Systematic Review

Association between Late Manifestations of Testicular Dysgenesis Syndrome and Anogenital Distance: A Systematic Review and Meta-analysis

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Abstract

Background: In 2001, Skakkebæk *et al*. proposed that certain male reproductive disorders might be grouped into a syndrome called testicular dysgenesis syndrome (TDS), as they all appear to be associated with disruption of the embryonic and foetal programming of gonadal development. TDS may be manifested in early life by the presence of genital malformations (hypospadias and cryptorchidism) and in adult life as disorders represented by low sperm counts and testicular cancer. Changes in androgen hormones during the foetal development, in addition to resulting in TDS, can also cause permanent changes in anopenile anogenital distance (AGDap) and anoscrotal anogenital distance (AGDas). **Aims:** The objective of this study was to determine whether there is a relationship between late manifestations of TDS and reduced anogenital/anoscrotal distance. **Materials and Methods:** The present study is a systematic review and meta-analysis. The research included papers from 2001 to 2020, comprising a total of 737 articles, and 13 articles were selected. **Results:** Linear regression analysis was performed to evaluate the relationship between the two anogenital distance measures, which showed a significant positive association (P = 0.039). A meta-analysis was also performed and compared AGDap and AGDas between control and case groups, with cases defined as men with any late TDS manifestation. These data showed a significant reduction in AGDas in the affected population (P = 0.04), but no differences in the AGDap measure (P = 0.59). **Conclusion:** Our study confirmed a significant relationship between reduced AGDas and late manifestations of TDS, providing further support to the association between prenatal androgen deficiency and late-onset reproductive disorders.

Keywords: *Anoscrotal distance, genital measures, male reproductive disorders, sperm counts, testicular cancer*

Introduction

Male reproductive disorders have shown a dramatic increase over the past five decades. Such disorders can occur in newborn males (cryptorchidism and hypospadias) as well as in adult men (impaired spermatogenesis and testicular germ cell cancer).[1] In 2001, Skakkebaek *et al*.[2] encompassed these manifestations into a common syndrome named testicular dysgenesis syndrome (TDS), since they seemed to be associated with similar aetiopathogenic mechanisms

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early in life, resulting from a disruption of the embryonic and foetal programming of gonadal development.[3,4]

The aetiology of TDS is presumed to be multifactorial, i.e. related to genetic factors and/or exposure to environmental aspects during pregnancy,[5] including infections, diet, environmental pollutants, medications, body weight and lifestyle habits.[4] Many substances called endocrine-disrupting chemicals have been related to the aetiopathogenesis of TDS because they are able to interfere with different kinetic (production, distribution, metabolism and excretion) and dynamic (receptor binding and activation) processes of natural hormones in the body, which are responsible for maintaining homeostasis and regulating reproductive development.[1] In this sense, when prenatal sexual development processes or gonadal tissue differentiation are impaired, resulting in Sertoli and Leydig cell dysfunction, TDS can occur.

Alterations in androgen hormone levels or action during the foetal masculinisation programming window (MPW), in addition to resulting in TDS, can also cause permanent changes in anogenital distance (AGD), which can be divided into anopenile AGD (AGDap), measured from the anterior base of the penis to the centre of the anus, and anoscrotal AGD (AGDas), measured from the posterior base of the scrotum to the centre of the anus.[6] This window refers to the 8th to 14th weeks of gestation in humans, in which the actions of androgens programme the subsequent development of all male reproductive organs, including their final adult size and function.[7]

AGD, defined as the distance between the anus and the external genitalia,[8] is considered a biomarker of the amount of androgen to which the male foetus is exposed during MPW.[9] Likewise, it also reflects intrauterine androgenic action throughout the development of the reproductive system,[8] being able to retrospectively indicate the interruption or reduction of foetal androgen signalling and predict late reproductive disorders in male offspring.[10] In addition to serving as a long-life marker of foetal testosterone production by the testicles,[11] in adults, AGD is associated with the serum circulation of this hormone, as well as its aromatisation to oestradiol.[12] Thus, this measurement has been used to show that agents that alter androgenic signalling can lead to abnormal genital length and even alter testicular function, as clinically assessed by testosterone and sperm production.[13]

After birth, AGD can be easily measured, and thus, it is possible to correlate the late manifestations of TDS with reduced AGD measurements in adults. In this sense, studies have shown a relationship between manifestations such as infertility, low concentration and total sperm count, changes in sperm motility and morphology and a shorter AGD when compared to men without the same changes.[13,14] Furthermore, some authors have shown that AGD can also predict final testicular size, as well as being related to penis size at birth and in adult men.[11] In the most severe cases, impaired spermatogenesis can lead to an increased risk of testicular cancer,[5] further illustrating the interrelationship between the TDS disorders. Some authors also point to a possible relationship between a shorter AGD and prostate cancer, which despite not being a TDS component, is allegedly associated with low exposures to androgens and/or higher exposures to oestrogens in the prenatal life.[15]

The knowledge of the existence of TDS is important for the clinical management of patients,[16] since a better understanding of the origin of the syndrome disorders may enable its prevention.[11] Since gonadal development is initiated *in utero* during this period and has the ability to influence reproductive function at adulthood,[14] it is essential to develop and apply markers of intrauterine hormonal changes such as AGD. Furthermore, AGD can be measured via ultrasound during gestation,[12] and because it is considered a useful biomarker for normal gonadal development and function, it might be able to act as an early identification method for TDS. Thus, such an artifice can further assist in understanding the role of the intrauterine environment, foetal reproductive programming and its consequences on adult reproductive health through non-invasive studies.[12] Thus, this systematic review aims to evaluate whether there is a relationship between late manifestations of TDS and reduced anogenital/scrotal distance measurements.

Materials and Methods

We used the Preferred Reporting Items for Systematic Reviews and Meta-Analyses guidelines to check the essential integral parts of a systematic review.[17,18]

The protocol for this research was registered on the International Prospective Register of Systematic Reviews platform under the registration number: CRD42020215583.

The research question was formulated according to the PICO tool, an acronym for Population, Intervention, Comparison and Outcomes.[19] The population group (P) was defined as patients with any late manifestation of TDS, and the intervention (I) as the measurement of anogenital or anoscrotal distance. The control group (C) comprised patients without late manifestations of TDS, and the outcome (O) was the reduction in anogenital/scrotal distance in patients with late manifestations of TDS. Thus, we aimed to determine whether there is a

relationship between late manifestations of TDS and reduced anogenital/anoscrotal distance.

The PubMed, Embase and Scopus databases were used to search for the articles. The search included articles published from 2001 to July 2020 in a different way in each database in order to obtain as many articles as possible, as described in Table 1. The first search year (2001) corresponds to the publication year of the TDS hypothesis paper by Skakkebaek *et al*.[2]

As inclusion criteria, the research participants should be males over 18 years of age and/or adult rodents with any late manifestations of TDS (changes in semen parameters, infertility and testicular cancer) or prostate cancer and who underwent anogenital or anoscrotal distance measurements. Prostate cancer was included in the searches since, although it does not make up the TDS, it is a parameter possibly associated with prenatal androgenisation and, consequently, AGD. Likewise, we also included penis size (penile length or diameter) in our search strategy, because similarly to AGD, the final size of the penis is also dependent on the androgen action during foetal life and may be negatively associated with the manifestations of TDS, as illustrated by the relationship between reduced penile length and TDS disorders.[13]

Furthermore, the articles should be complete, in Portuguese, English or Spanish, address the relationship between the anogenital or anoscrotal distance and late manifestations of TDS, penis size or prostate cancer and present as methodologies: cross-sectional studies, case–control, cohort and randomised controlled trials.

Studies that presented only analyses in females or female rodents, children or early manifestations of TDS (cryptorchidism and hypospadias) as well as populations with reproductive abnormalities with aetiologies unrelated to the TDS were excluded. Furthermore, articles that did not comprehend the relationship between TDS, penis size or prostate cancer and AGD or that did not perform the analysis in humans or rodents were excluded.

The 737 articles found were independently peer-reviewed and in case of conflict a third author was consulted. A total of 243 duplicate articles were analysed using Mendeley software's duplicate analysis tool; these were discarded, leaving 494 articles for title analysis. During this phase, 323 articles whose titles were not consistent with the proposed theme or included only females or female rodents were excluded, leaving 171 articles for abstract screening.

From this on, inclusion and exclusion criteria were instituted for the evaluation of the selected articles. The articles removed were classified according to the reason for their exclusion, and they were numbered from 1 to 5. Criterion number 1 corresponded to the absence of the relationship between TDS, penis size or prostate cancer and AGD, that is, the exclusion of articles that did not address the measurement of the AGD and the association of some late manifestations of TDS or articles that discussed only about the AGD but did not relate it to the syndrome. Number 2 included studies performed only in children or immature rodents, or even early manifestations of TDS. Criterion number 3 comprised articles with methodologies not selected as inclusion criteria for this review, criterion number 4 comprised articles that used experimental animals other than rodents, and finally, criterion number 5 comprised articles that did not present an abstract. Some articles presented more than one reason for exclusion, and for data presentation, only the most relevant reason was selected. After applying the above criteria, 49 articles were selected for full-text reading, of which 33 were conducted in humans and 16 in rodents. Most of the excluded articles (n = 64) comprised those with criterion number 2, i.e. that conducted studies only in children or immature rodents, or were related to the early manifestations of TDS. This was followed by

Table 1: Search strategy according to each database

Databases	Search strategy	Number of articles
PubMed	(Testicular Dysgenesis Syndrome OR Sperm count OR Sperm quality OR Semen Analysis OR Semen quality OR Azoospermia OR Testicular cancer OR Prostate cancer OR Testicular neoplasms OR Prostate neoplasms OR Penis length OR Penis diameter OR Penile length OR Penile diameter OR Micropenis OR Low fertility OR Fertility[MeSH Terms]) AND (Anogenital distance[Title/Abstract] OR Anoscrotal distance[Title/Abstract])	184
Embase	ALL (testicular AND dysgenesis AND syndrome OR sperm OR semen OR azoospermia OR fertility OR penis OR cancer) AND ('anogenital distance':ab, ti OR 'anoscrotal distance':ab, ti)	352
Scopus	(ALL (testicular AND dysgenesis AND syndrome OR sperm OR semen OR azoospermia OR cancer OR penis OR fertility) AND TITLE-ABS-KEY (anogenital AND distance OR anoscrotal AND distance))	201
Total		737

Font: Authors (2021)

articles that did not comprise the relationship between TDS, penis size or prostate cancer and AGD (n = 36), articles which methodology was not selected as inclusion criteria for this review (n = 13), articles with analysis in animals other than rodents (n = 5) and articles with no abstract (n = 4) for a total of 122 articles excluded at this stage. Although we initially aimed to select human and rodent studies, we chose to exclude articles that presented only rodent data, since the number of studies in human beings was sufficient for this study. That is, the reading of the full text was done in the 33 articles referring to humans, and the 16 that dealt with rodents were excluded from the results (exclusion reason number 6). During the full-text reading, the analysis criteria comprised the approach to the relationship between TDS, penis size or prostate cancer and anogenital measurements and the presence of the full article. Nine articles were excluded for not presenting data on the relationship between these parameters and 8 articles for the absence of the full text, leaving 16 articles for final analysis.

After careful analysis of the selected articles, we found some studies by the same authors that presented repeated populations in 2 different analyses, i.e. articles with the same study population. Thus, 3 articles were excluded for this reason, leaving 13 articles for the presentation of the results of the systematic review, as shown in Figure 1.

A quality analysis was performed in each of the articles used by means of the Newcastle–Ottawa Scale (NOS), which evaluated the criteria 'selection, comparability and exposure' for case–control studies and 'selection, comparability and outcome' for cohort studies. For each question observed, the articles received marks if they were in accordance with the criteria proposed by the scale, resulting in individual scores for each article, reaching a maximum of 9 points at the end of the analysis [Table 2].

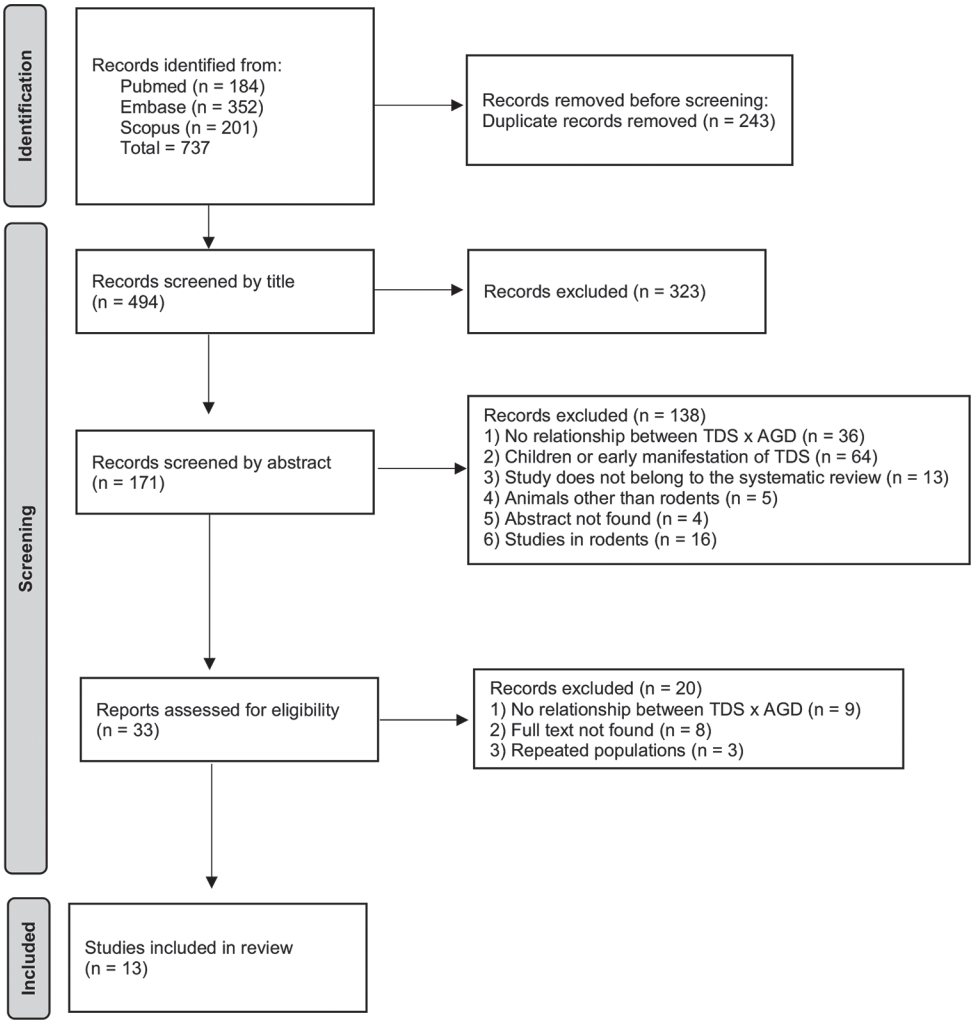

Figure 1: The Preferred Reporting Items for Systematic Reviews and Meta-Analyses flow chart. From: Page MJ, McKenzie JE, Bossuyt PM, Boutron I, Hoffmann TC, Mulrow CD, *et al*. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. BMJ 2021;372:n71. [doi: 10.1136/bmj. n71]. Font: authors (2022)

For the assessment of the relationship between the two AGD measures, AGDap and AGDas, and for the subgroup analysis, statistical programme R version 4.0.2[20] was used. This programme is a free and open-source software. RevMan (Review Manager 5.3) was used to perform the meta-analysis.

Results

According to the search made in the databases cited in Table 1, initial evaluation and elimination of articles incompatible with this systematic review, a total of 13 articles were obtained for analysis and discussion, which were submitted to quality analysis using the NOS [Table 2].

After selection, the articles were tabulated according to their authors, methodology, country of origin, total number of participants, average age and body mass index (BMI) [Table 3]. The studies were organised according to the date of publication, from the oldest to the most recent. Articles that had both a control and a case population were entered twice in the table, with these populations separated, as the data presented for each population were different. Case (affected) populations,

Table 2: Quality analysis using the Newcastle–Ottawa Scale

Article	Selection	Comparability	Exposure/outcome	Total (maximum 9)
Mendiola *et al*., 2011[6]	★★★★	-	★★★	7
Eisenberg *et al*., 2011[21]	★★★	★★	★★★	8
Castaño *et al*., 2012[22]	★★★★	★★	★★★	9
Eisenberg *et al*., 2012[23]	★★★	★★	★★★	8
Mendiola *et al*., 2015[24]	★★★	-	★★★	6
Parra *et al*., 2015[25]	★★★★	-	★★★	7
Zhou *et al*., 2016[26]	★★★★	-	★★★	7
López-Espín *et al*., 2018[14]	★★★★	-	★★★	7
Foresta *et al*., 2018[27]	★★★★	-	★★★	7
Oñate-Celdrán *et al*., 2018[15]	★★★	★★	★★	7
Priskorn *et al*., 2019[28]	★★★★	-	★★★	7
Sahin *et al*., 2019[29]	★★★★	★★	★★★	9
Moreno-Mendoza *et al*. 2020[30]	★★★	★★	★★★	8

Font: Authors (2021). Each star symbolizes a point in the quality assessment based on the New-Castle-Ottawa Scale (NOS). The more stars, the more points the article received, which means it is of higher quality

Table 3: Characteristics of the articles

Authors	Method	Country	Total of participants	Mean (±SD)	
				Age	BMI
Mendiola *et al*., 2011[6]	M1	USA	126	19.7 (±1)	24.6 (±3.5)
Eisenberg *et al*., 2011*[21]	M1	USA	117	34.3 (±6)	24.8 (±7.3)
Eisenberg *et al*., 2011*[21]	M1	USA	56	-	28.9 (±5.7)
Castaño *et al*., 2012*[22]	M2	Spain	60	65 (±7)	27.4 (±3.1)
Castaño *et al*., 2012[22]	M2	Spain	52	65 (±7)	27.3 (±3.8)
Eisenberg *et al*., 2012*[23]	M3	USA	29	32.8 (±4.8)	30.2 (±6.1)
Eisenberg *et al*., 2012[23]	M3	USA	69	44.2 (±9.2)	27.7 (±3.8)
Mendiola *et al*., 2015*[24]	M1	Spain	91	35.3 (±4.5)	27.5 (±4.1)
Parra *et al*., 2015[25]	M3	Spain	215	20 (±1.3)	23.9 (±3.4)
Zhou *et al*., 2016[26]	M3	China	656	20.1 (±1.6)	-
López-Espín *et al*., 2018[14]	M3	Spain	16	29 (±6.2)	25.3 (±2.8)
Foresta *et al*., 2018[27]	M3	Italy	794	18.7 (±0.7)	22.7 (±2.8)
Oñate-Celdrán *et al*., 2018*[15]	M2	Spain	125	50.2 (±12.4)	29.4 (±4.4)
Oñate-Celdrán *et al*., 2018[15]	M2	Spain	135	61.8 (±5.6)	28.2 (±4.4)
Priskorn *et al*., 2019[28]	M1	Denmark	1106	19.3 (±1.3)	22.4 (±2.8)
Sahin *et al*., 2019*[29]	M2	Turkey	52	67.7 (±7.7)	28.2 (±3)
Sahin *et al*., 2019[29]	M2	Turkey	60	67.03 (±7.8)	27 (±3)
Moreno-Mendoza *et al*. 2020*[30]	M2	Spain	166	-	24.8 (±2.7)
Moreno-Mendoza *et al*. 2020[30]	M2	Spain	110	-	26.8 (±3.7)

*Affected population. -=No data for this category, M1=Cross-sectional studies, M2=Case–control, M3=Cohort, USA=United States of America, SD=Standard deviation, BMI=Body mass index

defined as men with late TDS manifestations (or prostate cancer), are marked with an asterisk (*) next to the author's name, and Tables 5 and 6 follow the same pattern.

Regarding methodology, 4 (30.8%) articles were cross-sectional studies, categorised as M1 in Table 3, 4 (30.8%) were case–control (M2) and 5 (38.4%) were cohort studies (M3).

Concerning the countries of origin of the analysed articles, Spain had the largest number of studies, corresponding to 6 (46.1%), followed by the United States with 3 (23.0%) articles. The other countries, China, Italy, Denmark and Turkey, had only 1 article, corresponding to approximately 7.7% of the studies each.

The sample size in each article ranged from 16 to 1106, and the total number of participants analysed amongst the 13 articles was 4035 individuals. The average age ranged from 18.7 to 67.7 years, with two articles lacking age data. The average BMI of the participants ranged from 22.4 to 30.2 kg/m², and one of the articles did not present data on the average of BMI in its study.

Table 4 presents the 13 articles and their respective data about the main outcome, i.e. late TDS manifestations or prostate cancer, when available, and the position used for measuring the anogenital or anoscrotal distance, as well as the average of such measurements in millimetres (mm), separated in subgroups of affected (cases) or unaffected (control) populations, and their standard deviations. The affected population consisted of patients with altered semen parameters (subfertile or infertile), or who had prostate or testicular cancer. The unaffected group consisted of men without manifestations of TDS (or prostate cancer) or individuals randomly recruited from the general population.

Regarding the manifestations related to TDS, 9 (69.2%) articles presented data about semen parameter analysis, 4 (30.7%) data about penile length, 3 (23%) results on prostate cancer and 1 (7.7%) on testicular cancer. The position used to perform the AGD measurement was lithotomy in 7 (53.8%) of the articles and frog-legged in 6 (45.1%). Lithotomy consists of positioning the thighs at a 45° angle to the examination table, while the frog-legged position is performed in supination, with the legs abducted to allow the soles of the feet to meet. There was no significant difference between the use of lithotomy or frog-legged for the results.

The anogenital and anoscrotal distance measurements in studies reporting penis size, semen parameters (semen volume, sperm concentration, motile spermatozoa, sperm count and sperm morphology) are presented in Tables 5 and 6, respectively.

A linear regression analysis was performed to assess the correlation between the AGDap and AGDas measurements [Figure 2]. This analysis showed a significant positive correlation (P = 0.039) between AGDap and AGDas measurements in the clinically evaluated studies. Subgroup analysis was also performed using meta-analysis. This evaluation compared the measures of AGDap and AGDas between the affected and non-affected groups [Figures 3 and 4] and showed a significant reduction in the AGDas of the affected population (P = 0.04) when compared to the non-affected population [Figure 4], a result not present in the comparison of the AGDap, which showed no

Table 4: Parameters evaluated in the articles and anogenital distance and age according to the population studied

Authors	Parameters	Position	AGDap, mean (±SD) (mm)		AGDas, mean (±SD) (mm)	
			Affected	Non-affected	Affected	Non-affected
Mendiola *et al*., 2011[6]	1	A	x	128 (±13)	x	51.3 (±14.5)
Eisenberg *et al*., 2011[21]	1, 2	B	-	-	31.8 (±11.3)	44.6 (±14.1)
Castaño *et al*., 2012[22]	3	B	119.4 (±12.7)	124.9 (±13.7)	34.8 (±10.9)	35.6 (±12.6)
Eisenberg *et al*., 2012[23]	2	B	-	-	36.3 (±16.3)	41.9 (±11.3)
Mendiola *et al*., 2015[24]	1	A	140 (±24.8)	x	46.4 (±12.9)	x
Parra *et al*., 2015[25]	1	A	x	128 (±12)	x	48.3 (±11.6)
Zhou *et al*., 2016[26]	1	B	x	116.1 (±10.9)	x	39 (±10.7)
López-Espín *et al*., 2018[14]	1	A	x	121.4 (±18.8)	x	51.8 (±28.2)
Foresta *et al*., 2018[27]	1, 2	B	x	-	x	44 (±9)
Oñate-Celdrán *et al*., 2018[15]	3	A	121 (±12.5)	128 (±14.7)	29.5 (±13)	38.3 (±15.4)
Priskorn *et al*., 2019[28]	1	A	x	130.5 (±11.6)	x	60.5 (±2.9)
Sahin *et al*., 2019[29]	3	A	139 (±13.1)	125.8 (±17.3)	49.1 (±12)	49.6 (±8.6)
Moreno-Mendoza *et al*. 2020[30]	4, 1, 2	B	131.1 (±11.5)	140.9 (±12.7)	50.5 (±13.4)	55.5 (±12.8)

-=No data for this category, X=No data because of the non-existence of this population in the study, A=Lithotomy, B=Frog-legged, 1=Semen parameter analysis, 2=Penile length analysis, 3=Prostate cancer analysis, 4=Testicular cancer analysis, SD=Standard deviation, AGDap=Anopenile anogenital distance, AGDas=Anoscrotal anogenital distance

significant difference (P = 0.59) of this measure. The affected group combined men with any late TDS manifestation (or prostate cancer), because of the limited number of studies with data on the relationship between AGD and each individual TDS disorder (or prostate cancer).

Discussion

Anogenital/anoscrotal distance has been shown to reflect the amount of androgen to which a male foetus is exposed.[27] Increased exposure to androgens *in utero* results in longer and more masculine AGD, demonstrating that this measure can be a sensitive biomarker of prenatal exposure to these hormones.[6,25] In a study published by Juul *et al*.,[31] reduced AGD was even described as one of the TDS manifestations, whether late or early, since it is considered a permanent biomarker and shares a common pathophysiology of androgen insufficiency with all other TDS components. Proving the reliability of the relationship between AGD and male reproductive function would enable the prediction of the TDS hypothesis in humans.[26]

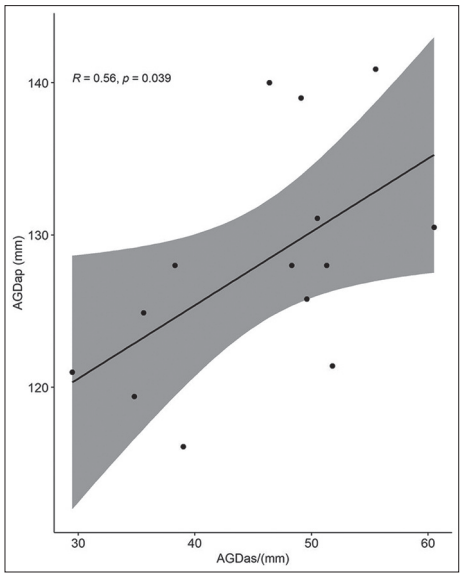

Figure 2: Linear regression to assess the correlation between the anopenile anogenital distance and anoscrotal anogenital distance measurements. Font: authors (2022). AGDap = Anopenile anogenital distance, AGDas = Anoscrotal anogenital distance

A systematic review published in 2018 by Hua *et al*.[3] evaluated the relationship between early manifestations of TDS and AGD in male infants and showed that boys who manifested cryptorchidism and/or hypospadias had lower AGD compared to boys without early manifestation of TDS. To the best of our knowledge, the present article is the first systematic review with meta-analysis to relate late manifestations of TDS to decreased anogenital and anoscrotal distances.

Sertoli cell dysfunction can alter the development and differentiation of spermatogenic cells, eventually leading to poor semen quality and testicular cancer.[1] In turn, Leydig cell malfunctioning can result in defective testosterone production and impaired masculinisation process.[8] Furthermore, these changes may negatively affect the control of foetal androgen production and testicular androgen maintenance throughout life,

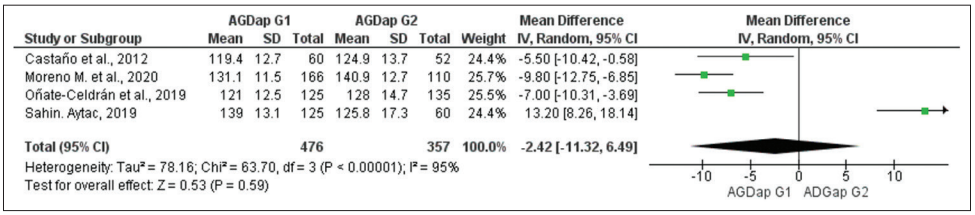

Figure 3: Meta-analysis anopenile anogenital distance comparing affected and non-affected groups. Font: authors (2022). AGDap = Anopenile anogenital distance, SD = Standard deviation, CI = Confidence interval, IV = Intravenous

Table 5: Data regarding penile length and anopenile anogenital distance/anoscrotal anogenital distance measurement

Authors	Length (mm)		Mean (±SD) (mm)	
	Mean (±SD)	Median (IQR)	AGDap	AGDas
Eisenberg *et al*., 2011*[21]	107.1 (±23)	-	-	31.8 (±11.3)
Eisenberg *et al*., 2011[21]	119.5 (±22.7)	-	-	44.6 (±14.1)
Eisenberg *et al*., 2012*[23]	123 (±25.2)	121 (90–165)	-	36.3 (±16.3)
Eisenberg *et al*., 2012[23]	125.4 (±20.3)	127 (103–157)	-	41.9 (±11.3)
Foresta *et al*., 2018[27]	89 (±17)	-	-	44 (±9)
Moreno-Mendoza, *et al*. 2020*[30]	-	116.9 (101.5–129.3)	131.1 (±11.5)	50.5 (±13.4)
Moreno-Mendoza, *et al*. 2020[30]	-	120.4 (109.9–129.3)	140.9 (±12.7)	55.5 (±12.8)

*Affected population. -=No data for this category, IOR=Interquartile range, SD=Standard deviation, AGDap=Anopenile anogenital distance, AGDas=Anoscrotal anogenital distance

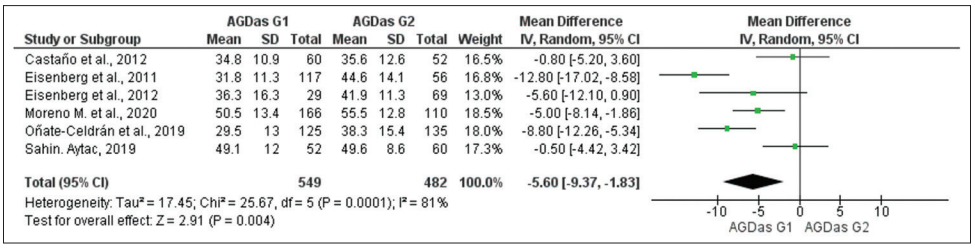

Figure 4: Meta-analysis anoscrotal anogenital distance comparing affected and non-affected groups. Font: authors (2022). AGDas = Anoscrotal anogenital distance, SD = Standard deviation, CI = Confidence interval, IV = Intravenous

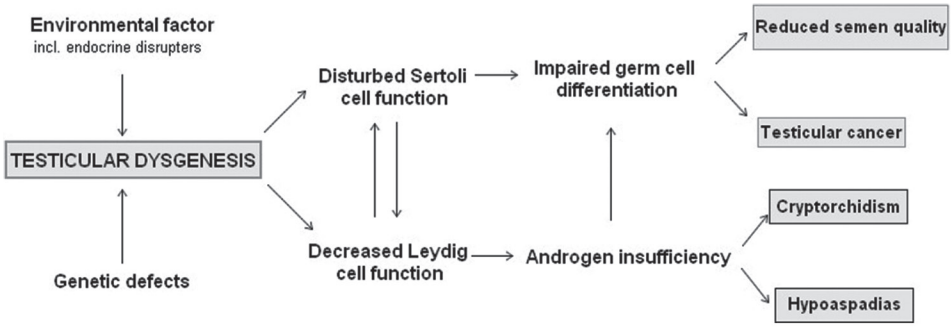

Figure 5: Schematic representation of pathogenetic links between the components and clinical manifestations of testicular dysgenesis syndrome. Adapted from Skakkebaek *et al*.[2] Font: authors (2022)

Table 6: Semen parameters and anopenile anogenital distance/anoscrotal anogenital distance

Authors	Semen volume (mL)		Sperm concentration (10^6/mL)		Motile spermatozoa (%)	
	Mean (±SD)	Median (IQR)	Mean (±SD)	Median (IQR)	Mean (±SD)	Median (IQR)
Mendiola *et al*., 2011[6]	3.3 (±1.6)	3.1 (2.1–4.3)	72.6 (±66.5)	53.5 (19.8–99.3)	57.4 (±15.5)	60.3 (49.3–69)
Eisenberg *et al*., 2011*[21]	2.7 (±1.2)	-	16.2 (±24)	-	24.4 (±20.2)	-
Eisenberg *et al*., 2011[21]	2.7 (±1.1)	-	33 (±27.9)	-	40.3 (±14.6)	-
Mendiola *et al*., 2015*[24]	3.6 (±2)	3.4 (2–5)	61.8 (±63.2)	44.8 (18.6–80)	39.4 (±20.5)	40 (30–50)
Parra *et al*., 2015[25]	3.3 (±1.7)	3 (1–6.4)	52.1 (±37.1)	44 (8.9–129)	56.5 (±10.9)	57.2 (38.9–74)
Zhou *et al*., 2016[26]	3.8 (±1.8)	3.5 (1.7–6.5)	69.3 (±61.2)	51.8 (13.7–194.3)	86 (±12.1)	89.4 (60–98.9)
López-Espín *et al*., 2018[14]	3.4 (±1.3)	-	35.2 (±28.7)	-	69.3 (±10.4)	-
Foresta *et al*., 2018[27]	2.7 (±1.5)	2.5 (1.5–3.5)	70.4 (±76.4)	51 (25.4–90)	-	-
Priskorn *et al*., 2019[28]	-	3.2 (1.3–5.9)	-	41 (3–147)	-	68 (32–88)
Moreno-Mendoza *et al*. 2020*[30]	-	-	-	-	-	-
Moreno-Mendoza *et al*. 2020[30]	-	-	-	-	-	-

Authors	Total sperm count (10^6)		Normal sperm morphology (%)		Mean (±SD) (mm)	
	Mean (±SD)	Median (IQR)	Mean (±SD)	Median (IQR)	AGDap	AGDas
Mendiola *et al*., 2011[6]	241 (±269)	157 (66.6–321)	8.4 (±4.6)	8.5 (5–12.4)	128 (±13)	51.3 (±14.5)
Eisenberg *et al*., 2011*[21]	-	-	-	-	-	31.8 (±11.3)
Eisenberg *et al*., 2011[21]	-	-	-	-	-	44.6 (±14.1)
Mendiola *et al*., 2015*[24]	199 (±190)	144 (54.4–288)	5.1 (±4.8)	4 (2–7)	140 (±24.8)	46.4 (±12.9)
Parra *et al*., 2015[25]	154 (±120)	121 (18–400)	10.3 (±3.3)	9 (2.8–23)	128 (±12)	48.3 (±11.6)
Zhou *et al*., 2016[26]	252.7 (±221.7)	193.4 (42.5–732.5)	11.9 (±7.4)	10 (2.3–27)	116.1 (±10.9)	39 (±10.7)
López-Espín *et al*., 2018[14]	115 (±99.6)	-	20.3 (±9.6)	-	121.4 (±18.8)	51.8 (±28.2)
Foresta *et al*., 2018[27]	179.8 (±211.8)	122.5 (51.1–224.8)	7.4 (±5.5)	6 (4–10)	-	44 (±9)
Priskorn *et al*., 2019[28]	-	125 (8–456)	-	6 (0.5–14.4)	130.5 (±11.6)	60.5 (±2.9)
Moreno-Mendoza *et al*. 2020*[30]	-	54.5 (14.8–111.3)	-	-	131.1 (±11.5)	50.5 (±13.4)
Moreno-Mendoza *et al*. 2020[30]	-	213 (119–399.6)	-	-	140.9 (±12.7)	55.5 (±12.8)

*Affected population.-=No data for this category, IOR=Interquartile range, SD=Standard deviation, AGDap=Anopenile anogenital distance, AGDas=Anoscrotal anogenital distance

as shown in Figure 5, adapted from the article by Skakkebaek *et al*.[2]

The studies selected for this review measured the anogenital and anoscrotal distances in two different

positions, called frog-legged and lithotomy, which did not show significant differences in the results. The frog-legged position was used to perform the measurements in 6 (46.15%) studies, while the lithotomy position was used in 7 (53.84%) studies. All studies used the stainless steel digital calliper as the measuring instrument.

The study by Moreno-Mendoza *et al*.[30] evaluated the measurements of AGDap and AGDas distances and penile length in populations with and without testicular cancer. This was the only article selected that evaluated the relationship of late manifestation of testicular cancer with these measurements. This study showed that patients with testicular cancer presented a significant reduction of the anogenital/anoscrotal distance when compared to individuals without cancer. Despite being the only article to address this manifestation, Rajpert-De Meyts *et al*.[32] stated that there is an association of testicular cancer with other presentations of TDS, implying that testicular cancer is encompassed in the syndrome.

From the 14 analyses performed in this systematic review, considering the study by Moreno-Mendoza *et al*.[30] as two different populations (regarding testicular cancer and regarding penile length), 10 (71.4%) studies showed reduced AGD in individuals with some late manifestations of TDS. Amongst the 6 studies that evaluated semen parameters, 4 (66.6%) showed altered seminal patterns in the subfertile or infertile population. In addition, 3 out of the 4 studies that evaluated penile length (75%) had positive results regarding the relationship with decreased AGD. Regarding the studies that evaluated patients with cancer, there was one on testicular cancer and three on patients with prostate cancer. Amongst these, only 1 (25%) study, by Sahin *et al*.,[29] found no relationship between prostate cancer and reduction in AGD measurements. These authors observed that the AGDap measurement was significantly higher in groups of men with prostate cancer (139 mm) compared to the control group (125.8 mm), composed of men with prostatic hyperplasia. In respect of AGDas, the authors found no significant changes; however, when observing the results, it is possible to point out that AGDas is slightly lower in patients with prostate cancer, a result that would be in agreement with the hypothesis of the present article. However, it is worth noting that prostate cancer is not part of the description of TDS proposed by Skakkebaek *et al*.[2] and its relationship with prenatal androgen insufficiency is less clear than in relation to the classic components of TDS.

In addition to the measures of anogenital and anoscrotal distances presenting a direct correlation between them, in the articles used to perform the meta-analysis, it was observed a significant relationship regarding the decrease in AGDas and the presence of late manifestations of TDS. On the other hand, there was no statistically significant relationship between these manifestations and reduced AGDap. This result is possibly directly related to the analyses presented by Sahin *et al*.,[29] since their article presented a relationship contrary to the others. Presumably, if this article was not considered for meta-analysis, the relationship between reduced AGDap measurements and the presence of late manifestations would also be significant. Another possibility, which needs further confirmation, is that AGDas may be a better indicator of prenatal androgenisation than AGDap.

Aydin *et al*.[12] stated that the AGD can be safely checked *in utero* during the second and third trimesters of pregnancy by ultrasonography (US), which demonstrates that early measurements by US may be a potential resource to be introduced as part of routine prenatal care. Considering that the decrease in AGD may be related to changes in hormonal patterns for the foetus during pregnancy, one of the aetiopathogeneses of the development of TDS, the possibility of measuring AGD during intrauterine development may be a tool for early diagnosis of possible late manifestations of TDS. Although not all articles in this review agreed on the relationship of decreased AGD with late manifestations of TDS, all articles that addressed AGD as an endocrine biomarker stated that this measure has the potential to assess intrauterine androgen exposure, which may reflect in alterations in foetal genital development or in adult life.

There are several potential limitations in conducting this systematic review with meta-analysis. First, the methodology of the included studies is not consistent, presenting several criteria for participant selection and several tools for measuring AGD, resulting in high heterogeneity and potentially impacting our results. In addition, some confounding factors, such as age, weight, BMI, ethnicity and abstinence time before semen collection, are different amongst the included studies. The number of studies found does not correspond to a large sample, and an analysis with a larger number of studies and participants may show different results from those presented here. In addition, not all the articles analysed in the systematic review could be included in the meta-analysis, since only six studies had both a case and a control population. Furthermore, not all studies measured the longer version of the AGD (AGDap), which is why only four articles had these data included in the meta-analysis. Furthermore, some articles addressed only one of the late manifestations of TDS,

while others evaluated more than one parameter. Thus, it was not possible to discriminate completely which manifestation is related to the anogenital changes presented. It is suggested that studies addressing all late manifestations of TDS be carried out in the evaluated participants, since patients who present one of the manifestations are more likely to present another one.

Conclusion

The study evidenced a significant relationship between reduced anoscrotal distance (AGDas) and late manifestations of TDS, supporting the view of prenatal androgen deficiency as one possible common pathogenic pathway linking all TDS disorders. However, further studies are needed to confirm the relationship between AGDap with such manifestations. As for the use of anogenital and anoscrotal measurements as an endocrine biomarker, all articles that addressed this relationship affirmed its potential as an evaluation of androgenic exposure during the foetal period, this being a parameter that can be evaluated by US during pregnancy and, therefore, capable of acting as an early identification method still during the prenatal period.

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Conflicts of interest

There are no conflicts of interest.

Data Availability

Not Applicable.

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