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Full Length Research Article

ESTIMATION OF FOETAL AGE USING ULTRASONOGRAPHIC BIOMETRIC PARAMETERS IN RABBITS

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ABSTRACT
Accurate estimation of foetal age in rabbits is essential for effective reproductive management, research planning, and timely clinical intervention. This study evaluated the use of ultrasonography measurements of key foetal parameters to predict gestational age in New Zealand White rabbit Does. Eight adult virgin Does were naturally mated individually using eight bucks, and scanned using a Medison S600V ultrasound machine equipped with a 6.5 MHz transcutaneous curvilinear probe. Ultrasonographic examinations were performed on days 4, 8, 12, 16, 20, 24, and 28 post-coitus. Crown-rump length (CRL), biparietal diameter (BPD), and embryonic vesicle (EV) diameters were recorded and analyzed. All parameters showed progressive increases with advancing gestation: CRL increased from 0.5 cm (day 8) to 4.5 cm (day 28); BPD from 0.4 cm (day 12) to 1.7 cm (day 28); and EV diameter from 0.39 cm (day 4) to 2.8 cm (day 24). Strong positive correlations were observed between gestational age and CRL (R = 0.991), BPD (R = 0.995), and EV (R = 0.994). The derived regression models GA = 4.840(CRL) + 7.027; GA = 12(BPD) + 8; GA = 7.753(EV) + 0.999 demonstrate high predictive value and statistical significance (p≤0.01). These findings confirm that CRL, BPD, and EV are reliable biometric indicators for estimating foetal age in rabbits. The study provides practical reference data that can enhance reproductive monitoring, improve timing of interventions, and support biomedical research involving rabbit gestation.

Keywords: Ultrasonography; Gestation; Rabbit; Foetal age; Measurement.

INTRODUCTION
Ultrasonography, while widely used in human pregnancy, is less common in laboratory animal studies (Stasinopoulou *et al.,* 2014). Until recently, ultrasonography was restricted to larger non-rodent species (Beccaglia & Luvoni, 2006; Carr *et al.,* 2012; Quintela *et al.,* 2012). For example, ultrasound was used to estimate gestational age in sheep and goats (Gouda *et al.*, 2021), and parturition day in sheep was predicted using sonographic assessment of foetal abdominal circumference (AC) and foetal renal volume (Carr *et al.*, 2011). Sonography was also used to estimate litter size and gestational age in pregnant bitches (Lenard *et al.,* 2007). The detection of foetal abnormalities is another important goal of ultrasound in reproduction (Sahli *et al.*, 2019). Commercially available ultrasound systems now have the spatial and temporal resolution to produce precise images of the hearts, kidneys, and other organs of rats and mice, owing to advances in ultrasound imaging technology (Stypmann, 2007; Golden *et al.,* 2011). Furthermore, ultrasound is a useful technique for diagnosing pregnancy in agouti mice (Sousa *et al.,* 2012), staging gestation, and tracking intrauterine growth in rats (Brown *et al.,* 2006; Mu *et al.,* 2008; Nguyen *et al.,* 2012; Pallares & Gonzalez-Bulnes, 2009; Ypsilantis *et al.,* 2009).

Monitoring gestation in rabbits is important because rabbits are commonly used in biomedical research to investigate human diseases (Coombs *et al.*, 2020) or to manage the species' overall health (Akkuş & Erdoğan, 2019). Radiology is ineffective for diagnosing early pregnancy in rabbits, owing to foetuses' very late ossification (DeSesso & Scialli, 2018). In rabbits, pregnancy is diagnosed using abdominal palpation (Idris *et al.*, 2016), B-mode and Doppler ultrasound (El-Gayar *et al.*, 2014; Amran *et al.*, 2019), and microultrasound and magnetic resonance imaging (Coombs *et al.*, 2020). Biochemical procedures may also help detect pregnancy in rabbits (Mazandarani *et al.*, 2021). Several studies used New Zealand White (NZW) rabbits (Chavatte-Palmer *et al.*, 2008), Japanese White rabbits (Mazandarani et al., 2021), and Dutch rabbits (Cavanaugh *et al.*, 2014). Rabbits are widespread and have a diverse range of breeds worldwide. They play important roles in human life, including nutrition (Michalik *et al.*, 2006), entertainment, and biomedical modelling (Suckow *et al.*, 2010). Phylogenetic analysis reveals that domestic rabbits belong to the Lagomorpha order, the Leporidae family, and the *Oryctolagus cuniculus* species. The English lop is the oldest domesticated lop rabbit in the world (Suckow *et al.*, 2010).

Currently, reproductive biotechnologies like cloning (Chesné *et al.*, 2002) and transgenesis (Bosze & Houdebine, 2006) are being developed using rabbits as model species. It is also a useful model for studies of how maternal nutrition affects placental development and offspring health, especially regarding nutrients involved in lipid metabolism (Chavatte-Palmer *et al.*, 2008) or maternal hypertension (Denton *et al.*, 2003). Given the similar metabolic requirements and rate of embryonic genome activation in the early stages of development between the human and rabbit embryo (Chavatte-Palmer *et al.*, 2008), as well as the fact that the rabbit's hemochorial placentation is more similar to the human placenta than that of other domestic species, the rabbit seems like an intriguing model to investigate the long-term effects of disruptions of the early embryonic life (i.e., in vitro production) or changed maternal conditions (Foote & Carney, 2000; Soares *et al.*, 2018). The rabbit also has the advantage of being larger than laboratory

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rodents, allowing the use of traditional veterinary scanners to study foetal development via ultrasound. Additionally, the researcher may be able to examine foetal development in the two distinct groups of foetuses within the same maternal environment by transferring embryos from different origins or treatment groups into each horn (with separate cervices).

Additionally, timely intervention to prevent or minimize reproductive losses can be achieved through accurate prediction of parturition in rabbits (Akkuş & Erdoğan, 2019). This is to reduce kit mortality and monitor animals at high risk for dystocia using precise parturition dates. A precise determination of gestational age can help with treatment decisions for rabbits with a history of abortion, embryonic resorption, or inadequate luteal phase. Lastly, precise estimation of ovulation, gestational age, and parturition date is necessary for the advancement of assisted reproductive methods in rabbits, such as oestrus synchronization and embryo transfer (Akkuş & Erdoğan, 2019). Digital palpation of the abdomen is unquestionably the traditional method of diagnosing pregnancy in rabbits. According to reports, abdominal palpation is accurate between 14 and 21 days following mating (Akkuş & Erdoğan, 2019). Individual amniotic gestational sacs are tiny and difficult to palpate before approximately 7 days, particularly in high-intensity or obese rabbits. According to reports, B-mode ultrasonography can diagnose pregnancy in rabbits with 94 – 98% accuracy when used after 14 – 21 days of gestation and 99% accuracy when used more than 22 days after the last breeding (Ghi *et al.*, 2017). According to reports, foetal movement can be seen as early as 12 days of gestation, while foetal heartbeats can be detected as early as 15 days (Ghi *et al.*, 2017). It has been shown that measuring the bi-parietal head diameter of foetuses, either with or without measuring the dorsoventral trunk diameter, can accurately estimate gestational age in dogs (Schröder, 2003); however, this has not yet been established in rabbits. Additionally, there are currently no published sonographic measurements of the various gestational structures in rabbits. This study used ultrasonic measurements of various foetal parameters in Does to estimate the age of the foetus.

MATERIALS AND METHODS

Ethics:

All procedures were approved by the ethics committee of animal experimentation at the University of Nigeria, Nsukka, with ethical number FVM-UNN-IACUC-2025-03/214. All procedures were conducted in accordance with internationally recognized ethical guidelines. Ultrasonographic measurements were performed in accordance with the professional standards outlined by the Society of Radiographers (SoR) and the British Medical Ultrasound Society (BMUS) to ensure patient and operator safety (SoR & BMUS, 2021). Animal handling and experimental procedures adhered to the ethical principles for animal research recommended by the International Bioethics Study Group, ensuring minimal distress and discomfort while maintaining scientific validity (Kiani *et al.,* 2022).

Animals

Sixteen (16 – 8 bucks and 8 does) New Zealand white rabbits, mean age of 8 ± 0.5 months (SEM, n=16) and mean weight of 1.5 ± 0.13 kg (SEM, n=16) were provided by a breeder in Nsukka, Enugu State. They were housed individually in 16 wooden hutches with internal dimensions of 1.0m x 1.0m x 1.0m (L x W x H) in the Animal House of the Department of Veterinary Obstetrics and Reproductive Diseases, University of Nigeria, Nsukka, between May and July 2025 (a total duration of 60 days). +++ The animals were exposed to natural environmental conditions, with ambient temperatures ranging from 21.6 - 31.3℃, relative humidity of 76-86%, and a near-constant photoperiod of approximately 12.4 - 12.5h of light per day. They were fed *Panicum maximum* grass and supplemented with a commercial poultry grower feed (Vital Grower Feed from Grand Cereals Ltd, Ogbaru, Nigeria) containing 15% crude protein, 7% fat, 10% crude fibre, 1% calcium, 0.35% phosphorus, 2550 kg metabolizable energy, and water was provided *ad libitum*.

Mating

Following an adaptation period of two weeks, one buck of the same breed was introduced into the hutch of a doe; the selection criteria were random, and they were monitored for the coupling event. Three mating frequencies were used for each doe + buck. The mated females were maintained individually in cages throughout the pregnancy.

Ultrasound examination

Ultrasound scans were performed using a Medison SonoAce SA-600 Portable Ultrasound Machine (*2725 Westinghouse* Boulevard*, Suite 1100, Charlotte, North Carolina 28273*, USA) with a 6.5 MHz transcutaneous curved-linear probe, ultrasound gel, and gloves. Each doe was properly restrained and placed on dorsal recumbency. Furs from the level of the xiphoid cartilage down to the pelvic region were then liberally shaved and thoroughly cleaned with cotton wool soaked in an antiseptic solution. Thereafter, the surface was dried, and Aquasonic gel was applied to the shaved skin. The portable ultrasound machine was used to scan the abdominopelvic region, with the bladder as a landmark. The probe was gently placed transversely on the skin and tilted longitudinally until a descriptive echographic image appeared on the screen. This process was carried out on the 4th day post coitus and thereafter on days 8, 12, 16, 20, 24, and 28. After obtaining a descriptive echocardiographic image, the freeze button on the keyboard was used to freeze it, and the image was then printed on thermal paper for documentation. Each doe was insonated for an average of 5 minutes.

Data Analysis

Data obtained were analyzed using SPSS Statistics for Windows, Version 20.0 (IBM Corp., USA). The relationship between the gestational age and the foetal biometric parameters (crown-rump length, biaparietal diameter, and embryonic vesicle diameter) was evaluated using Pearson's product-momentum correlation coefficient analysis. Simple linear regression (y = a + bx) was performed to derive predictive equations for estimating gestational age from each biometric parameter. Statistical significance was set at p≤0.01. y = Gestational age (days), x = value of the respective biometric parameter (mm), n = exponent and b = regression coefficients.

RESULTS

Measurements of f0etal development parameters showed progressive increases with advancing gestational age.

Foetal Crown-Rump Length (CRL): CRL increased steadily from 0.5 ± 0.03 cm at day 8 of gestation to 4.5 ± 0.02cm by day 28.

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Specifically, measurements were 0.5 ± 0.03 cm at day 8, 0.9 ± 0.02 cm at day 12, 1.8 ± 0.03cm at day 16, 2.4 ± 0.04cm at day 20, 3.5 ± 0.01cm at day 24, and 4.5 ± 0.02cm at day 28 (Table 1). The increase in CRL showed a strong correlation with gestational age (R=0.991; p≤0.01) (Figure 1).

Biparietal Diameter (BPD): The BPD showed a consistent upward trend from 0.4 ± 0.01cm at day 12 to 1.7 ± 0.01 cm at day 28. Values recorded were 0.4 ± 0.01 cm at day 12, 0.6 ± 0.01 cm at day 16, 1.0 ± 0.03cm at day 20, 1.3 ± 0.01cm at day 24, and 1.7 ± 0.01cm at day 28 (Table 2). The biparietal diameter exhibited a strong positive correlation with gestational age (R=0.995; p≤0.01) (Figure 2).

Embryonic Vesicle (EV): The size of the embryonic vesicle increased from 0.39 ± 0.02 cm at day 4 to 2.8 ± 0.01cm at day 24. Measurements included 0.39 ± 0.02 cm at day 4, 0.87 ± 0.02 cm at day 8, 1.4 ± 0.01 cm at day 12, 2.0 ± 0.01 cm at day 16, 2.6 ± 0.02 cm at day 20, and 2.8 ± 0.01 cm at day 24 (Table 3). Embryonic vesicle diameter was also strongly and positively correlated with gestational age (R=0.994; p≤0.01) (Figure 3).

Simple linear regression analysis revealed significant predictive relationships between gestational age and each foetal parameter, yielding the following equations:
The derived growth equations were:

- GA = 7.027 + 4.840(*crl*)
- GA = 8 + 12(*bpd*)
- GA= 0.999 + 7.753(*ev*)

where GA is the gestational age in days, CRL is the Crown Rump Length, BPD is the Biparietal Diameter, and EV is the Embryonic Vesicle, all in millimeters.

Table 1: The Foetal crown-rump length and its corresponding gestational ages**.**

DAYS	8	12	16		20	24	28
CRL(cm)	0.5 ± 0.03	0.9 ± 0.02	1.8 ± 0.03		2.4 ± 0.04	3.5 ± 0.01	4.5 ± 0.02

Table 2: The Biparietal Diameter and its corresponding gestational ages.

Days	12	16	20	24	28
BPD(cm)	0.4 ± 0.01	0.6 ± 0.01	1.0 ± 0.03	1.3± 0.01	1.7 ± 0.01

Table 3: The Embryonic Vesicle and its corresponding gestational ages

Days	4	8	12	16	20	24
EV(cm)	0.39 ± 0.02	0.87 ± 0.02	1.4 ± 0.01	2.0 ± 0.01	2.6 ± 0.02	2.8 ± 0.01

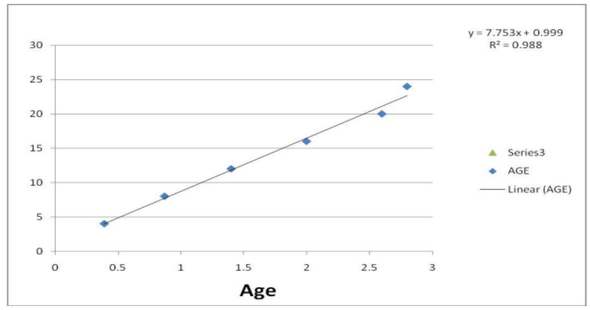

Figure 1: Relationship between (CRL) and foetal age of New Zealand white Rabbit.

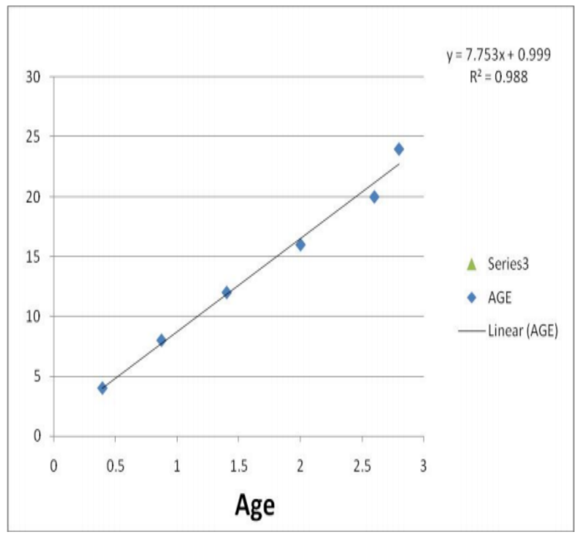

Figure 2: Relationship between Biparietal Diameter (BPD) and foetal age of New Zealand white

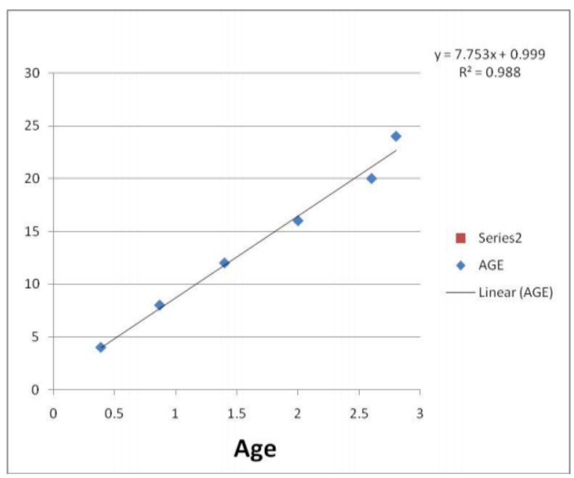

Figure 3: Relationship between Embryonic Vesicle (EV) and foetal age of New Zealand white Rabbit

DISCUSSION

The present study investigated the progressive development of foetal parameters: crown-rump length (CRL), biparietal diameter (BPD), and embryonic vesicle (EV), during gestation. It showed a

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consistent, significant increase as gestational age advanced. These findings support the fundamental principle that embryonic and fetal growth occur in a predictable and orderly fashion, in which structural dimensions expand progressively over time as organogenesis and tissue differentiation proceed (Sadler, 2018; Torchia & Persaud, 2024). The strong positive correlations observed between gestational age and each measured parameter indicate that CRL, BPD, and EV diameters are reliable indices for estimating fetal age during the early stages of pregnancy.

Foetal Crown Rump Length (CRL): The observed steady increase in CRL with advancing pregnancy is consistent with the existing literature, which identifies CRL as one of the most dependable measures of early fetal growth (Coombs *et al.*, 2020; Juárez *et al.*, 2021).

CRL reflects the distance from the top of the head (crown) to the base of the buttocks (rump) and is considered a direct indicator of longitudinal embryonic development (Malik *et al.*, 2017). According to Xu *et al.* (2022), CRL correlates strongly with gestational age in both human and animal models, particularly during the first trimester when fetal growth is relatively uniform. The progressive pattern found in this study is consistent with the findings of Pexsters *et al.* (2010), who demonstrated that CRL follows an exponential growth curve until approximately the end of the first month of gestation. The derived growth equation further confirms this close relationship, suggesting that small increments in CRL correspond to predictable advances in gestational age. Such a relationship is critical for accurate gestational age estimation, especially in species or clinical contexts where the exact date of conception may be uncertain (Ohuma *et al.*, 2013), and can be used to predict embryonic development and detect deviations indicative of delayed or accelerated growth. These deviations may suggest underlying developmental or endocrine abnormalities (Baken *et al.,* 2017).

Biparietal Diameter (BPD): Similarly, the biparietal diameter (BPD) exhibited a consistent upward trend. BPD measures the transverse diameter of the fetal head and is widely used as a standard biometric parameter in ultrasonographic evaluation of fetal maturity (Chitty *et al.*, 1994). The pattern of increase observed in the current study is consistent with the findings of Adiri et al. (2015), who reported comparable increases in fetal BPD in mammalian models. The derived regression equation demonstrates a linear relationship between gestational age and BPD, further validating its predictive value. The gradual increase in BPD may reflect the rapid brain and skull development characteristic of the late embryonic and early fetal periods. During this time, neural tube closure and early neurogenesis significantly contribute to cranial expansion (Moore *et al.*, 2020). Thus, the measured increases in BPD not only represent overall somatic growth but also provide insight into central nervous system development. Moreover, deviations from expected BPD growth trajectories can serve as early indicators of potential anomalies such as microcephaly or growth retardation (Hasegawa *et al.*, 2015).

Embryonic Vesicle (EV): The embryonic vesicle diameter increased, displaying a progressive and regular expansion pattern consistent with embryonic sac development reported in similar studies (Mazandarani *et al.*, 2021; Keshavarz *et al.*, 2022). The derived equation closely aligns with previous observations that vesicular diameter correlates with gestational age in the earliest weeks of pregnancy (Topie *et al.*, 2015). According to Lee *et al.* (2006), the growth rate of the embryonic vesicle reflects not only embryonic development but also amniotic fluid accumulation, trophoblastic proliferation, and early placental function. The strong correlation observed in this study supports the view that EV measurement is a sensitive indicator of early pregnancy progression. Furthermore, the parallel increases in CRL, BPD, and EV underscore the synchrony of embryonic and extraembryonic development. The expansion of the vesicle provides the physical and biochemical environment necessary for fetal differentiation and growth. This coordination between structural growth and physiological maturation has been reported in both animal and human studies (Santos *et al.*, 2014; Thowfeequ & Srinivas, 2022).

Comparative and Biological Implications: The consistent growth trends across the three parameters reveal that fetal development during early gestation proceeds in a predictable linear or near-exponential manner. This reflects tightly regulated genetic, hormonal, and environmental influences that govern embryonic morphogenesis (Rossant & Tam*,* 2002). The close agreement between the derived regression equations and observed data indicates a high degree of biological precision in fetal growth control. These findings have practical implications for both veterinary and human obstetrics. In veterinary contexts, such growth equations can assist in pregnancy dating and monitoring of intrauterine development in experimental models or livestock species (Vannucchi *et al.,* 2019). In clinical human applications, similar measurements form the basis of early obstetric ultrasound assessments, enabling accurate dating and detection of developmental irregularities such as intrauterine growth restriction (IUGR) or early embryonic demise (Pexsters *et al.*, 2010; Nusken *et al*., 2024).

Conclusion

This study provides strong quantitative evidence that fetal CRL, BPD, and EV diameter increase predictably with advancing gestational age. The high statistical correlations and derived regression equations confirm the reliability of these parameters as diagnostic and developmental markers during early gestation. These findings are consistent with established embryological knowledge and reinforce the use of biometric indices as essential tools in both research and clinical evaluation of pregnancy progression. Future studies could extend these findings by exploring how maternal factors, species variations, or pharmacological interventions influence the growth trajectories of these parameters.

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