

Anogenital distance, male factor infertility and time to pregnancy

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Abstract

Background: Anogenital distance (AGD), the distance between the anus and genitals, is in rodents a well-established marker of early androgen action and has been suggested to be so in humans as well. Thus, a link between human AGD and semen quality and potentially fecundity may exist.

Objective: The aim of this study was to assess the association between AGD and male factor infertility and among proven fertile men also time to pregnancy (TTP).

Material and methods: All included men were recruited from and examined at Copenhagen University Hospital - Rigshospitalet, Denmark ($N = 388$). Men with impaired semen quality were included from infertile couples ($N = 128$), and men with naturally conceived pregnant partners were invited to participate when their partners had their routine second trimester examination ($N = 260$). All men underwent a physical examination, completed a questionnaire (including TTP for the fertile men), delivered a semen sample and had a blood sample drawn. The primary exposure was AGD_{AS} measured from the centre of the anus to the posterior base of the scrotum. Associations between AGD and fertility status as well as between AGD and TTP among the fertile men were calculated using multiple logistic regression adjusted for covariates.

Results: AGD did not show a statistically significant association with fertility status. In adjusted logistic regression models, the odds of infertility per 1 cm increase in AGD_{AS} were 1.02 (95% confidence interval [CI]: 0.88; 1.19). Among fertile men, a 1-cm increase in AGD_{AS} was associated with an 8% non-statistically significantly reduced odds of having a longer (>3months) TTP (adjusted odds ratio (OR) = 0.92, 95% CI: 0.76–1.11).

Conclusion: Our study showed that the clinical application of AGD as a predictor of fertility and fecundity seems to be limited as no associations were observed between AGD and fertility status, nor was the decreased risk of experiencing a longer TTP with longer AGD_{AS} statistically significant.

KEYWORDS

anogenital distance, fecundity, fertility, male factor infertility, time to pregnancy

1 | INTRODUCTION

Reduced androgen action during foetal life affecting the development of the male reproductive system is a suspected cause of some cases of male factor infertility, which may be a result of early exposure to chemical environmental factors or other foetal exposures such as maternal exposure to stressful life events.^{1,2} Anogenital distance (AGD), the distance between the anus and genitals, is a well-established marker of early androgen action in rodents and has been suggested to be so in humans as well. Thus, a link between human semen quality and AGD has been suggested,³ but this is not well-established in human studies.

In rodents, various studies have demonstrated that androgen-driven masculinization is determined in the early period of pregnancy, called the masculinization programming window.⁴ One marker of rodent masculinization is AGD, which becomes longer with higher prenatal androgen exposure. Like in rodents, it is hypothesized that AGD can be a non-invasive, lifelong marker of the androgenic action in the masculinization programming window in humans and may predict reproductive disorders such as infertility.⁵ Hence, AGD measurements may provide functional insights into the hidden process of foetal androgen exposure in adulthood, where fertility issues are detected. Once a better understanding is in place, AGD may be proven to be a useful tool in clinical situations.⁶

Studies of men who were not selected on the basis of their semen quality or fertility status are conflicting; three studies report a negative association between AGD and semen parameters,⁷⁻⁹ while two others report no association between AGD and either semen parameters or reproductive hormone levels,^{10,11} which might be explained by the varied environmental background exposures of the studied population (Italy, USA, Denmark, and Spain and China, respectively). In men selected according to their fertility status, three studies report associations between longer AGD and better semen parameters, higher testosterone level and larger testis size.^{3,6,12} Only one of the three studies examined whether AGD was associated with fertility status, defined by proven fatherhood.³ The study showed that the infertile men had significantly shorter mean AGD compared to the proven fertile controls.³ Thus, there is a lack of studies examining this association, and the few studies conducted show ambiguous results, hindering the clinical application of AGD as a marker of male factor infertility.

Time to pregnancy (TTP) has been proposed as a sensitive method to study delays in pregnancy¹³ as very fecund couples have a higher probability of conception within a few months of beginning unprotected intercourse,¹³ but no studies have investigated the association between AGD and TTP.

Based on the assumption that AGD is determined at foetal stage and is a lifelong marker of prenatal endocrine disruption and subsequent reduced reproductive function, a difference in AGD length is expected between fertile and infertile men and between men with short and long TTP.

Therefore, the aims of this study were first to investigate the association between AGD and fertility status and second to investigate the association between AGD and TTP among proven fertile men.

2 | MATERIALS AND METHODS

2.1 | Study population

The study population consisted of a total of 393 men recruited to an either fertile or infertile study group as described elsewhere.^{14,15} In brief, all men were recruited at Rigshospitalet (University Hospital in Copenhagen, Denmark), and inclusion criteria in both groups were age between 20 and 45 years, and that the man and his mother were born and raised in Denmark, as testicular function is hypothesized to be partly determined prenatally and depending on the environment in which the mother lived.^{16,17} Thus, the included men were mainly Caucasian.

The fertile men were recruited from late 2012 through 2014, from couples who had naturally achieved pregnancy, without any kind of medically assisted reproduction. These men were invited to participate when their pregnant partners had their routine second trimester examination. The participation rate was 38%.¹⁸

The group of infertile men was recruited from the out-patient clinic at Department of Growth and Reproduction, Rigshospitalet between 2013 through 2016, where the men were referred to andrological examination because of male factor infertility. Inclusion criteria for participation in the infertile study group were planned intracytoplasmic sperm injection (ICSI) as well as a semen sample with sperm concentration <20 mill/ml, progressive motility <50% or less than 12% morphologically normal spermatozoa during routine clinical workup and <2 mill progressively motile spermatozoa in a following purified semen sample. Exclusion criteria in the infertile study group were azoospermia, genetic disorders (Klinefelter syndrome, microdeletions of the Y chromosome), history of orchitis, epididymitis, testicular torsion, varicocele, vasectomy, orchiectomy, chemotherapy or radiation therapy and the presence of chronic diseases requiring treatment. The number of invited infertile men was not known; thus the participation rate cannot be calculated.

Initially, 417 men were included and examined but 29 (fertile/infertile: 11/18) were excluded: 21 due to missing AGD measurements and eight due to lack of information on covariates. In total, 128 infertile and 260 fertile men were included in the analysis (Figure 1).

2.2 | Ethical approval

The study was approved by the local ethical committee (protocol number for fertile men: H-2-2012-090 and for infertile men: H-2-2012-091). All participants gave written informed consent at enrolment.

2.3 | Physical examination, including AGD measurement

Each man underwent a physical examination at Department of Growth and Reproduction at Rigshospitalet. AGD was measured in two ways

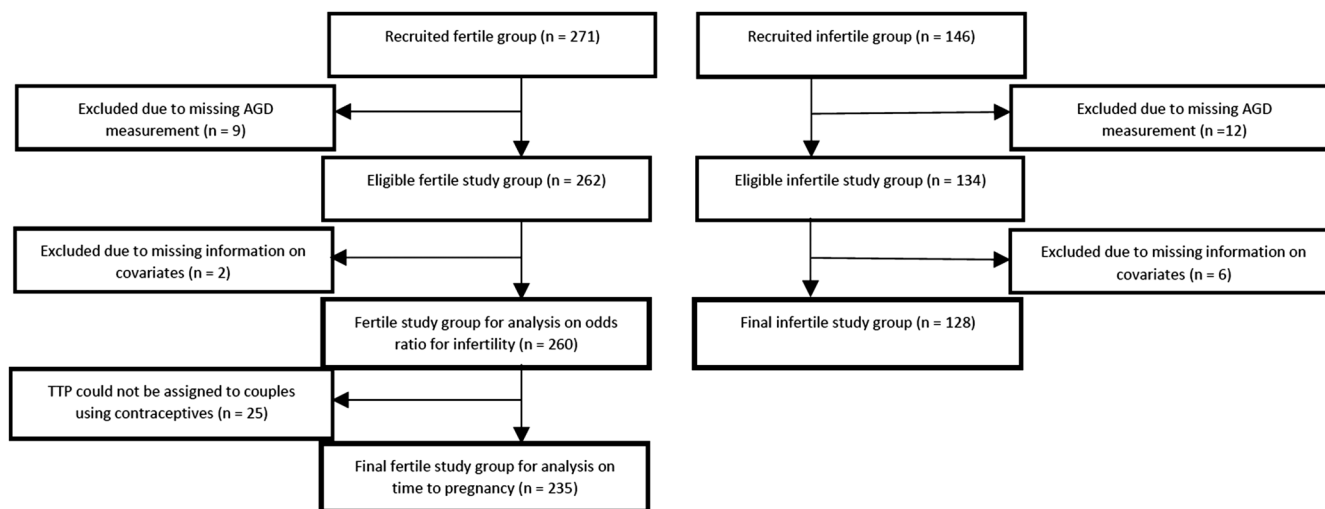


FIGURE 1 Flowchart of men in each group after exclusion of missing variables and no possible assignment of time to pregnancy (TTP)

by a physician trained in andrology. AGD_{AS} (anoscrotal distance) was measured from the centre of the anus to the posterior base of the scrotum and AGD_{AP} (anopenile distance) measured from the centre of anus to the cephalad insertion of the penis. Both measurements were done while the man was in the lithotomy position with his thighs in a 45° degree angle to the examination table.⁸ AGD was measured in millimetres, using a stainless-steel digital calliper with the numbers facing away from the examiner. To improve precision, the physician made each of these measurements three times, and the mean of the three measurements was used as the estimates of AGD in the statistical analysis. Four physicians performed 90.5% of the AGD measurements with a total of nine physicians across the study years.

During the physical examination, the physician also assessed testis size by ultrasound. Body weight and height were measured, and body mass index (BMI) was calculated. Finally, the men delivered a semen sample and had a venous blood sample taken.

2.4 | Questionnaire data

Both the fertile and infertile men completed a questionnaire that included information on lifestyle factors (smoking, alcohol, physical fitness and self-rated health). All men also completed a questionnaire pertaining to reproductive health and were asked to consult their mothers regarding information from the index pregnancy, including smoking, preterm delivery and birth weight.

The fertile men additionally provided information regarding TTP. They were asked (in Danish): 'Were you or your partner doing anything to avoid pregnancy at the time your partner became pregnant?'. If the answer was 'No', the men were asked: 'How many months did it take before your partner became pregnant (thus, having intercourse without doing anything to avoid pregnancy)?'. Consequently, TTP in this study is defined as the number of months of having unprotected intercourse before conceiving. Since TTP cannot be assigned to couples using contraceptives when pregnancy occurred, these men were excluded in the TTP

analyses.¹⁹ A total of 235 of the 260 fertile men had information about TTP (Figure 1). The men's reported TTP was furthermore validated by comparing with the pregnant partner's reported TTP.

2.5 | Statistical analysis

First, descriptive statistics on anthropometric measures, lifestyle and variables related to the man's mother's pregnancy with him were calculated, stratified by fertile and infertile men. Differences between the fertile and infertile men were tested using the Kruskal–Wallis test for continuous variables and chi-square for categorical variables. Similar descriptive statistics related to semen parameters and reproductive hormones are included in the [Supplementary Material](#).

The association between AGD and infertility as well as AGD and TTP was estimated using logistic regression analysis. TTP was dichotomized as more or less than 3 months, as a pregnancy leading to a live birth has been calculated statistically to happen after 3 months of regular unprotected intercourse.²⁰ The exposure, AGD, was included in the analyses both as a continuous, linear variable and divided into quartiles. In the logistic regressions where AGD was modelled as a continuous, linear term, the OR and 95% confidence interval (CI) estimate the odds of infertility or longer TTP, respectively, per 1 cm increase in AGD. Both AGD_{AS} and AGD_{AP} was analyzed in different models, with AGD_{AS} as the main exposure of interest as this is the variant previously associated with semen quality in adult men.^{7–9,12} AGD_{AP} was included as per tradition in human studies of AGD as a secondary exposure. All results are presented as crude and adjusted OR (aOR) with corresponding CIs.

The covariates were selected a priori based on previous studies^{9,21} and included height (linear, cm), BMI (kg/m², linear), age (years, linear) and physician (categorical (5 levels): four individual physicians and a separate single category (other) pooling the remaining five physicians). For the analyses of TTP, models were further adjusted for female partner's age (years, linear) at the time of conception due to the decline in female fecundity with increased age.²²

Additionally, five sensitivity analyses were performed to ascertain whether variation in physicians performing AGD, extremes in fertile or infertile men, or outliers were driving any of the associations, thus ascertaining the robustness of the estimates, using the following approaches: (a) analyses of the association between AGD and infertility stratified on the four primary physicians performing the physical examination (excluding the “Other”-group); (b) the association between AGD and infertility excluding infertile men with sperm concentration >15 mill/ml; (c) the association between AGD and infertility after exclusion of infertile men with sperm concentration >15 mill/ml and fertile men with sperm concentration <15 mill/ml; (d) the association between AGD and TTP applying a cut-off of 2 months and 6 months for TTP; (e) removing 12 outliers in the analysis of AGD_{AS} (cm) and infertility and TTP as well as removing nine outliers in analyses of AGD_{AP} (cm) and infertility and TTP, respectively.

All statistical analyses were performed in IBM SPSS 25.0 (IBM Corporation, Armonk, NY, USA). *p*-Values <0.05 were considered as statistically significant.

3 | RESULTS

The basic characteristics of the 388 included men according to fertility status are presented in Table 1. Median AGD_{AS} among the infertile (*n* = 128) and fertile men (*n* = 260) was 6.1/5.9 cm (*p* = 0.3), and AGD_{AP} was 13.5/13.3 cm (*p* = 0.1). Among men with proven fertility (*N* = 235), the median TTP was 2.0 months.

Infertile men were significantly older (median age, 33.6 vs. 32.4 years), heavier (median weight, 82.8 vs. 80.5 kg), had higher BMI (median BMI, 24.3 vs. 23.7 kg/m²), smaller testis size (median size, 12.9 vs. 14.6 ml), were less likely to smoke (6.3 vs. 13.8%), drank less alcohol per week (8.0 vs. 9.0 units/week), but were more likely to be born <37 weeks of gestation and more likely to be exposed to maternal smoking in utero (32.7 vs. 27.1%) compared to the group of fertile men.

Infertile men had lower semen quality (volume, concentration, total sperm count, progressive motility and morphology) than fertile men. Testosterone levels were similar in both groups, but infertile men had higher levels of luteinizing hormone (LH) and follicle-stimulating hormone (FSH), lower inhibin B, as well as lower inhibin B/FSH and testosterone/LH ratios (markers of Sertoli cell and Leydig cell function, respectively) (Table SA).

The results of the logistic regression analyses (crude and adjusted) are presented in Tables 2 and 3. In general, the 95% CIs were wide as well as overlapping unity, and the ORs were subject to large uncertainties.

The aORs and 95% CI for the risk of infertility per 1 cm increase in AGD_{AS} and AGD_{AP} were 1.02 (95% CI: 0.88; 1.19) and 0.93 (95% CI: 0.75; 1.17), respectively. In the analyses including AGD_{AS} in categories, a higher odds of infertility in quartiles 2–4 compared to the first quartile were observed; the highest odds were observed for the second AGD_{AS} quartile with an aOR of 1.62 (95% CI: 0.78; 3.35). In contrast, we observed a U-shaped relationship between the odds of infertility and

AGD_{AP} quartiles, with the lowest odds for men with an AGD in the second quartile (aOR = 0.70, 95% CI: 0.34; 1.47) (Table 2). Overall, results based on crude and adjusted models were similar in direction and magnitude for AGD_{AS} while the direction of the association reversed for AGD_{AP} after adjustment. The estimates did not change notably in the sensitivity analyses stratified by the four main physicians, excluding infertile men with sperm concentration >15 mill/ml nor excluding infertile men with sperm concentration >15 mill/ml along with fertile men with sperm concentration <15 mill/ml or after removal of AGD outliers (data not shown).

The validation of TTP reported by men and their pregnant partner indicated that there was excellent comparability and no statistically significant differences in the median (*p* = 0.6), and showed a statistically significant correlation coefficient (*r*_s = 0.88, *p* ≤ 0.001) and a very high degree of correspondence in the outcome category based on male and female data. Longer AGD was associated with a lower odds for experiencing a longer TTP (>3 months), though not statistically significant. Per 1-cm increase in AGD_{AS}, aOR for TTP >3 months was 0.92 (95% CI: 0.76; 1.11) and for AGD_{AP} 0.85 (95% CI: 0.64; 1.12). In the categorical analyses, lower OR for longer TTP was observed in AGD_{AS} quartiles 2, 3 and 4 compared with quartile 1. For AGD_{AP}, no specific pattern was seen in the aOR between the quartiles (Table 3). Estimates were similar in unadjusted analyses. Likewise, the sensitivity analysis using a cut-off of 2 months and 6 months for TTP did not change the results notably (data not shown).

4 | DISCUSSION

In this cross-sectional study, we examined the association between AGD and male factor infertility as well as TTP among the proven fertile men. Although subject to significant uncertainty, our results indicated that neither AGD_{AS} nor AGD_{AP} are markers of infertility. We detected a non-statistically significant association between longer AGD and reduced odds of long TTP among fertile men.

Only one other study has examined whether AGD is associated with fertility status, measured by fatherhood. Eisenberg et al. showed that infertile men had a significantly shorter mean AGD_{AS} compared to the fertile controls (31.8 vs. 44.6 mm).³ Eisenberg et al. included 117 infertile and 56 fertile men, while our study included 128 infertile and 260 fertile men. Despite including around four times as many fertile and around a dozen more infertile men, our study could not confirm those previous results. AGD measurements in the two studies cannot be directly compared since the men were placed in frog-legged position in the Eisenberg study, previously reported to result in shorter measurements compared to the lithotomy position, as applied in our study.²³ None the less, it is possible to compare the measurements within each study, and there are—based on the study design applied in the two studies—no obvious explanations for the conflicting results. The fertile men in the American study were older (mean: 43.6 years) than the infertile men (mean: 34.3 years),³ while in our study, the infertile men (mean: 34.1 years) were slightly older than the fertile men (mean: 32.7 years), but this is unlikely to have influenced the results

TABLE 1 Characteristics of the participants ($n = 388$) according to fertility status

	Fertile ($n = 260$)			Infertile ($n = 128$)			<i>p</i> -Value*
	<i>n</i>	Mean (standard deviation (SD))	Median (5-95 percentile (pctl))	<i>n</i>	Mean (SD)	Median (5-95 pctl)	
Anthropometric measures							
AGD _{AS} (cm)	260	6.0 (1.7)	5.9 (3.5–8.8)	128	6.2 (1.7)	6.1 (3.3–8.8)	0.3
AGD _{AP} (cm)	260	13.4 (1.5)	13.3 (11.2; 15.6)	128	13.6 (1.4)	13.5 (11.2–15.8)	0.1
Age (years)	260	32.7 (4.1)	32.4 (26.5–39.8)	128	34.1 (4.5)	33.6 (27.1–41.6)	0.006
Height (cm)	260	183 (7)	183 (174–195)	128	184 (7)	184 (174–196)	0.2
Weight (kg)	260	81.2 (11.2)	80.5 (65.6–102.3)	128	84.9 (13.2)	82.8 (68.2–112.3)	0.01
BMI (kg/m ²)	260	24.2 (2.9)	23.7 (20.1–29.7)	128	25.0 (3.5)	24.3 (20.6–31.9)	0.04
Testis size (both testes) (ml)	251	15.0 (3.8)	14.6 (9.2–22.0)	127	13.3 (3.9)	12.9 (7.4–21.3)	<0.001
TTP (months)	235	3.3 (3.8)	2.0 (0.0–12.0)	–	–	–	–
TTP >3 months, <i>n</i> (%)	235	164 (69.8)	–	–	–	–	–
Lifestyle measures							
Physical activity (hours/week)	260	6.7 (7.6)	4.5 (0.0–22.9)	128	7.0 (12.4)	3.0 (0.0–27.2)	0.001
Alcohol (units/week)	260	10.3 (9.7)	9.0 (0.0–29.0)	128	8.9 (8.1)	8.0 (0.0–25.0)	0.2
Daily smokers, <i>n</i> (%)	260	36 (13.8)		128	8 (6.3)		0.03
Self-rated health, <i>n</i> (%)	260			128			
Good		232 (89.2)			117 (91.4)		0.8
Average		26 (10.0)			10		
Bad		2 (0.8)			10 (0.8)		
Self-rated physical fitness, <i>n</i> (%)	259			128			0.9
Good		149 (57.5)			78 (60.9)		
Average		89 (34.4)			40 (31.3)		
Bad		21 (8.1)			10 (7.8)		
Pregnancy and birth characteristics							
Gestational age, <i>n</i> (%), <37 weeks	235	13 (5.5)		89	8 (9.0)		0.3
Maternal smoking, <i>n</i> (%)	240	65 (27.1)		113	37 (32.7)		0.3
Size at birth, <i>n</i> (%), (<2.500 g)	239	6 (2.5)		110	4 (3.6)		0.8

Abbreviations: AGD, anogenital distance; BMI, body mass index; TTP, time to pregnancy.

*Kruskal–Wallis was used for continuous and χ^2 for categorical variables.

since AGD has been reported not to differ with age in adulthood,²⁴ and adjustment for age in our analyses did not change the estimates. Average sperm concentration in our Danish population of infertile men was, however, higher than in the American infertile population, indicating that the latter constitutes a group of more severely infertile men, which could potentially explain that a difference in AGD was detected in that study while not in ours. However, the sensitivity analyses with exclusion of infertile men with sperm concentration >15 mill/ml and fertile men with sperm concentration <15 mill/ml, potentially increasing the contrast in fertility potential between the groups, did not change the estimates appreciably. Since AGD is only hypothesized to be shorter in infertile men with prenatal origin of their disease, the lack of differ-

ences in AGD could indicate that more men in our study were infertile due to other causes playing a role later in life. For example, for men with prior testicular cancer, which is known to be of prenatal origin and to be related to infertility, AGD is significantly shorter compared to controls.^{25,26}

We detected a reduced risk of longer TTP associated with longer AGD, which was in line with our hypothesis, although the associations were not statistically significant. For the 2nd, 3rd and 4th ADG quartiles, the odds for a TTP above 3 months were around 30% less than for the first AGD quartile, which could indicate that men with shortest AGD have prolonged TTP, while there are no further differences with higher AGD. This is in line with our previous study on AGD and

TABLE 2 Odds ratio (OR) for infertility according to anogenital distance (AGD) and AGD quartiles (N = 393)

			Crude	Adjusted ^a
		Infertile/fertile (n)	OR (95% CI)	OR (95% CI)
AGD _{AS}	Continuous (cm)	128/260	1.05 (0.93; 1.19)	1.02 (0.88; 1.19)
	Q1: 4.4 (2.3–4.90) ^b	24/71	1 (ref.)	1 (ref.)
	Q2: 5.4 (5.0–5.9) ^b	39/60	1.82 (0.99; 3.44)	1.62 (0.78; 3.35)
	Q3: 6.6 (6.0–7.1) ^b	31/65	1.48 (0.80; 2.75)	1.56 (0.75; 3.23)
	Q4: 7.9 (7.1–12.1) ^b	34/64	1.50 (0.81; 2.80)	1.38 (0.66; 2.90)
AGD _{AP}	Continuous (cm)	128/260	1.09 (0.94; 1.25)	0.93 (0.75; 1.17)
	Q1: 11.9 (10.61–12.47) ^b	28/69	1 (ref.)	1 (ref.)
	Q2: 13.0 (12.6–13.4) ^b	30/60	1.23 (0.67; 2.26)	0.70 (0.34; 1.47)
	Q3: 13.8 (13.45–14.34) ^b	33/65	1.16 (0.63; 2.13)	0.81 (0.38; 1.67)
	Q4: 15.2 (14.5–17.0) ^b	37/64	1.60 (0.87; 2.91)	1.19 (0.46; 3.07)

Abbreviation: CI, confidence interval.

^aAdjusted for BMI (kg/m², linear), height (cm, linear), age (years, linear) and physician performing AGD assessments.

^bAGD quartile median (5–95 pctl), cm.

TABLE 3 Odds ratio (OR) for time to pregnancy >3 months by AGD among fertile men (N = 235)

			Crude	Adjusted ^a
		≤3/> 3 months, n	OR (95% CI)	OR (95% CI)
AGD _{AS}	Continuous (cm)	164/71	0.93 (0.79; 1.10)	0.92 (0.76; 1.11)
	Q1: 4.4 (2.435–4.85) ^b	38/21	1 (ref.)	1 (ref.)
	Q2: 5.4 (4.9–5.9) ^b	43/16	0.67 (0.31; 1.47)	0.62 (0.27; 1.41)
	Q3: 6.5 (5.9–7.1) ^b	41/18	0.79 (0.37; 1.71)	0.73 (0.32; 1.66)
	Q4: 7.9 (7.1–11.7) ^b	42/16	0.69 (0.32; 1.51)	0.58 (0.24; 1.42)
AGD _{AP}	Continuous (cm)	164/71	0.90 (0.74; 1.10)	0.85 (0.64; 1.12)
	Q1: 12.6 (11.0–15.3) ^b	38/21	1 (ref.)	1 (ref.)
	Q2: 13.2 (11.2–15) ^b	43/16	1.17 (0.54; 2.53)	1.25 (0.54; 2.94)
	Q3: 13.4 (11.7–15.3) ^b	41/18	0.88 (0.40; 1.94)	0.85 (0.32; 2.22)
	Q4: 14.3 (12.0–15.6) ^b	42/16	0.91 (0.41; 2.03)	1.01 (0.34; 3.54)

Abbreviations: AGD, anogenital distance; CI, confidence interval.

^aAdjusted for BMI (kg/m², linear), height (cm, linear), age (years, linear), female partners age (years, linear) and physician performing AGD assessments (categorical).

^bAGD quartile median (5–95 pctl) for fertile men only with known time to pregnancy, cm.

semen quality in which only the 30% men with the shortest AGD_{AS} had increased risk of impaired semen quality.⁹ In parallel to the data collection in the present study, the previous study on young men from the general population, unselected regarding semen quality, was conducted using the same study setup, including measurement of AGD by the same physicians.⁹ The study reported a median AGD_{AS} of 6.0 cm among 1106 young men from the general population, which was largely comparable to the 6.1 and 5.9 cm among infertile and fertile men, respectively, observed in the present study. Based on our hypothesis, we would have expected to find a difference in AGD_{AS} between the two study populations such that lowest AGDs were detected in infertile men followed by AGD in the general population, while highest AGD lengths would hypothetically be expected in fertile men. However, despite observing an association between AGD_{AS} and semen quality

in the study of men from the general population, a huge variation in AGD_{AS} among men with the same sperm concentration (as well as other semen parameters) was also detected. Thus, although a relevant endpoint in a research context, based on our study and the previous findings by Priskorn et al.,⁹ the use of AGD as a clinical marker for fertility may not be appropriate due to the inconsistencies reported. Further studies comparing subgroups of infertile men and larger studies of AGD in relation to TTP are needed to reach firm conclusions.

4.1 | Strengths and limitations

Our study has several strengths. It compares infertile men at the time of initiating fertility treatment to a well-characterized group of

fertile men from couples with an ongoing pregnancy. Both groups were evaluated for AGD and reproductive function, as well as potential confounders, according to the same questionnaires and detailed protocols and direct thorough assessment of their reproductive function. Inclusion of fertile men rather than men from the general population increased the likelihood of detecting a difference in AGD between the two groups. Furthermore, men with male factor infertility and planned ICSI based on a low sperm motility or morphology were also included, rather than limiting inclusion criteria to men with low total sperm count or low sperm concentration only. Lastly, we excluded men with azoospermia, genetic disorders, history of orchitis, epididymitis, testicular torsion, varicocele, vasectomy, orchiectomy, chemotherapy or radiation therapy, or the presence of diseases requiring permanent treatment, as these forms of male factor infertility are not expected to be related to AGD. Although this is a valid exclusion, it is important to note that around 50% of azoospermia patients have an identifiable etiology, and it could have been pertinent inviting these men and basing exclusion on genetic testing, history and physical examination. Azoospermia represents around 10%–15% of infertility cases; and the inclusion of these men would represent an absolute inclusion of around six additional infertile men (azoospermia with identifiable etiology).

As women show a decline in fecundity as they pass through the reproductive years,²² the association between AGD and TTP could be influenced by the female partners age. Thus, another strength of this study is the adjustment for the female partner's age at the time of conception. Additionally, the validation of the men's reported TTP to the TTP reported by his pregnant partner further strengthens the validity of the estimated association between AGD and TTP in our study. The average TTP was, however, skewed to the left, that is, rather low, and having a more bell-shaped distribution of TTP would have been beneficial for these analyses.

Certain limitations warrant mentioning. In total, nine physicians carried out the physical examinations (AGD measures) with four physicians carrying out a combined 90.5% of these. Even though the physicians received specific training in measurement of AGD and measured AGD three times in each person, there is a risk of between-physician difference in AGD measurements. Furthermore, physicians were not blinded to fertility status of the men when measuring AGD. However, in our sensitivity analyses stratifying by these four main physicians, we did not detect any differences in our estimates.

Initially, 271 fertile men and 146 infertile men were recruited and examined, but we did not have information about how many infertile men were approached, that is, the participation rate in this group. Thus, the participation rate may have been different in the two groups. However, we believe that participation from both infertile and fertile men are expected to be unrelated to our exposure of interest, since most people are not aware of their AGD, and thus potential differences in participation should not bias the estimates of the association between AGD and infertility.²⁷ As only men referred to the out-patient clinic at the Department of Growth and Reproduction, Rigshospitalet, were included in the study, we acknowledge that the group of infertile men in our study may not be representative of all infertile men in Denmark.

Small study size may have limited the ability to detect any associations in the present study, and future larger studies are recommended. Effect estimates from the analyses of AGD and infertility did not indicate any associations, while a suggestion of an association between AGD and TTP was found.

5 | CONCLUSIONS

In conclusion, our study did not find a clear association between AGD and fertility status or TTP, nor was the decreased risk of experiencing a longer TTP with longer AGD_{AS} statistically significant. This study used a thorough and standardized setup with several strengths supporting the validity and reliability of the results. Based on our findings, AGD may not be an appropriate clinical marker for fertility. Additionally, this study is to our knowledge the first to investigate the association between AGD and TTP. Along with the conflicting results of studies, there is a need for larger studies comparing AGD in fertile and infertile men including subgroups of these as well as studies examining AGD and TTP.

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AUTHOR CONTRIBUTIONS

L. Priskorn and N. Jørgensen were involved in conception and design of the study. L. Priskorn was involved in data collection. F. Madvig and M.K. Pedersen analyzed the data. F. Madvig, M.K. Pedersen and L. Priskorn drafted the manuscript. All authors were involved in data interpretation, revising the manuscript critically for important intellectual content and gave final approval of the manuscript.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

DATA AVAILABILITY STATEMENT

The data underlying this article cannot be shared publicly due to the privacy of the individuals that participated in the study. The data will be shared upon reasonable request to the corresponding author provided that ethical permission can be obtained for the specific request.

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