defined hypoechoic region; septation with well-defined hyperechoic curvilinear echogenicity; scattered hyperechoic foci; a mildly hypoechoic region with smooth curved well-defined cranioventral margins; well-defined hypoechoic curvilinear echogenic-

ity. Examples of these other sonographic features of the regression yolk sac are shown in Figure 6A–M

The median (IQR) height, length, and width of the yolk sac of brown kiwi chicks aged 1–5, 6–9, 10–15, and 16+ days are outlined in Table 2. A Box and whisker graph of approximate ellipsoid

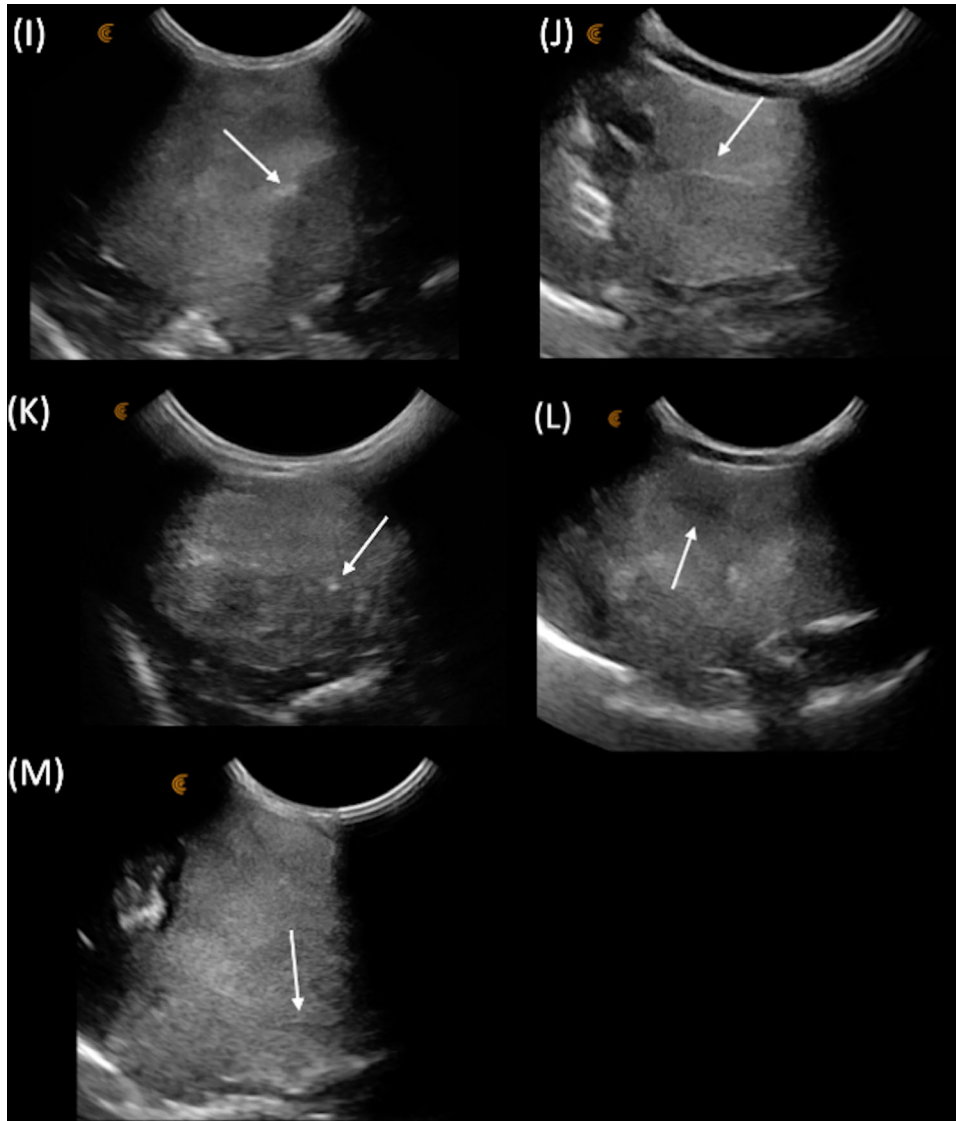


FIGURE 5 Continued

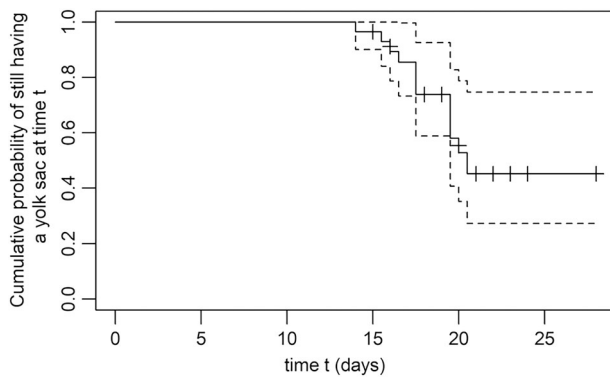


FIGURE 6 Cumulative probability of still having a yolk sac at time t (days of age) in captive brown kiwi chicks ($n = 29$). Survival function assuming that if the yolk sac was seen at one visit but not the one after, it disappeared halfway.

volume (mL) of the yolk sac of brown kiwi chicks aged 1–5, 6–9, 10–15, and 16+ days, measured via ultrasound is shown in Figure 7. The median approximate yolk sac volume for chicks aged 1–5, 6–9, 10–15, and 16+ days are outlined in Table 2. The best-fit equation to model the approximate yolk sac volume as a function of age was $71.72 - 8.94 \times \text{age} + 0.38 \times \text{age}^2 - 0.006 \times \text{age}^3$ (Figure 8).

In chicks where the yolk sac was observed to be less than 1 mL in approximate ellipsoid volume or was not identified and presumed completely absorbed, this occurred between 13 and 21 days of age (Figure 6). The probability of a chick still having a yolk sac by 21 days was 45.2% (95% confidence interval 27.4%–74.7%), assuming the rate of disappearance of the yolk sac was the same in the kiwis lost to follow-up and those that remained in the sample until the end of the period of data collection. Yolk sac resorption was more rapid in younger chicks and slowed with increasing age (Figure 8).

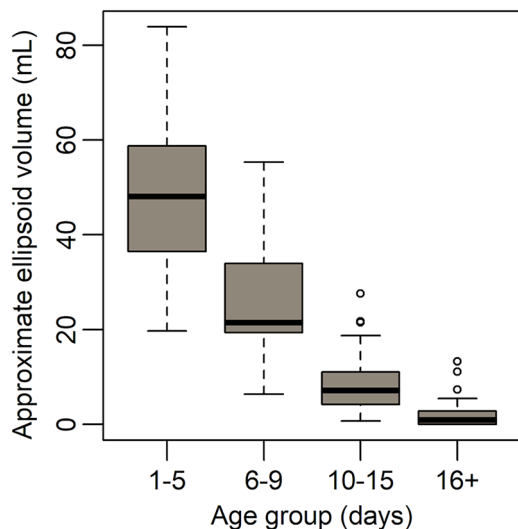


FIGURE 7 Box and whisker plots of the approximate ellipsoid volume (mL) of the yolk sac of brown kiwi chicks aged 1–5, 6–9, 10–15, and 16+ days, measured via ultrasound. [Color figure can be viewed at [wileyonlinelibrary.com](https://onlinelibrary.wiley.com)]

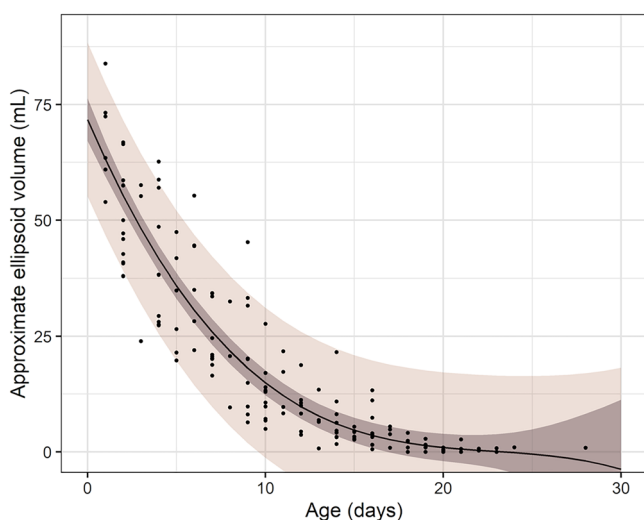


FIGURE 8 Predicted volume of the yolk sac of brown kiwi chicks ($n = 29$) as a polynomial function of age. In the plot, the dots are chicks (observed), the line is the predicted average volume, the darker ribbon is the 95% confidence interval for this predicted average, and the lighter ribbon is the prediction band (i.e., we predict that for a given age the volume will be in this range). [Color figure can be viewed at [wileyonlinelibrary.com](https://onlinelibrary.wiley.com)]

4 | DISCUSSION

The results of this study fulfilled the study objectives including description of the sonographic appearance of the yolk sac throughout regression in a group of clinically healthy brown kiwi chicks hatched and raised in captivity, description of the rate of regression, and age of complete regression of the yolk sac in these chicks. On sonographic evaluation, the yolk sac was predominantly hyperechoic

relative to adjacent intracoelomic structures, had rounded/ovoid or invaginated/lobulated margins, was homogeneous, mildly or moderately heterogeneous in echogenicity, and had variable evidence of flow within small vessels within or adjacent to the yolk sac margins. This study identified a pattern of a decreasing rate of absorption over time. Brown kiwi chick yolk sacs reduced in volume with time and were resorbed (<1 mL or not identified) between 13 and 21 days.

Based on our review of the literature, a description of the sonographic appearance of the yolk sac has not been published for any avian species. There is a report of sonographic evaluation of the yolk sac of ostrich chicks (*Struthio camelus*) which involved regular measurement of the size of the yolk sac throughout regression; however, the sonographic appearance of the yolk sac was not reported in that study.¹⁷ All yolk sacs examined in the present study were predominantly hyperechoic relative to adjacent coelomic structures. This is likely attributed to the high lipid content of brown kiwi yolk sac.¹⁸ Small anechoic pockets within the yolk sac were occasionally documented. The authors postulate that these anechoic fluid pockets may represent a small amount of normal anechoic free coelomic fluid between folds or invaginations of the yolk sac margin. Sampling and analysis of this fluid was not performed; however, we suspect this represents a transudate.

Thirteen “other sonographic features” of the regressing yolk sac were seen and described. Without additional diagnostics involving tissue sampling, the exact cause of these sonographic findings remains undetermined. The regions of differing echogenicity of the yolk may be associated with regions of differing composition of the yolk sac content, and the intrasac curvilinear echogenicities seen in some yolk sacs may represent an invaginated or folded yolk sac margin.

In the majority of the older kiwi chicks, a dorsal recumbency hold was required to identify the yolk sac by positioning the transducer left parasagittal to the umbilicus. It is likely this is associated with progressive caudal positioning of the avian yolk sac within the coelom as the chicks get older and the yolk content regresses, as described previously in ostrich chicks.¹⁷

A study involving postmortem examinations of ostrich chicks found that there was a precipitous fall in the mass of the yolk sac within the first 48 h period posthatch, followed by a gradual decline occurring from days 3 to 10.¹¹ A similar rate of resorption was seen in the present study, reducing more rapidly in size initially and slowing down with age (Figure 8.).

We found that where the yolk sac was observed to be less than 1 mL, or was not identified and presumed completely absorbed, this occurred by 21 days of age (Figure 6). This was greater than the 4–15 day period for yolk utilization described for other species in the literature which utilized postmortem examination for quantification of yolk sac size.^{6,12,15,16} The precocial biology of brown kiwi, the large size of the kiwi egg and the greater yolk sac percentage may contribute to the presence of yolk for a longer period in this species.

A previous study investigating the effects of environmental and nutritional stressors on yolk sac utilization in commercial broiler chicks showed moderate changes in the optimal environmental temperature,

and deprivation of food and water, influenced yolk sac resorption rate. The highest rate of resorption was observed in chick groups on the day food was withheld for 12 h.¹⁰ Although beyond the scope of this study, we anticipate environmental and nutritional factors for captive kiwi chicks differ from chicks in the wild and are therefore likely to influence the rate of yolk absorption. In addition, individual variability in feed intake between kiwi chicks may influence yolk absorption and could be the subject of future research.

Because the present study was performed within the normal management regimen of a functional and very busy kiwi hatchery, this research had multiple limitations. The interval between sonographic examinations of each chick was determined by sonographer access to the site, and no more frequently than every second day. With COVID-19 restrictions in place, the sonographer was predominantly limited to accessing the sites in alternating 4-day blocks. The reduced frequency of access to the sites meant that less data was available for determining the rate of regression and age of complete regression of the yolk sac and that the scanning intervals were not identical for all chicks.

The kiwi chicks remained on the premises until they were individually selected to be moved off-site by hatchery management after reaching an individual weight target. This meant some chicks left the study premises before a small or absent yolk sac was documented sonographically and further evaluations were not logistically feasible. For statistical robustness, it would be preferred that every chick was scanned until the yolk was small/not seen.

To limit any deformation of the yolk sac secondary to restraint, it was initially planned that chicks would be held in sternal recumbency, as described in ostrich chicks by Blue-McLendon and Homco (1995). However, brown kiwi chicks resisted restraint in this position making it unfeasible, and left lateral or dorsal recumbency holds were used instead. Imaging the yolk in left lateral and dorsal recumbency may have resulted in deformation of the yolk sac due to gravity and external compression, which may have resulted in positional variation in measurements of height, length, and width.

In conclusion, this study describes the sonographic appearance of the regressing yolk sac of normal brown kiwi chicks that are hatched and raised in captivity. This information can be used as a reference of normal during sonographic assessment of captive brown kiwi chicks with suspected yolk sac disease. Importantly, these sonographic findings do not exclude an abnormal yolk sac, and therefore future research could include description of the sonographic appearance of yolk sac disease in brown kiwi chicks.

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LIST OF AUTHOR CONTRIBUTIONS

Category 1

- (a) Conception and design: Short, Owen, Morgan, Bolwell
- (b) Acquisition of data: Short
- (c) Analysis and interpretation of data: Short, Bolwell, Vallee, Owen, Morgan

Category 2

- (a) Drafting the article: Short, Morgan, Vallee
- (b) Revising article for intellectual content: Short, Owen, Morgan, Bolwell, Vallee

Category 3

- (a) Final approval of the completed article: Short, Owen, Morgan, Bolwell, Vallee

Category 4

- (a) Agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved: Short, Owen, Morgan, Bolwell, Vallee

CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

PREVIOUS PRESENTATION OR PUBLICATION DISCLOSURE

Portions of this work have been presented at the Australia and New Zealand College of Veterinary Scientists (ANZVCS) Science Week Conference 2022 and the Massey University Wildbase Post-graduate Research Symposium 2022.

REPORTING GUIDELINE DISCLOSURE

STROBE-Vet statement checklist was followed for this study.

DATA AVAILABILITY STATEMENT

The data is available from the corresponding author upon request.

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