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Association between Birth Weight and Testicular Development in Wagyu Calves

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Abstract

Reproductive traits in Wagyu cattle have been less extensively studied than meat quality characteristics. This study investigated the association between birth weight and testicular development in purebred Wagyu bulls. Thirty-four purebred Wagyu male calves from a single herd were monitored from birth until orchiectomy at approximately 200 days of age. At orchiectomy, the testes were weighed and measured. Histological samples were processed using hematoxylin and eosin staining to evaluate the diameter, height, and area of the germinal epithelium. Pearson's correlation and linear regression models were used to assess relationships among birth weight, age, and histomorphometric parameters. A positive, though not statistically significant, correlation was identified between birth weight and germinal epithelium área ($r = 0.312$, $P = 0.072$). A significant sire effect was also observed for the assessed testicular traits. These findings provide limited evidence of an association between birth weight and testicular development; they do not establish a direct or causal relationship. Further studies are needed to clarify these relationships and their relevance to male fertility.

Keywords: Bos taurus, histomorphometry, reproductive efficiency, spermatogenesis.

Introduction

The growing appreciation and demand for high-quality beef have driven interest in specialized cattle breeds, such as Wagyu, whose meat is widely recognized for its organoleptic characteristics, including its high degree of marbling, juiciness, unique flavor, and elevated concentration of monounsaturated fatty acids (Gotoh et al. 2018, Gallas et al. 2025). Originally from Japan, the Wagyu breed was introduced to Brazil in 1992 and is currently being incorporated into national production systems to meet demanding markets seeking premium, high-value products (Gallas et al. 2025).

Despite the breed's prominence for production traits, especially regarding meat quality, the reproductive characteristics of Wagyu males remain poorly explored. Studies indicate that these animals exhibit sexual precocity, early onset of puberty, higher libido, elevated hormone levels, and satisfactory sperm production (Sosa et al. 2002, Moura et al. 2023). However, the Wagyu bulls are known to have smaller scrotal circumference (SC) and lower body weight (BWH) compared to other taurine breeds of the same age (Sosa et al. 2002), raising concerns about their reproductive efficiency and possible physiological and genetic implications of their lineage (Marchi et al. 2023, Moura et al. 2023, Camargo et al. 2024, Silva et al. 2015).

Reproductive and productive efficiency in livestock are key components of zootechnical performance within animal production systems (Barcelos et al. 2025, Gallas et al. 2025). Consequently, selecting sires with superior sperm production capacity may be an efficient strategy for enhancing herd productivity (Kastelic 2013, Chechin et al. 2025). In swine, Flowers (2022) observed that birth weight (BW) was positively correlated to the sperm concentration per ejaculate in males, as well as with lifetime productivity in females. Thus, BW may serve as an early indicator of reproductive performance in cattle, as it is directly associated with reproductive traits (Mendonça et al. 2003).

Previous studies have identified substantial reproductive and hormonal variability in both males and females of the Wagyu breed (Facioli et al. 2020, Marchi et al. 2023). Marchi and colleagues (2023) reported a wide variation in Anti-Müllerian Hormone (AMH) levels in males. AMH is a hormone directly involved in reproductive development in males (Xu et al. 2018) and serves as a fertility marker in females (Marchi et al. 2023). Considering the growing use of the Wagyu breed, especially in

crossbreeding systems, identifying males with greater sperm production potential becomes essential. Therefore, our study aimed to evaluate the parameters associated with testicular development in a population of Wagyu bulls.

Materials and Methods

The experiment was approved by the Animal Ethics Committee of the University of Passo Fundo (CEUA-002/2024). The study was conducted in a traditional Wagyu herd located in Paim Filho, Rio Grande do Sul, Brazil (27°41'11.0"S, 51°43'21.2"W). The farm maintains approximately 150 animals, including bulls, cows, heifers, and calves, managed under an extensive grass-fed system with mineral supplementation as described by Gallas et al. (2025).

A total of 34 intact, purebred Wagyu male calves were included in this study. The animals were monitored from birth until surgical castration, which was performed at approximately 200 days of age. Calves originated from either artificial insemination (AI) or natural mating (NM), using semen from bulls of four different Wagyu lineages. At birth, their umbilical cords were disinfected with iodine, and the animals were individually identified using numbered ear tags. Further, each calf also received 10 mL of Toltrazuril orally and was weighed using a digital scale. Tail hair samples were collected for DNA-based parentage verification using microsatellite markers at a commercial laboratory to ensure their genealogy. Animals' body weight and health status were assessed monthly.

Prior to castration, scrotal circumferencewas measured. For the orchiectomy procedure, chemical restraint was induced using an intramuscular injection of 0.05 mg/kg Xylazine Hydrochloride (2%). Following sedation, the animals were physically restrained using ropes. The scrotal sac was cleaned with 2% iodine solution, and the open technique was used for orchiectomy, as described by Barros (2024). After testicle removal, each testis was individually weighed, and its dimensions (length and diameter) were measured using a caliper. At the end of the orchiectomy, each animal received 1 mL/10 kg of an antibiotic and anti-inflammatory combination (Penicillin, Streptomycin, and Piroxicam) and a topical application of a healing and repellent spray (Fipronil Silver Sulfadiazine) at the surgical site.

Histological samples were obtained from the right testis of each animal by making a longitudinal incision through the visceral vaginal tunic, tunica albuginea, and testicular parenchyma. Two or three testicular fragments, approximately 2 x 2 x 2 mm in size, were collected, avoiding the mediastinum region. The fragments were immediately placed in histological cassettes containing Bouin's fixative solution. After 24 hours, all samples were transferred to 70% ethanol and stored at 4ºC until further processing. Histological analysis was restricted to the right testis for standardization. This approach was supported by a previous study in bull calves aged 5-8 months, which reported no significant differences in histological parameters both between the right and left testes and among different sampling locations within the testis (Rohländer et al., 2020).

Histological processing of the testicular tissue included fixation, sectioning, dehydration, clearing, paraffin embedding, microtomy, and Hematoxylin & Eosin (H&E) staining, as described by Slaoui & Fiette (2011). Slides were evaluated under a light microscope equipped with an image analysis system for morphometric evaluation. Measurements of the diameter, height, and area of the germinal epithelium of the seminiferous tubules were obtained according to Silvia et al. (2015). Histomorphometric measurements of seminiferous tubuleswere performed using a light microscope (Zeiss Axio Observer Z1), and images were captured and analyzed using ZEN Blue® software (version 3.8). Tubule diameter, germinal epithelium height, and germinal epithelium area were measured as illustrated in representative H&E-stained photomicrographs (Figure 1). The boundaries used for these measurements are indicated in Figure 1, and scale bars are provided to indicate the magnification-independent dimensions of the structures. Germinal epithelium area was calculated using the circular crown area formula: Area = $\pi \times (R^2 - r^2)$, where R is the tubule radius and r is the luminal radius (R − epithelial height).

Descriptive statistics (mean ± standard deviation) were calculated for all variables. Pearson's correlation coefficients (r) were used to assess relationships among continuous variables, including BW, age, SC, testicular weight, and histomorphometric parameters of the seminiferous tubules. To evaluate the effects of BW and age on the area of the germinal epithelium, a dependent variable and functional marker of testicular activity, simple and multiple linear regression models were fitted. The effect of paternal genetics on morphometric traits and BW was assessed using analysis of variance

(ANOVA). The goodness-of-fit of the regression models was evaluated using the coefficient of determination (r^2), which represents the proportion of variance explained by the independent variables. All statistical analyses were performed using the R software, with significance set at $p< 0.05$.

Results

The mean gestation length in the evaluated animals was 286.62 ± 5.63 days, while the mean birth weight (BW) was 23.17 ± 5.88 kg. No significant effect of the reproductive technique (AI or natural mating) on birth weight was observed ($p = 0.09$). Similarly, there was no significant correlation between BW and gestation length ($p = 0.1061$).

Castration was performed at a mean age of 200.79 ± 52.43 days. At this time, the calves exhibited a mean scrotal circumference of 41.84 ± 27.09 mm and a mean body weight of 154.70 ± 43.39 kg.

The mean total testicular weight was 119.02 ± 81.96 g, with the right and left testes weighing 60.67 ± 40.79 g and 58.35 ± 41.34 g, respectively, with no significant difference between them ($p = 0.81$).

Following the correction of histomorphometric measurements and the exclusion of obliquely sectioned tubules (where epithelial height exceeded tubule radius), 30 out of 34 animals (88.2% of the sample) were considered valid for analysis. Of the total 170 fields analyzed (5 fields per animal), 66.5% were considered valid, with a mean of 3.3 valid fields per animal.

Histological analysis was performed on the right testis, with five fields evaluated per animal to determine epithelial height, diameter, and area of the germinal epithelium. After data reassessment, the seminiferous tubules exhibited a mean diameter of 102.38 ± 33.47 μm, a mean epithelial height of 43.85 ± 20.18 μm, and a mean germinal epithelium area of 8,482 ± 4,132 μm^2.

A strong positive correlation was observed between epithelial height and tubule diameter ($r = 0.88$; $p = 3.483 \times 10^{-12}$). However, no significant correlations were found between BW and either epithelial height ($p = 0.53$) or tubule diameter ($p = 0.09$).

Conversely, the germinal epithelium area showed a strong positive correlation with both epithelial height and tubule diameter ($r = 0.7316$; $p < 8.864 \times 10^{-7}$).

A positive, though not statistically significant, correlation was identified between BW and germinal epithelium area ($r = 0.312$; $p = 0.072$) (Figure 2B). Linear regression analysis indicated a positive trend in which, for each 1 kg increase in BW, the germinal epithelium area increased by an estimated **226.4 µm²**. The model explained 9.7% of the variation in the dependent variable ($R^2 = 0.097$).

Birth weight showed a strong correlation with scrotal circumference (SC) at castration ($r = 0.6729$; $p < 0.0001$), indicating that heavier calves at birth exhibited greater scrotal development at castration, regardless of age (Figure 2A). Furthermore, BW was significantly correlated with left testis weight ($r = 0.5939$; $p < 0.0002$) and total testicular weight ($r = 0.4764$; $p = 0.0044$).

Body weight at castration was strongly correlated with right testis weight ($r = 0.8269$) and left testis weight ($r = 0.4647$; $p < 0.0001$), as well as with SC ($r = 0.5132$; $p < 0.0019$). Scrotal circumference was strongly correlated with total testicular weight ($r = 0.7688$; $p < 0.0001$) and with germinal epithelium area ($r = 0.6505$; $p < 0.0001$) (Figure 2C), supporting its use as a non-invasive marker of testicular function.

Animal age was positively correlated with total testicular weight ($r = 0.8845$) and scrotal circumference ($r = 0.8365$), consistent with testicular maturation. A moderate correlation was observed between age and germinal epithelium area ($r = 0.389$; $p = 0.022$), with an estimated mean increase of **12.4 µm²** per additional day of age. Age alone explained 15.1% of the variation in germinal epithelium area ($R^2 = 0.151$).

A significant sire effect was detected for germinal epithelium area ($p < 0.001$), tubule height ($p < 0.03$), tubule diameter ($p < 0.01$), and birth weight ($p < 0.004$). Analysis of variance (ANOVA) revealed that sire explained 59% of the variation in BW ($R^2 = 0.59$; $p = 0.004$), 95% of the variation in SC ($R^2 = 0.95$; $p < 0.001$), and 61% of the variation in germinal epithelium area ($R^2 = 0.61$; $p = 0.002$) (Figure 3).

Simple linear regression for predicting SC indicated that BW was a significant predictor (coefficient = 3.11; $p < 0.001$), with an adjusted R^2 of 0.47. When age was

added to the model, both predictors remained significant (BW: coefficient = 1.68; p = 0.001; age: coefficient = 0.34; p < 0.001), with an adjusted R^2 of 0.76.

For germinal epithelium area, the model including BW and age (Table 1) yielded an adjusted R^2 of 0.085, with a coefficient for BW of 304.19 (p = 0.278) and for age of 34.54 (p = 0.310), indicating that the full model was not statistically significant (p = 0.114).

When SC was included in the predictive model for germinal epithelium area (SC + BW + age), SC became the main predictor (coefficient = 342.3; p = 0.001), while BW lost significance (coefficient = -270.0; p = 0.335) and age showed a trend (coefficient = -82.0; p = 0.061). The adjusted R^2 of this model was 0.376.

To test the central hypothesis that the effect of BW on germinal epithelium area is mediated by SC, a mediation analysis was performed. The indirect pathway (BW → SC → Area) was significant (indirect effect = 639.0; p = 0.007; 95% bootstrap CI = 119.3 to 1,162.4), while the direct effect of BW on area, controlling for SC, was not significant (c' = -189.4; p = 0.513). These results suggest that the effect of birth weight on functional testicular development occurs indirectly, with scrotal development (SC) acting as a statistical mediator of this association.

Discussion

To meet the growing demand for animal protein and reduce reliance on meat imports, strategies aimed at improving beef production and quality are essential. One such strategy is the use of artificial insemination, which facilitates genetic improvement and herd expansion. As interest in the Wagyu breed continues to grow, driven by its exceptional carcass quality, an increasing number of breeders have adopted the use of Wagyu bulls. However, these animals exhibit distinctive characteristics compared to other beef breeds, particularly regarding reproductive parameters and fertility, which remain insufficiently characterized (Gotoh et al. 2018). A notable concern is the frequent misinterpretation by veterinarians during breeding soundness evaluations, who may fail to account for the inherent differences in semen quality and scrotal circumference between Wagyu and more traditional British or Continental cattle breeds (Sosa et al. 2002, Moura et al. 2023). Therefore, this study evaluated the effect of BW,

age, body and testicular traits, and sire lineage on the development of the testicular germinal epithelium in Wagyu cattle.

The mean birth weight of the animals was 23.17 ± 5.88 kg, slightly lower than the 27.2 kg reported by Camargo et al. (2024) and the 30.5 kg reported by Hearnshaw et al. (2001) in crossbred Wagyu calves. This discrepancy may be attributed to the extensive management system employed on the farm in the present study, which contrasts with the intensive systems used in the aforementioned studies. The mean gestation length was 286.62 ± 5.63 days, slightly longer than the 283.8 ± 5.91 days reported by Camargo et al. (2024). This variation may reflect genetic differences among sires, as well as the nutritional status of dams during gestation (Chechin et al. 2025).

Pietruszka et al. (2017) identified an association between BW and sperm production in swine. In the present study, BW showed a positive correlation with scrotal circumferenceat castration ($r = 0.6729$; $p < 0.0001$), left testicular weight ($r = 0.5939$; $p < 0.0002$), and total testicular weight ($r = 0.4764$; $p < 0.004$). Similarly, Bautista et al. (2023) reported that BW could predict scrotal circumference in growing lambs raised under tropical conditions. These findings support the hypothesis that intrauterine growth influences testicular development by promoting early formation of spermatogenic function.

A positive trend was observed between BW and germinal epithelium area ($r = 0.312$; $p = 0.072$), suggesting that calves with higher birth weight tend to exhibit more advanced functional testicular development, although this association did not reach statistical significance in our sample. The limited explanatory power of BW alone ($R^2 = 0.097$) reinforces the notion that birth weight should be regarded as a supplementary indicator rather than a standalone definitive predictor for sire selection.

Age also showed a significant correlation with germinal epithelium area ($r = 0.389$; $p = 0.022$), as expected for testicular maturation (Miranda et al. 2018, Aissanou et al. 2022). Collinearity analysis revealed a moderate correlation between BW and age ($r = 0.55$; $p < 0.0009$), which may have influenced the estimates of independent effects. The multiple regression model with BW and age (Table 1) yielded an adjusted R^2 of 0.085, confirming the limited direct predictive capacity of these variables on germinal epithelium area.

Another important finding was the correlation between SC and testicular development (Pérez et al. 2014). Scrotal circumference showed a strong positive correlation with total testicular weight ($r = 0.7688$; $p < 0.0001$) and with germinal epithelium area ($r = 0.6505$; $p < 0.0001$). These results suggest that, in addition to being a practical marker of pubertal development, SC is functionally associated with spermatogenic capacity, as previously identified by Menegassi et al. (2019). This supports its utility as an early selection marker in breeding programs. Scrotal circumference is a useful tool for predicting the onset of puberty and improving reproductive performance in beef cattle (Menegassi et al. 2019). Furthermore, it is well established that SC correlates with sperm quality and production (Corbet et al. 2013).

The significant sire effect identified for germinal epithelium area ($p < 0.001$) indicates a substantial genetic influence on the functional maturation of testicular tissue. These results reinforce previous studies reporting moderate to high heritability for reproductive traits in cattle (Olasege et al. 2021). Identifying sires whose offspring exhibit superior testicular epithelial development may contribute to genetic improvement programs aimed at enhancing fertility (Marchi et al. 2023, Carvalho et al. 2025).

The mediation analysis revealed that the effect of BW on germinal epithelium area is indirect, mediated by SC (indirect effect = 639.0; $p = 0.007$; 95% bootstrap CI = 119.3 to 1,162.4). The direct effect of BW on area, controlling for SC, was not significant ($c' = -189.4$; $p = 0.513$), indicating that BW influences testicular histology through scrotal development rather than through a direct mechanism. This finding is central to understanding the reproductive physiology of young Wagyu bulls: higher birth weight promotes greater testicular development (SC), which, in turn, is associated with greater germinal epithelium area.

The multiple regression model including SC, BW, and age explained 37.6% of the variation in germinal epithelium area (adjusted $R^2 = 0.376$), with SC as the only significant predictor ($p = 0.001$). This result strengthens the conclusion that SC is the most important proximal predictor of functional spermatogenic capacity, while BW acts as a distal predictor, mediated by SC.

A limitation of this study is the histological analysis restricted to the right testis, under the assumption of bilateral symmetry. This approach was supported by a previous

study in bull calves aged 5–8 months, in which no significant differences were detected between the right and left testes for the histological parameters evaluated (Rohländer et al., 2020). In the present study, right and left testicular weights also did not differ significantly (60.67 ± 40.79 g and 58.35 ± 41.34 g, respectively; $p = 0.81$). However, because histological parameters were not assessed in the left testis, bilateral histological equivalence could not be verified in the Wagyu calves included in this study. Future studies should confirm this assumption for Wagyu cattle at this developmental stage and also evaluate the correlation between the histomorphometric measures obtained here and semen quality parameters, to validate the use of germinal epithelium area as a predictor of reproductive capacity.

In summary, the data presented here suggest that birth weight is a promising supplementary marker for future testicular development, including the functional activity reflected by germinal epithelium growth. However, its effect is indirect, mediated by scrotal development. These findings have practical implications for early selection strategies in genetic and reproductive improvement programs, especially for breeds with longer production cycles, such as Wagyu. The effects of age, BW, and SC should be considered in the overall assessment of pubertal development and the reproductive potential of young males.

The findings of this study indicate that birth weight was positively associated with scrotal circumference and testicular weight in Wagyu calves. However, the association between birth weight and germinal epithelium area did not reach statistical significance, and the model including birth weight and age did not significantly explain variation in this histomorphometric parameter. The mediation analysis identified a statistically significant indirect association through the proposed mediator, which should be interpreted cautiously. Further studies incorporating bilateral histological assessment and semen quality measurements are needed to determine whether birth weight can serve as a useful supplementary indicator of reproductive potential in Wagyu bulls. Although birth weight showed a positive trend, its limited predictive power suggests it should be considered only as a supplementary indicator, rather than a standalone definitive predictor, for selecting males with superior reproductive performance in Wagyu breeding systems.

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Declaration of Competing Interests

The authors declare that Eraldo Zanella and Ricardo Zanella are owners of Agropecuária Zanella. This relationship is disclosed for transparency and did not influence the study design, data collection, analysis, interpretation of results, or preparation of the manuscript. The remaining authors declare no competing financial or non-financial interests.

Data Availability Statement

The datasets generated and/or analyzed during the current study are available from the corresponding author upon reasonable request.

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Figure 1 Representative photomicrograph of seminiferous tubules from a Wagyu calf (Hematoxylin and Eosin staining, 400× magnification). The image demonstrates scale calibration and morphometric delineation of tubule diameter (A: 243.790µm) (yellow dashed line), germinal epithelium height (B: 289.266µm) (red double-headed arrow), and epithelial area (C: 76.322µm) (green dotted contour). Scale bar = 50 µm.

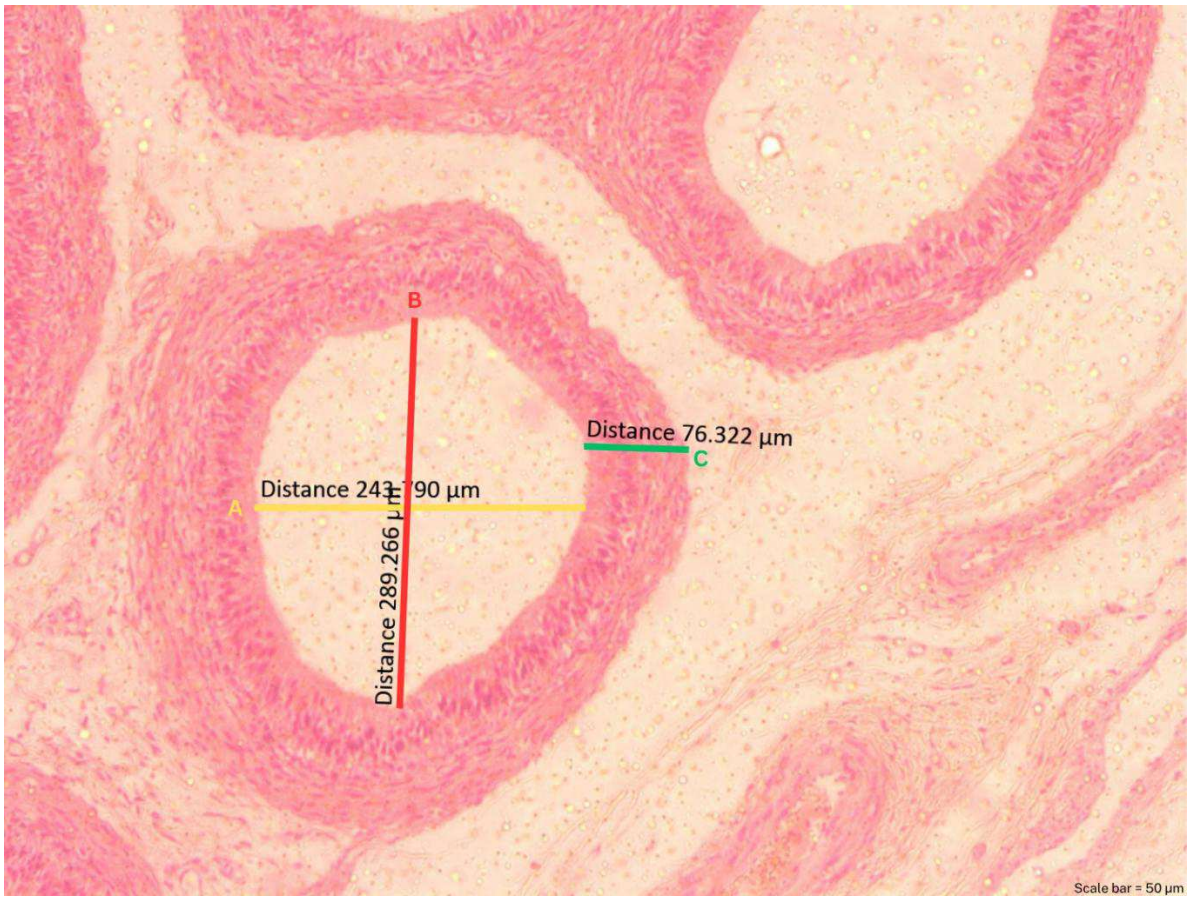
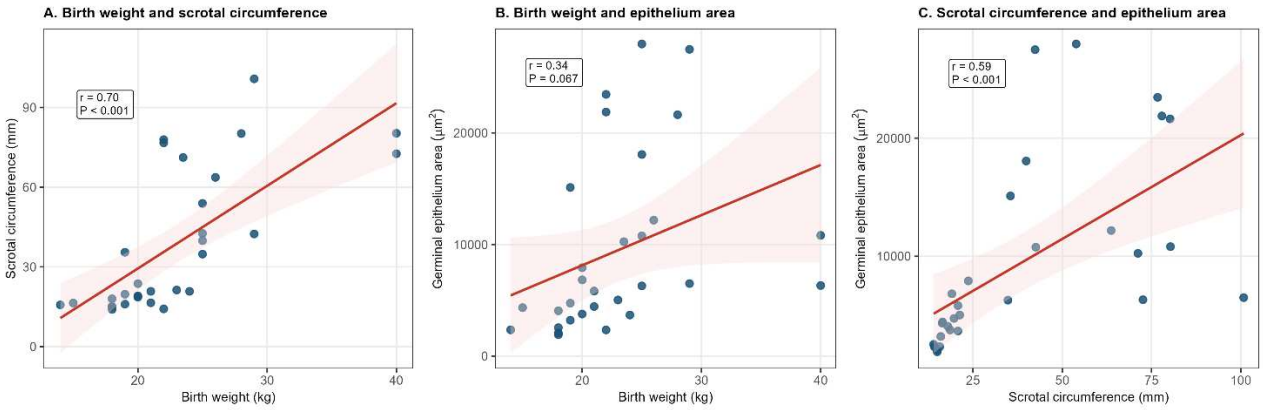


Table 1: Multiple linear regression model predicting germinal epithelium area (μm²) as a function of birth weight (kg) and age (days) in Wagyu calves. Overall model p = 0.114; adjusted R^2 = 0.085. VIF = Variance Inflation Factor, indicating no significant collinearity between predictors.

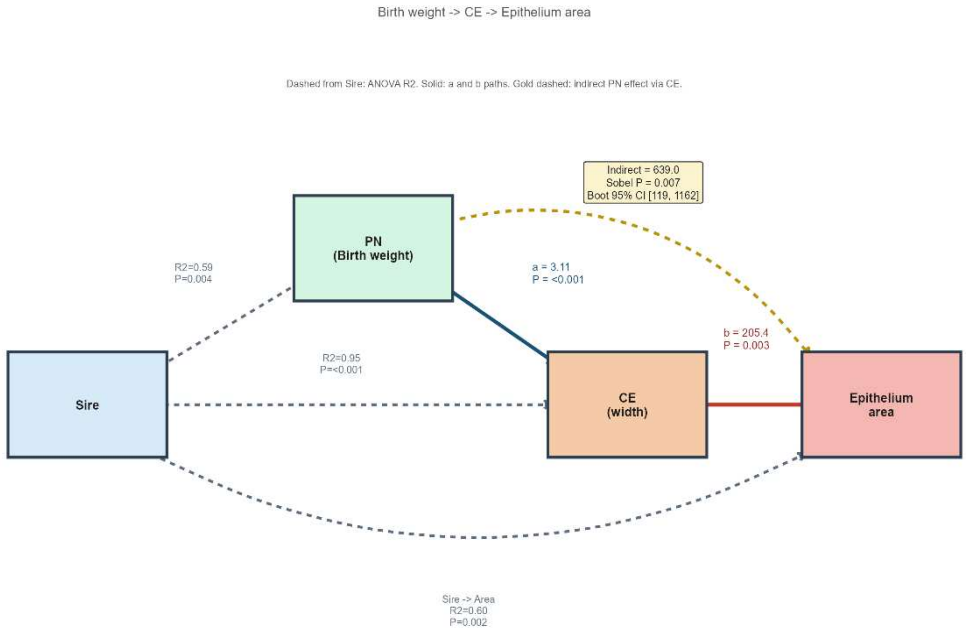
PREDICTOR	B	SE	t	p	Adjusted_R2	VIF
Intercept	-4253,99	6477,94	-0,657	0,517	0,085	—
Birth weight (kg)	304,19	274,55	1,108	0,278		1.35
Age (days)	34,54	33,36	1,035	0,31		1.35

Figure 2: Associations among birth weight, scrotal circumference, and germinal epithelium area in Wagyu calves. (A) Relationship between birth weight and scrotal circumference at castration in Wagyu calves. Data were adjusted for age. Each point represents an individual animal. The solid line represents the linear regression fit, and the shaded area represents the 95% confidence interval ($r = 0.70$; $p < 0.001$). (B) Relationship between birth weight and germinal epithelium area of the seminiferous tubules in Wagyu calves. Data were adjusted for age. Each point represents an individual animal. The solid line represents the linear regression fit, and the shaded area represents the 95% confidence interval ($r = 0.34$; $p = 0.067$, non-significant). (C) Relationship between scrotal circumference at castration and germinal epithelium area of the seminiferous tubules in Wagyu calves. Data were adjusted for age. Each point represents an individual animal. The solid line represents the linear regression fit, and the shaded area represents the 95% confidence interval ($r = 0.59$; $p < 0.001$).



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458 **Figure 3:** Path analysis and mediation model showing sire effect and indirect pathways
459 to germinal epithelium area in Wagyu calves. Dashed grey lines represent the effect size
460 (R^2) and significance (p-value) of the sire ANOVA on birth weight (PN), scrotal
461 circumference/width (CE), and germinal epithelium area. Solid lines indicate the direct
462 regression paths (a and b) between variables. The gold dashed curved line shows the
463 indirect effect of birth weight (PN) on germinal epithelium area mediated by scrotal
464 circumference (CE). Indirect effect statistics are provided with Sobel test p-value and
465 95% bootstrap confidence intervals.



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