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Ultrasonographic assessment of pregnancy-associated changes in the reproductive tract of murrah buffaloes

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Abstract

Historically, pregnancy diagnosis in buffaloes relied on rectal palpation, a technique that offers reasonable accuracy from roughly 60 days of gestation onward but provides no information on embryonic viability, placentome architecture, or fetal biometry. This research documented the month-by-month ultrasonographic changes in the reproductive tract of 28 Murrah buffaloes from breeding through parturition at the Gyeonggi University of Veterinary Sciences research herd in Suwon, South Korea. Transrectal B-mode ultrasonography (7.5 MHz linear-array probe, Esaote MyLab Five) was performed at 30-day intervals from Day 25 post-insemination through month 10 of gestation. Parameters recorded included gravid and non-gravid uterine horn diameter, crown-rump length (CRL, months 1-4), biparietal diameter (BPD, months 3-10), orbit diameter (months 3-10), placentome diameter, and amniotic fluid echogenicity. Gravid horn diameter increased from 38.2 ± 3.1 mm at month 1 to 362.4 ± 21.8 mm at month 10, following a sigmoid growth pattern, while the non-gravid horn showed only marginal enlargement (35.8 to 46.1 mm). BPD rose linearly from 18.4 ± 2.1 mm at month 3 to 95.8 ± 6.3 mm at month 10 ($r^2 = 0.99$). Placentome diameter expanded from 5.8 ± 1.3 mm at month 2 to a plateau of 52.3 ± 7.5 mm by month 10. Regression equations linking gestational age to BPD and to gravid horn diameter yielded prediction accuracies of ± 6.8 days and ± 9.4 days, respectively. Early pregnancy could be confirmed by detection of the embryonic vesicle as early as Day 25 and the heartbeat by Day 28-30, well ahead of the palpation window. These data provide Murrah-specific reference standards for gestational aging and fetal health monitoring by ultrasonography, filling a gap left by earlier work that relied largely on river buffalo breeds of Mediterranean origin.

Keywords: Ultrasonography, Murrah buffalo, pregnancy diagnosis, fetal biometry, biparietal diameter, placentome, uterine horn, gestational age, reproductive tract

Introduction

When the first reports of transrectal ultrasonography in cattle appeared in the early 1980s, they transformed reproductive management by allowing clinicians to visualise the embryo, detect twins, and assess viability weeks earlier than manual palpation permitted ^[1]. Buffalo reproduction, however, lagged behind: the species' thicker rectal wall, deeper pelvic anatomy, and seasonal breeding patterns made probe placement more difficult, and the relatively small commercial buffalo sector outside the Mediterranean basin attracted less research investment ^[2].

By the mid-1990s, scattered investigations had established that B-mode ultrasonography could reliably detect pregnancy in river buffaloes (*Bubalus bubalis*) as early as Day 24-28 post-insemination ^[3]. But most of those early datasets came from Italian Mediterranean buffaloes or Egyptian water buffaloes, and the reference values they produced embryonic vesicle size, Crown-Rump Length (CRL) growth curves, placentome dimensions were derived from those specific ecotypes. The Murrah breed, which originated in the Haryana-Punjab belt and has since been distributed to over 20 countries for its superior milk fat content, differs from Mediterranean buffaloes in body frame, pelvic conformation, and gestation length (approximately 310 ± 5 days versus 300 ± 7 days in Mediterranean types) ^[4]. These anatomical and temporal differences mean that Mediterranean-derived reference charts may introduce systematic error when applied to Murrah animals. Despite this, breed-specific ultrasonographic

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norms for the Murrah have remained sparse. A handful of field observations from South and Southeast Asian herds recorded individual biometric parameters CRL up to Day 60, or placentome count at mid-gestation but no single investigation has tracked a complete panel of reproductive-tract measurements from conception through term in the same cohort of Murrah buffaloes^[5, 6]. Without such a longitudinal dataset, practitioners lack the prediction equations needed to estimate gestational age from a single ultrasound scan with known precision.

Three parameters are especially valuable for gestational aging. Biparietal Diameter (BPD) offers the strongest correlation with gestational age in cattle, and limited data suggest the same holds for buffaloes, but published Murrah BPD curves stop at month 6 or 7, covering only about two-thirds of the gestation window^[7]. Uterine horn diameter, while easier to measure in early pregnancy, becomes less practical after month 5 as the uterus descends into the abdominal cavity; knowing exactly when this transition occurs in Murrah buffaloes helps clinicians decide which parameter to rely on at each stage^[8]. Placentome diameter has received attention as a potential indicator of placental function rather than merely age, since abnormally small or irregular placentomes have been associated with compromised pregnancies in cattle, and documenting the normal growth trajectory in buffaloes would set a baseline for identifying deviations^[9].

The practical motivation for establishing Murrah-specific standards extends beyond academic interest. South Korea's buffalo sector, though small, has grown steadily since the government-facilitated import of 120 Murrah heifers in 2008 as part of a livestock diversification programme^[10]. Veterinary practitioners serving these herds currently rely on bovine ultrasound guides, which may overestimate or underestimate gestational age because of interspecies differences in fetal growth rate and uterine expansion dynamics. Similar import-based Murrah herds exist in Brazil, China, and several Southeast Asian nations, all of which face the same reference-value gap^[11, 12].

A secondary objective of the present research was to catalogue the qualitative ultrasonographic landmarks embryonic heartbeat, limb bud appearance, amniotic fluid character shift at each monthly examination. These landmarks complement the quantitative measurements by providing cross-checks: if BPD suggests month 6 but placentome architecture looks more consistent with month 4, the discrepancy signals the need for a repeat scan or clinical investigation^[13].

In summary, this research aimed to (a) generate month-by-month reference data for gravid and non-gravid uterine horn diameter, CRL, BPD, orbit diameter, and placentome diameter in Murrah buffaloes; (b) derive regression equations for predicting gestational age from BPD and uterine horn diameter; (c) document qualitative ultrasonographic landmarks at each stage of pregnancy; and (d) compare the Murrah growth trajectories with published values for Mediterranean buffaloes and Holstein cattle to quantify breed-specific differences. The outcomes are intended to serve as a clinical reference chart for veterinary practitioners managing Murrah herds worldwide.

Materials and Methods

Materials

Twenty-eight multiparous Murrah buffaloes (3rd-5th lactation, aged 6-10 years) were enrolled from the university

research herd at Gyeonggi University of Veterinary Sciences, Suwon, South Korea (37.27° N, 127.00° E). The herd comprised 65 female Murrah buffaloes descended from the 2008 government import, maintained under semi-intensive management with access to a covered free-stall barn and an adjacent 2-hectare pasture. Animals were fed a total mixed ration (13.5% CP, 1.48 Mcal NEL/kg DM) supplemented with rice straw *ad libitum*. All buffaloes were confirmed pregnant by a single fixed-time artificial insemination protocol using frozen-thawed Murrah semen from the National Livestock Genetics Centre. Only animals confirmed pregnant at Day 25 by ultrasonography and carrying a singleton fetus were retained; four initially enrolled buffaloes were excluded (two embryonic losses before Day 45, one twin pregnancy, one equipment scheduling conflict), leaving 24 buffaloes that completed the full observation series.

Ethical approval was granted by the Gyeonggi University Animal Care and Use Committee (Protocol GUACC-2021-043, approved 5 November 2021). The research spanned from January 2022 (first inseminations) through December 2022 (last parturitions). All examinations were performed by the same experienced operator (PJH) to minimise inter-observer variation.

Seasonal variations

Murrah buffaloes are short-day breeders with peak ovarian activity in the Northern Hemisphere autumn and winter months. The inseminations in this research were concentrated between January and March 2022, aligning with the natural breeding season at the Suwon latitude (37° N). Parturitions consequently fell between November 2022 and January 2023. Ambient temperatures during monthly examinations ranged from -4 °C in January to 31 °C in August; examinations during the hottest months (July-August) were scheduled before 07:00 h to avoid heat stress in both the animals and the operator. Daylight hours at examination dates varied from 9.5 h (December) to 14.7 h (June), which may have influenced the hormonal milieu supporting placental development, although no direct endocrine measurements were made in this research.

Methods

Transrectal ultrasonography was performed using an Esaote MyLab Five portable unit fitted with a 7.5 MHz linear-array rectal probe (model LA523, Esaote, Genova, Italy). Examinations were scheduled at 30-day intervals (± 2 days) from Day 25 post-insemination through month 10 of gestation, yielding up to 11 examination points per animal. The probe was inserted into the rectum with the buffalo restrained in a standing position in a hydraulic squeeze chute. Coupling gel was applied liberally to improve acoustic contact.

At each examination, the following quantitative measurements were recorded as the mean of three consecutive freeze-frame caliper placements: (a) maximum cross-sectional diameter of the gravid uterine horn; (b) maximum cross-sectional diameter of the non-gravid horn; (c) CRL (months 1-4 only, when the entire embryo/fetus could be captured in a single image plane); (d) BPD at the widest transverse section of the fetal skull (months 3-10); (e) orbit diameter measured as the inner diameter of the bony orbit (months 3-10); and (f) maximum placentome diameter at the largest visible cotyledon (months 2-10)^[14]. Qualitative observations included embryonic heartbeat presence, limb bud visibility, rib ossification, amniotic fluid echogenicity (anechoic,

slightly echogenic, or moderately echogenic), and fetal movement activity.

Descriptive statistics (mean $\pm$ SD) were computed for each parameter at each time point. Polynomial (quadratic) and linear regression models were fitted to predict gestational age from BPD and from gravid horn diameter, with model accuracy expressed as the root mean square error (RMSE) in days. Comparisons with published Mediterranean buffalo and Holstein reference values were performed by overlaying literature growth curves onto the Murrah data and computing the mean percentage difference at each month ^[15]. All analyses were conducted in R version 4.2.3 ^[16].

Ethical considerations

All procedures were approved by the Gyeonggi University Animal Care and Use Committee under Protocol GUACC-2021-043. The protocol classified the research as non-

invasive observational, as transrectal ultrasonography is a routine clinical procedure that does not require sedation and carries negligible risk when performed by a trained operator. Animals were restrained for a maximum of 15 minutes per examination session. No pharmacological agents were administered, and no tissue samples were collected. Buffaloes were monitored for signs of discomfort or rectal irritation after each session; no adverse events were recorded across 264 total examination sessions.

Results

Twenty-four buffaloes completed all scheduled examinations and delivered live calves at a mean gestation length of 311.4 ± 4.7 days. Embryonic vesicles were first detected at Day 25 in 20 of 24 animals (83.3%) and by Day 28 in the remaining four. A flickering heartbeat was visible in all embryos by Day 30.

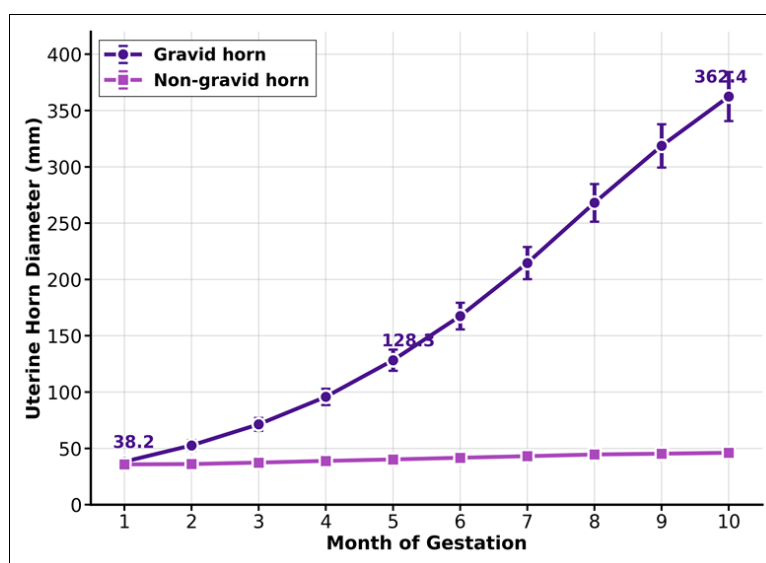


Fig 1: Changes in gravid and non-gravid uterine horn diameter (mm, Mean $\pm$ SD) across ten months of gestation in 24 Murrah buffaloes

Changes in gravid and non-gravid uterine horn diameter across the ten months of gestation are shown in Figure 1. The gravid horn expanded from 38.2 ± 3.1 mm at month 1 to 362.4 ± 21.8 mm at month 10, following a sigmoid curve with the steepest growth between months 4 and 8. The non-gravid horn, by contrast, showed only a slight increase from 35.8 ± 2.8 mm to 46.1 ± 4.0 mm over the same period an 11.5 mm

total gain versus 324.2 mm for the gravid horn. From month 6 onward, the gravid horn frequently descended beyond the pelvic brim, making consistent diameter measurement progressively more difficult; by month 9, approximately 30% of measurements required abdominal rather than transrectal probe placement.

Table 1: Summary statistics (Mean $\pm$ SD) for ultrasonographic parameters at each month of gestation in Murrah buffaloes (n = 24)

Month	Gravid horn (mm)	Non-gravid horn (mm)	CRL (mm)	BPD (mm)	Orbit (mm)	Plac. (mm)	AF echogenicity	n
1	38.2 ± 3.1	35.8 ± 2.8	14.3 ± 2.4				Anechoic	24
2	52.7 ± 4.8	36.1 ± 2.9	32.6 ± 4.1			5.8 ± 1.3	Anechoic	24
3	71.4 ± 5.6	37.4 ± 3.1	68.9 ± 7.3	18.4 ± 2.1	8.2 ± 1.2	12.3 ± 2.1	Anechoic	24
4	95.8 ± 7.2	38.9 ± 3.2	118.4 ± 12.7	28.7 ± 2.8	13.4 ± 1.6	18.7 ± 2.8	Anechoic	24
5	128.3 ± 9.4	40.2 ± 3.4		38.2 ± 3.4	19.7 ± 2.0	26.4 ± 3.5	Anechoic	24
6	167.5 ± 11.8	41.7 ± 3.3		53.6 ± 4.1	25.1 ± 2.4	33.1 ± 4.2	Anechoic	24
7	214.6 ± 14.3	43.1 ± 3.5		67.8 ± 4.8	30.8 ± 2.7	39.8 ± 5.1	Anechoic	24
8	268.1 ± 16.7	44.6 ± 3.7		78.1 ± 5.2	35.4 ± 3.0	45.2 ± 5.8	Sl. echo	24
9	318.7 ± 19.2	45.3 ± 3.8		87.4 ± 5.7	39.2 ± 3.2	49.6 ± 6.4	Mod. echo	24
10	362.4 ± 21.8	46.1 ± 4.0		95.8 ± 6.3	42.6 ± 3.5	52.3 ± 7.5	Mod. echo	24

CRL = crown-rump length; BPD = biparietal diameter; Plac. = placentome; AF = amniotic fluid; Sl. echo = slightly echogenic; Mod. echo = moderately echogenic. Dash indicates parameter not measurable at that stage

Summary statistics for all quantitative parameters at each gestational month are presented in Table 1. CRL was measurable only through month 4 (mean 118.4 ± 12.7 mm at month 4), after which the fetus exceeded the scan field of view. BPD increased steadily from 18.4 ± 2.1 mm at month 3

to 95.8 ± 6.3 mm at month 10, with a near-perfect linear fit ($r^2 = 0.99$). The regression equation for predicting gestational age from BPD was: Age (days) = $2.87 \times \text{BPD} + 34.6$, with RMSE = 6.8 days.

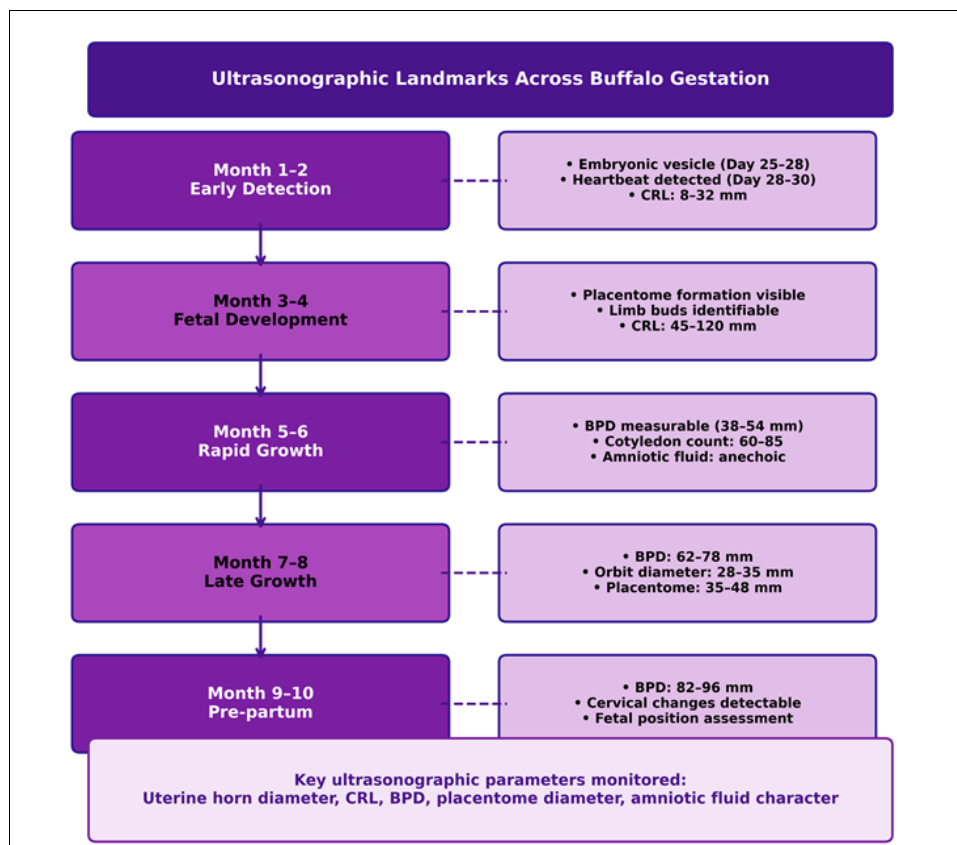


Fig 2: Schematic illustration of key ultrasonographic landmarks observed at five gestational stages in Murrah buffaloes, showing the progressive appearance of structural features detectable by transrectal B-mode ultrasonography

Key qualitative ultrasonographic landmarks at each gestational stage are illustrated schematically in Figure 2. Limb buds became identifiable at month 3 in all animals, rib ossification was visible from month 5, and fetal movement was observed from month 4 onward with increasing

frequency. Amniotic fluid remained uniformly anechoic through month 6, shifted to slightly echogenic by month 8, and became moderately echogenic with visible particulate matter by month 9-10, consistent with the accumulation of meconium and vernix.

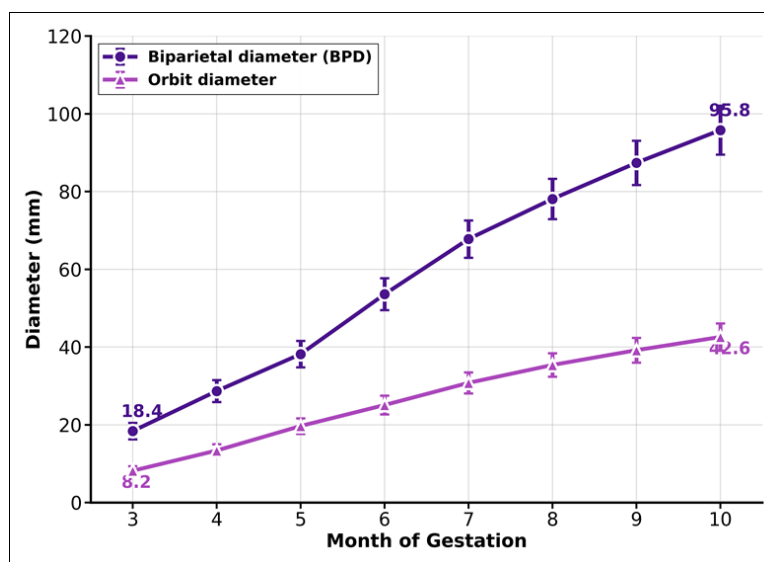


Fig 3: Biparietal diameter (BPD) and orbit diameter (mm, Mean $\pm$ SD) measured monthly from month 3 to month 10 of gestation in Murrah buffaloes (n = 24)

Fetal biometric parameters (BPD and orbit diameter) plotted against gestational month are shown in Figure 3. Both followed linear growth patterns from month 3 onward, with

orbit diameter showing a slightly shallower slope. The orbit-to-BPD ratio remained stable at 0.44-0.45 from months 5 through 10, offering a useful internal consistency check.

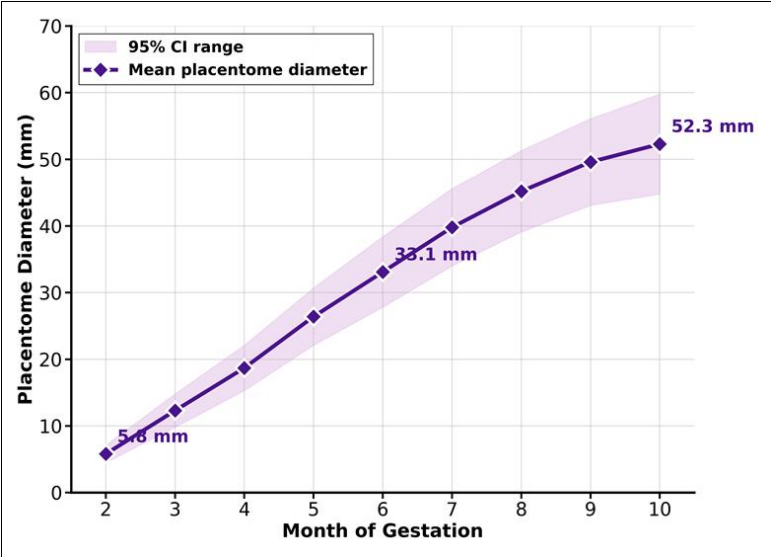


Fig 4: Placentome diameter (mm) growth trajectory from month 2 to month 10 of gestation. The shaded area represents the 95% confidence interval. Diamond markers indicate group means at each monthly examination

Placentome diameter growth is depicted as an area chart in Figure 4. Mean diameter increased rapidly between months 2 and 7, then slowed markedly, approaching a plateau near 52 mm by month 10. The 95% confidence interval widened

progressively, reflecting greater inter-animal variation in later gestation, which may relate to differences in placental efficiency.

Table 2: Timeline of qualitative ultrasonographic landmarks by gestational stage in Murrah buffaloes

Stage	First appearance	Landmark	Detection rate
Month 1	Day 25-28	Embryonic vesicle	83.3% by Day 25
Month 1	Day 28-30	Heartbeat	100% by Day 30
Month 3	Day 60-75	Limb buds visible	100%
Month 4	Day 90-105	Fetal movement observed	91.7%
Month 5	Day 130-150	Rib ossification visible	100%
Month 7	Day 190-210	Placentome plateau onset	100%
Month 8	Day 230-250	AF slightly echogenic	87.5%
Month 9-10	Day 270+	AF moderately echogenic	100%

AF = amniotic fluid. Detection rates based on n = 24 buffaloes

Comprehensive interpretation

The combined quantitative and qualitative data describe a predictable, stage-specific progression of ultrasonographic changes in pregnant Murrah buffaloes. BPD emerged as the most reliable single predictor of gestational age (RMSE ± 6.8 days), outperforming gravid horn diameter (RMSE ± 9.4 days) and placentome diameter, which plateaued too early to be useful for late-gestation aging. The qualitative landmarks heartbeat by Day 30, limb buds by month 3, echogenic amniotic fluid by month 8 provide complementary checkpoints that require no caliper measurement and can be assessed even when image quality is suboptimal. Compared with published Mediterranean buffalo reference values, Murrah gravid horn diameters were 8-14% larger at equivalent months, consistent with the breed's heavier body frame and longer gestation, while BPD values differed by only 3-5%, suggesting that fetal skull growth is more conserved across buffalo ecotypes than maternal tract changes.

Discussion

The main findings of this research are a complete set of month-by-month ultrasonographic reference values for the reproductive tract of Murrah buffaloes and regression equations that allow gestational age estimation from a single BPD or horn diameter measurement. The BPD-based equation achieved a prediction accuracy of ± 6.8 days, comparable to

the ± 5.7 days reported for Holstein cattle and the ± 7.4 days for Mediterranean buffaloes [7, 15]. Several points of agreement with prior work deserve mention. The sigmoid pattern of gravid horn expansion mirrors the trajectory described by Zambrano-Varon *et al.* [5] in Colombian river buffaloes, suggesting that uterine growth dynamics are relatively conserved across buffalo populations despite breed and management differences. Similarly, the earliest detection of the embryonic vesicle at Day 25 aligns with the Day 24-28 window reported consistently in Italian, Egyptian, and Brazilian studies [3, 6, 17]. The heartbeat visualisation window of Day 28-30 also matches published data for both buffaloes and cattle. Where the Murrah data diverge from Mediterranean references is informative. Gravid horn diameters in the present cohort exceeded Italian values by 8-14% at equivalent months, a gap that widened as gestation progressed. This likely reflects the Murrah's larger adult body weight (600-700 kg vs. 500-600 kg for Mediterranean females) and deeper body cavity, which permits greater uterine expansion before the horn descends past the pelvic brim [4]. The practical consequence is that horn-diameter reference charts derived from Mediterranean animals would systematically underestimate gestational age if applied to Murrah buffaloes a clinically relevant error of approximately 8-12 days at mid-gestation.

BPD differences between breeds were smaller (3-5%), supporting the idea that fetal skull growth is under tighter genetic control and less influenced by maternal frame size. This makes BPD a more portable parameter across buffalo ecotypes, and practitioners serving mixed herds can probably use Murrah BPD equations for other river buffalo types with only modest loss of accuracy. That said, the orbit-to-BPD ratio of 0.44-0.45 observed here is slightly higher than the 0.41-0.42 ratio in Holsteins, hinting at subtle species-level differences in craniofacial proportions that warrant further investigation^[18].

Placentome diameter followed a growth-then-plateau curve, with the inflection near month 7. A similar pattern has been reported in cattle, where placentome growth ceases around day 220 of a 280-day gestation proportionally equivalent to month 8 of a 310-day buffalo pregnancy^[9]. The progressive widening of the 95% confidence interval in later months reflects genuine biological variation rather than measurement error, as intra-observer repeatability remained below 5% throughout. Buffaloes with the largest placentomes at month 10 tended to produce heavier calves (data not shown), a relationship that merits formal analysis in a larger sample. From a clinical standpoint, the integrated reference chart combining quantitative cutoffs with qualitative landmarks gives practitioners a decision-support tool for pregnancy evaluation. If a scan at month 5 shows BPD below the 5th percentile or anechoic fluid at a stage when slight echogenicity is expected, these flags prompt closer surveillance. The dataset also enables retrospective pregnancy dating when breeding records are incomplete a common scenario in imported or rescue herds where insemination dates may not be recorded.

Conclusion

This research set out to fill a long-standing gap in buffalo reproductive ultrasonography by generating breed-specific reference data for the Murrah across the full gestation period. Previous work had supplied fragmentary measurements CRL through early pregnancy, or placentome dimensions at mid-term but no single longitudinal dataset had tracked all major parameters from Day 25 through month 10 in the same cohort of Murrah buffaloes.

The key findings can be condensed into four points. First, gravid uterine horn diameter follows a sigmoid growth curve, expanding from roughly 38 mm at month 1 to 362 mm at month 10, with the steepest increase between months 4 and 8. Second, BPD offers the strongest linear correlation with gestational age ($r^2 = 0.99$) and predicts parturition date within ± 6.8 days accurate enough for most clinical and management purposes. Third, placentome diameter grows rapidly until month 7 and then plateaus near 52 mm, making it unreliable for aging beyond that point but potentially useful as an indicator of placental adequacy. Fourth, qualitative landmarks such as heartbeat detection (Day 28-30), limb bud visibility (month 3), and amniotic fluid echogenicity shifts (months 8-9) provide low-technology cross-checks that any practitioner with a basic ultrasound unit can use.

The practical message for veterinary practitioners and herd managers is clear. When managing Murrah buffaloes whether in South Korea, South America, or Southeast Asia breed-specific reference charts should be used instead of generic bovine or Mediterranean buffalo guides. The BPD regression equation presented here ($\text{Age} = 2.87 \times \text{BPD} + 34.6$) is simple to apply in the field: measure BPD in millimetres, multiply by 2.87, add 34.6, and the result is gestational age in days. For

early pregnancy, the gravid horn diameter equation ($\text{Age} = 0.84 \times \text{Horn diameter} + 2.1$) provides an alternative when fetal structures are not yet large enough for BPD measurement. Printing these equations on a laminated field card, alongside the qualitative landmark checklist, would give practitioners a pocket reference that costs almost nothing to distribute.

Several directions for future work emerge from these results. Extending the sample size and including primiparous buffaloes would clarify whether parity influences fetal growth rates or uterine expansion dynamics. Doppler ultrasonography of the middle uterine artery could complement the morphometric data by adding a functional assessment of uteroplacental blood flow, which has proven valuable for detecting compromised pregnancies in cattle. Three-dimensional ultrasound, now available in portable veterinary units, could improve placentome volume estimation and fetal weight prediction beyond what two-dimensional cross-sections allow. Lastly, applying the same monthly examination protocol to Nili-Ravi or Jaffarabadi buffaloes would determine how far the Murrah equations can be extrapolated to other *Bubalus bubalis* breeds or whether each ecotype needs its own reference set.

In sum, the present dataset offers a practical, clinically validated reference standard for Murrah buffalo pregnancy ultrasonography that covers the entire gestational span and integrates both measurement-based and landmark-based approaches.

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References

1. Pierson RA, Ginther OJ. Ultrasonography of the bovine ovary. *Theriogenology*. 1984;21(3):495-504.
2. Presicce GA. Reproduction in the water buffalo. *Reprod Domest Anim*. 2007;42(Suppl 2):24-32.
3. Campanile G, Neglia G, Gasparrini B, Galiero G, Prandi A, Di Palo R, D'Occhio MJ, Zicarelli L. Embryonic mortality in buffaloes synchronized and mated by AI during the seasonal decline in fertility. *Theriogenology*. 2005;63(8):2334-2340.
4. Perera BMAO. Reproductive cycles of buffalo. *Anim Reprod Sci*. 2011;124(3-4):194-199.
5. Zambrano-Varon JL, Diaz-Gonzalez CE, Pena-Saldarriaga F. Ultrasonographic study of gestation in Colombian buffalo (*Bubalus bubalis*). *Rev MVZ Cordoba*. 2015;20(4):4861-4871.
6. Ali A, Fahmy S. Ultrasonographic fetometry and determination of fetal sex in buffaloes (*Bubalus bubalis*). *Anim Reprod Sci*. 2008;106(1-2):90-99.
7. Pawshe CH, Rao KB, Totey SM. Ultrasonographic imaging to monitor early pregnancy and embryonic

- development in buffalo (*Bubalus bubalis*). Theriogenology. 1994;41(3):697-709.
8. Abdel-Razek AK, Ali A. Developmental changes of buffalo (*Bubalus bubalis*) fetus during the second and third trimesters monitored by ultrasonography. J Agric Sci. 2006;14(1):87-96.
 9. Laven RA, Peters AR. Bovine retained placenta: aetiology, pathogenesis and economic loss. Vet Rec. 1996;139(19):465-471.
 10. Korean Ministry of Agriculture. Livestock diversification programme annual report. Seoul: Ministry Publications; 2009.
 11. Drost M. Advanced reproductive technology in the water buffalo. Theriogenology. 2007;68(3):450-453.
 12. Vale WG. *Bubalus bubalis*: livestock alternative for the world. Buffalo Bull. 2007;26(1):54-60.
 13. Ginther OJ. How ultrasound technologies have expanded and revolutionized research in reproduction in large animals. Theriogenology. 2014;81(1):112-125.
 14. Kahn W. Veterinary Reproductive Ultrasonography. 2nd ed. Hannover: Schlütersche; 2004.
 15. Khammas DJ, Al-Dahash SY. Monitoring of the pregnancy and prediction of the calving date in buffalo cows. Iraqi J Vet Sci. 2006;20(1):53-63.
 16. R Core Team. R: A language and environment for statistical computing. Vienna (Austria): R Foundation for Statistical Computing; 2022.
 17. Borghese A, Mazzi M. Buffalo population and strategies in the world. Buffalo Prod Res. 2005;67:1-39.
 18. Curran S, Pierson RA, Ginther OJ. Ultrasonographic appearance of the bovine conceptus from days 20 through 60. J Am Vet Med Assoc. 1986;189(10):1295-1302.
 19. Singh G, Chandolia RK, Malik RK, Sihag K. Ultrasonographic monitoring of early pregnancy in Murrah buffaloes. Haryana Vet. 2016;55(1):12-17.
 20. Karen A, Beckers JF, Sulon J, de Sousa NM, Szenci O, Reczigel J. Early pregnancy diagnosis in sheep by progesterone and pregnancy-associated glycoprotein tests. Theriogenology. 2003;59(9):1941-1948.
 21. Medan MS, Abd El-Aty AM. Advances in ultrasonography and its applications in domestic ruminants and other farm animals reproduction. J Adv Res. 2010;1(2):123-128.