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The heritability of pampiniform plexus vessel size and varicocele in boars

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Contents

Ultrasonography was used to capture a coronal-sagittal image of the veins of the pampiniform plexus (PP) and the testicular artery of 327 maternal-line boars at approximately 6 months of age at the University of Nebraska-Lincoln. Varicocele was diagnosed by two methods. Method 1 diagnosed varicocele when the average vessel area on one side of the scrotum was 1.5 times larger than the average vessel area on the other side of the scrotum. Method 2 diagnosed varicocele when the average vessel area on one side of the scrotum of a boar was 1.5 times larger than the average vessel on the same side of the scrotum of the boar's cohorts (same population and year). Varicocele was diagnosed in 23.17% and 15.1% of boars measured using method 1 and method 2, respectively. Ultrasonography showed to be an effective means to measure PP vessel size in boars and may even allow for earlier detection of varicocele than by using palpation. Animal models were employed to estimate the heritability for: average area of right PP vessels (0.52), average area of the left PP vessels (0.46), varicocele presence using method 1 (0.26) and varicocele presence using method 2 (0.25). These heritability estimates suggest that vessel size and varicocele could be selected against in breeding programmes to potentially improve boar semen quality.

KEYWORDS

heritability, pampiniform plexus, swine, varicocele

1 | INTRODUCTION

Varicocele is an abnormality of the pampiniform plexus (PP), where vascular lesions and/or tortuous dilated veins occur. The presence of varicocele has known associations with male infertility (Jarow, 2001). Countercurrent heat exchange of the PP is hindered when varicocele is present due to improper cooling of abdominal blood entering the testicle with varicocele. The abnormally warm blood causes the testicle with varicocele to have a higher than normal

temperature, which can lead to impaired testicular function (Hsiung, Nieva, & Clavert, 1991).

Varicoceles are the most common physical abnormality reported in human men with reduced semen quality parameters and fertility issues (Sigman & Howards, 1998; Sigman & Jarow, 1997). Reproductive tracts of boars that were reported to have poor semen quality for at least 5 weeks prior to culling were examined in a study conducted by Úbeda et al. (2014). Twenty-two per cent of these boars were reported to have varicoceles (Úbeda et al., 2014). In a group of boars examined for testicle abnormalities, 23.4% of the adult boars were diagnosed by palpation with varicocele (Kleefeld, Riesenbeck, Beyersbach, & Waberski, 2015). A significant effect

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on sperm membrane integrity, as well as a significant increase in sperm morphology abnormalities, was reported in the boars affected by varicocele (Kleve-Feld et al., 2015).

To the authors' knowledge, the heritability of PP vessel size, as well as the presence of varicocele, has not previously been estimated in any species. Incidence of varicocele in men that have first-degree relatives with varicocele has been examined to evaluate the familial risk. When compared to the control population, a significant threefold to eightfold increase in varicocele was reported in the group of men with first-degree relatives affected by varicocele (Gökçe et al., 2010; Raman, Walmsley, & Goldstein, 2005). This previous research indicates a genetic component in the control of varicocele in men. The degree to which genetics control varicocele in swine is unknown. Therefore, the objective of this study was to estimate preliminary heritability estimates for PP vessel size and varicocele presence in boars.

2 | MATERIALS AND METHODS

Research protocols and methods were approved by the University of Nebraska Institutional Animal Care and Use Committee.

2.1 | Boars utilized

A total of 327 boars of approximately 6 months of age (= 180 days of age 15 days) were measured via ultrasonography for average PP vessel size at the University of Nebraska—Lincoln research farm and

used in the analysis. Group 1, measured in 2016, consisted of 127 boars from 16 sires, with an average of 7.9 boars per sire. Group 2, measured in 2017, consisted of 112 sons from 11 of the previously measured boars (group 1) for an average of 10.2 boars per sire. Group 1 and group 2 were from the Nebraska Index Line, which is a composite of the breeds Landrace and Large White (Petry & Johnson, 2004). The third group was measured in 2018 and contained 88 boars. Group 3 consisted of sons of full-sibling females to the boars measured in 2017, sired by 14 industry Yorkshire sires, for an average of 6.2 boars per sire. Boars in groups 1 and 2 had a known pedigree of four generations through both the dam and the sire. Boars in group 3 had a known four-generation pedigree through their dam, but only boar's sire was known on the sire side of the pedigree.

2.2 | Pampiniform plexus vessel measurement and varicocele diagnosis

Ultrasonography was performed for each side of the scrotum by orientating the transducer towards the lower half of the testicle at approximately a 45° angle. This technique captured a coronal-sagittal image of the testicular artery and the veins of the PP. An Ibex EVO ultrasound (E.I. Medical Imaging, Loveland, CO) with a 6.5 MHz linear endorectal probe (L6E) was utilized. Bioquant image system (Bioquant Image Analysis Corp., Nashville, TN) was used to compile all images into a collage. Using the Bioquant image system, the area (mm²) of 3 to 10 vessels of the PP was measured and the average area of right PP vessels (AAR) and of the left PP vessels (AAL), as well as the standard deviation for each PP, was derived.

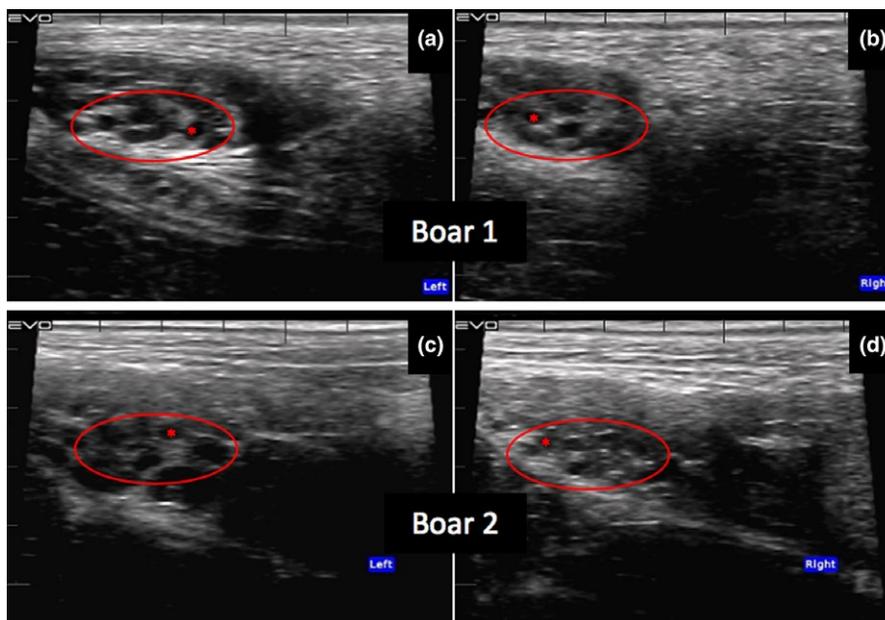


FIGURE 1 Ultrasound images of a boar without varicocele (Boar 1) and a boar with varicocele in the left pampiniform plexus (Boar 2). Red ovals on each ultrasound image indicate the cross section of the pampiniform plexus (PP) vessels. One representative vessel per image is identified with an asterisk (*). Using the Bioquant image system, the area (mm²) of 3 to 10 vessels of the PP was measured and the average area of right PP vessels (AAR) and of the left PP vessels (AAL) was derived. Panels a and b represent the left and right PP vessel images for a boar, respectively. The left and right vessel ratios (AAL/AAR or AAR/AAL, respectively) of Boar 1 were <1.5; therefore, the boar was identified as non-varicocele. Panels c and d are representative images from a boar (Boar 2) with varicocele in the left plexus (AAL/AAR >1.5)

To assess the presence or absence of varicocele, ratios of vessel size were utilized. A within-boar vessel ratio was estimated for each PP. The within-boar ratios were as follows: (AAL of boar X/AAR of boar X) and (AAR of boar X/AAL of boar X), and if either ratio exceeded 1.5, the boar was considered suspect varicocele via method 1. Method 1 allowed for the identification of boars with unilateral varicocele. Individual boars were also compared against their contemporaries and considered varicocele if a ratio exceeded 1.5 via method 2. The following ratios were utilized for method 2: (AAL of boar X/average AAL of the population) and (AAR of boar X/average AAR of the population). Method 2 was performed in order to identify: (a) boars in which both PP vessel areas were enlarged and a difference within boar would not have been observed, suggesting bilateral varicocele, and (b) boars that had a marginal difference (less than 1.5) between their own PP vessel areas, and however, when compared to their contemporaries, a difference (>1.5) was identified. The presence or absence of varicocele was treated as a binary trait. For each method, a boar suspected of varicocele on either the left or right side was assigned a 1, otherwise they were assigned a 0. Example ultrasound images of a boar with varicocele and a boar without varicocele, diagnosed via method 1, are provided in Figure 1.

2.3 | Statistical analysis

A paired *t* test was used to test for a significant difference between AAL and AAR vessel size for all boars sampled. These analyses were conducted in R (R Core & Team, 2018).

A bivariate animal model was fitted using ASReml 3 (Gilmour et al., 2008) to estimate (co)variance components for AAL and AAR, as well as the genetic correlation, phenotypic correlation and narrow-sense heritabilities. Fixed effects included weight at time of measurement, year of measurement and line nested within year. Random effects included the additive genetic component and residual. The genetic correlation was estimated to be 1.0. As this results in a singular covariance matrix, the genetic correlation was fixed to 0.99.

Univariate threshold animal models were utilized to estimate variance components for the presence of varicocele for methods 1 and 2. The threshold model was fitted using a logit cumulative probability model, and contained the fixed effects of weight at time of measurement, year of measurement and line nested within year, with the random effect of animal. Heritabilities are reported on the underlying scale, utilizing the logistic distribution.

3 | RESULTS

3.1 | Relationship between AAL and AAR

The AAL and AAR were significantly different ($p < 0.001$), with AAL being an average of 1.18 times larger than AAR (Table 1). The estimate of phenotypic correlation between AAL and AAR was 0.55 ± 0.22 , and the estimate of genetic correlation was 1.0.

TABLE 1 Average area of right pampiniform plexus vessels (AAR) and of the left pampiniform plexus vessels (AAL) with their respective standard deviations (SD)

	Mean area (mm ²)	SD
AAR	6.28 [*]	2.90
AAL	7.39 [*]	3.47

^{*}Statistically different as determined from the paired *t* test ($p < 0.001$)

TABLE 2 Percentage of boars determined to be suspect varicocele using two methods of varicocele diagnosis

	Percentage
L/R ^a	18.89
R/L ^b	4.28
L/Lpop ^c	9.66
R/Rpop ^d	9.92
Method 1 ^e	23.17
Method 2 ^f	15.1
Method 2: bilateral ^g	4.4
Method 1 & 2 overlap ^h	4.7

^aL/R is the percentage of boars exceeding a 1.5 ratio of the average left pampiniform plexus vessel size (AAL) of boar X/average right pampiniform plexus vessel size (AAR) of boar X. ^bR/L is the percentage of boars exceeding a 1.5 ratio of (AAR of boar X/AAL of boar X). ^cL/Lpop is the percentage of boars exceeding a 1.5 ratio of (AAL of boar X/average AAL of the population given year of measurement). ^dR/Rpop is the percentage of boars exceeding a 1.5 ratio of (AAR of boar X/average AAR of the population given year of measurement). ^eSummation of L/R & R/L (within-boar method) ^fSummation of L/Lpop and R/Rpop. Boars with bilateral varicocele are only included once. ^gPer cent of boars with bilateral varicocele determined from L/Lpop and R/Rpop. ^hBoars classified as suspect varicocele under both methods of diagnosis.

3.2 | Varicocele presence

Results for the presence of varicocele are presented in Table 2. Varicocele occurred more often in the left PP than the right PP utilizing method 1. When the presence of both right side and left side varicoceles was summed, 23.17% of the boar population showed varicocele with method 1. Method 2 permits varicocele diagnosis on the left side, right side and/or bilaterally. Under method 2, left or right side varicocele was diagnosed for approximately 10% of the population each, and approximately 4% of boars were determined to have bilateral varicocele. When varicocele was considered a binary trait regardless of diagnosis side, 15.1% of the population was considered suspect varicocele under method 2, as bilateral boars are only counted once. Boars that were classified as suspect varicocele using both methods consisted of 4.7% of the population.

3.3 | Heritability estimates

Heritability estimates for AAL and AAR are presented in Table 3. Both AAL and AAR were found to be highly heritable, 0.52 ± 0.13

and 0.46 ± 0.13 , respectively. Heritability estimates for the presence of varicocele are also presented in Table 3, and were moderately heritable, method 1 (0.26 ± 0.03) and method 2 (0.25 ± 0.03).

4 | DISCUSSION

The genetic correlation of 1.0 between AAL and AAR implies that these are the same trait genetically. By selecting against large vessel size in one PP, the opposing PP would also be influenced. If selected against, special care should be taken that PP vessel size, especially on the right where varicocele occurs less often, is not reduced to a point that could potentially hinder the countercurrent heat exchange of the PP.

The larger AAL compared to AAR was not unexpected and explained the higher incidence of varicocele on the left side when utilizing the within-boar ratio. This scenario has previously been described in men with a higher reported predominance of varicocele on the left side of the scrotum. This is thought to be caused by the long course of entry of the left testicular vein into the left renal vein (Sabanegh & Agarwal, 2012). When men are diagnosed with varicocele, it is either done with a subjective diagnosis via a physician through palpation or ultrasonographically (Jarow, 2001; Pryor, 2003). When palpation is used, diagnosis is based on how visible the vascular lesions are on a scale of highly visible and easy to palpate, to difficult to palpate and not visible, and vessel size has not been compared to any other person/group (Jarow, 2001). With ultrasound, varicocele is diagnosed when the vein/testicular vein is greater than a threshold value (Pryor, 2003). The palpation method has previously been used to diagnose varicocele in live boars by Kleve-Feld et al., (2015), where 23.4% of the boar population were considered suspect varicocele. This estimate matches closely to the method 1 estimate in the current study of 23.17% of boars being considered suspect varicocele. However, the 23.4% estimate was for adult boars in the Kleve-Feld et al. (2015) study, whereas in the

current study young boars were measured (approximately 6 months of age). Kleve-Feld et al., (2015) also reported prevalence of varicocele in young boars (7–12 months of age) at 7.2%, which is lower than what the current study estimated. This could indicate that the use of ultrasound to diagnosis varicocele allowed for earlier diagnosis than palpation would allow. These results also suggest that regardless of breed, the prevalence of varicocele in the boar population remains approximately the same. In the study conducted by Kleve-Feld et al., (2015), the terminal Pietrain breed was used to estimate varicocele occurrence compared to the current study, which utilized only maternal-based lines. The incidence of varicocele in boar populations is slightly higher than what has been reported in human populations at approximately 15% (Gökçe et al., 2010; Gorelick & Goldstein, 1993; Raman et al., 2005).

When using method 2, approximately 15% of boars were found to be suspect varicocele, which is lower than what was previously reported in boars but similar to what has been found in humans (Gökçe et al., 2010; Gorelick & Goldstein, 1993; Kleve-Feld et al., 2015; Raman et al., 2005). Method 2 allows boars with bilateral varicocele to be captured, unlike method 1 that can only diagnosis varicocele unilaterally. The diagnosis of varicocele in boars is still a novel practice, which is why two methods were utilized in this study. More research needs to be conducted analysing semen quality of boars diagnosed with varicocele under the different methods to determine which method does a more precise job of identifying boars with varicocele that impacts semen quality.

Pampiniform plexus vessel size was found to be highly heritable. To the authors' knowledge, no other studies have reported heritability estimates for PP vessel size in any species. However, a study conducted in humans which assessed the heritability of the central retinal artery and the central retinal vein reported estimates of 0.70 and 0.83, respectively (Taarnhøj et al., 2006). Though the vessel size heritability estimates reported by Taarnhøj et al. (2006) are higher than what is reported in the current study, this supports the finding of vessel size being a highly heritable trait.

The current study found the presence of varicocele to be moderately heritable. Previous research in relation to varicocele heritability in all species is very limited. In human studies, a significant increase in the presence of varicocele occurred in first-degree relatives in men with a known varicocele compared to a control population. Among the first-degree relatives of men with known varicocele, 36.2% (Gökçe et al., 2010), 45.5% (Mokhtari, Pourreza, Falahatkar, Kamran, & Jamali, 2008) and 56.6% (Raman et al., 2005) have been reported with palpable varicocele. This indicates a genetic component to the presence of varicocele, which is in agreement with the current study.

The presence of varicocele in boars as well as humans has been shown to negatively influence semen quality (Kamal, Jarvi, & Zini, 2001; Kleve-Feld et al., 2015; Pasqualotto et al., 2005). As the results of the current study indicate that varicocele presence is a heritable trait, this suggests an opportunity to select against varicocele in boars to potentially improve semen quality. When considering that varicocele effects roughly 20% of boars across breeds, as

TABLE 3 Heritability and standard error (SE) estimates for the average area of the right pampiniform plexus vessels (AAR), the average area of the left pampiniform plexus vessels (AAL) and the presence of varicocele

	Heritability (SE)
AAR	0.52 (0.13)
AAL	0.46 (0.13)
Within: Presence ^a	0.26 (0.03)
Population: Presence ^b	0.25 (0.03)

^aPresence of varicocele determined from the following ratios: (AAL of boar X/AAR of boar X) or (AAR of boar X/AAL of boar). When the ratio exceeded a 1.5 threshold, varicocele was considered suspect. ^bPresence of varicocele determined from the following ratios: (AAR of boar X/average AAR of the population given year of measurement) and (AAL of boar X/average AAL of the population given year of measurement). When the ratio exceeded a 1.5 threshold, varicocele was considered suspect. This includes boars with unilateral varicocele and bilateral varicocele.

determined by the current study and Kleve-Feld et al., (2015), the presence of varicocele could be unknowingly having substantial negative effects on semen quality industrywide.

5 | CONCLUSION

The use of ultrasound to diagnosis varicocele in boars proved an effective method and potentially allows for diagnosis of varicocele prior to what could be diagnosed via palpation. Pampiniform plexus vessel size and the presence of varicocele were found to be moderately to highly heritable traits indicating that varicocele could be selected against in the boar population to reduce its incidence and potentially improve semen quality. More research is needed to determine how semen quality is influenced by varicocele under the two methods of diagnosis described in this study. Future studies should also investigate whether a difference exists in arterial/venous blood flow in boars with and without varicocele and the testicular temperature relationship.

CONFLICT OF INTEREST

None of the authors have any conflict of interest to declare.

AUTHOR CONTRIBUTIONS

Tasha Gruhot assisted in ultra-sounding the boars, conducted the statistical analysis and drafted the manuscript. Benny Mote assisted in ultra-sounding the boars, designing study and revising the manuscript. Lea Rempel assisted in ultra-sounding the boars, designing study, statistical analysis and revising the manuscript. Matthew Spangler and Stephen Kachman assisted in the statistical analysis and revising the manuscript.

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