Vulvar Developmental Stages During Puberty : A Systematic Review

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Contents

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Vulvar Developmental Stages During Puberty : A Systematic Review

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Abstract

Background : Puberty is associated with important changes in secondary sexual characteristics, but the changes occurring in female external genitalia are not thoroughly described. The aim of this systematic review is to summarize and assess the current scientific knowledge regarding vulvar changes and development during puberty.

Methods : PubMed, Embase, Web of Science, and Cochrane were searched, using keywords related to "puberty", "vulva", and "morphology". Inclusion criteria were observational studies describing vulvar development in individuals between the ages of 8 and 16 years. The outcomes of interest were quantitative and/or qualitative descriptions of the vulva, including anatomical, physiological and histological changes.

Results : Of the 1658 articles screened, 10 were included. The mean clitoral glans diameter increases during puberty, as does the length of the clitoral hood. The clitoral hood changes, to become more retractile and rugose. The inner labia width and length increases throughout puberty, and the development of inner labia varies between individuals, with labial asymmetry being a common characteristic. The most frequent hymenal configuration found during puberty is the crescentic form, and features such as hymenal mounds, notches and longitudinal intravaginal ridges are common and physiological.

Conclusion : Reliable data on pubertal development of the external female genital organs is scarce. Future research is needed in order to provide more precise data to help categorize vulvar development into stages. A detailed description of vulvar maturation into sequential stages throughout puberty (as done by the Tanner scale for the male homologous structures) can increase knowledge of its morphological diversity and help reach clinical consensus on the nature of pathological variants. In addition, better knowledge of vulvar diversity is of importance to both healthcare professionals and individuals, and may empower and promote self-esteem.

Introduction

Puberty induces important anatomical and functional changes in secondary sexual characteristics and external genital organs. This development is partially described using the Tanner scale, which was developed by British pediatric endocrinologist James M Tanner, and first published in 1955 (1). In individuals with a penis, the Tanner scale describes development of pubic hair and changes in genitalia, including the enlargement of testes, penis and scrotum. In girls, the stages describe pubic hair growth and distribution, and breast development (1, 2). Tanner stages do not include changes in ovarian size, or clitoris, inner and outer labia size and color. Despite its wide use, the Tanner scale lacks an accurate description of vulvar anatomy and its modification through puberty. To this day, there are surprisingly few descriptions of vulvar maturation during puberty, and largely insufficient detail to categorize this development into stages (3, 4).

Such lack of data is not without consequences for individuals born with a vulva and healthcare professionals (HCP). Studies show an increase in requests for female genital cosmetic surgery (FGCS) amongst adolescents and young adults. For example, labiaplasty numbers in the National Health Service, in the United Kingdom, have increased five-fold between 2001 and 2010 (5), and similarly in Australia, demands have more than doubled over the same time period (6). Several studies have shown that the most frequently reported reason for surgery is an aesthetic concern (7). The increasing demand for FGCS is an indirect indicator of increasing genital dissatisfaction (8); this could be a socio-cultural consequence of increased media and pornography exposure, which largely portray adult vulvas without hair and with small inner labia, resembling prepubertal genitals (7, 9). In a study of 21 young women, all subjects identified the hairless genitalia with no visible inner labia as the "socially accepted ideal" vulva (10). The widespread portrayal of these "prepubertal-like vulvas" in mass-media is particularly deleterious amongst young adolescents, who use the internet as an important or only source for sexual education and information (11). Furthermore, adolescence is a particularly vulnerable period in terms of self-esteem construction. The comparison of one's body to images portrayed in the media can have a significant impact of psychological and physiological well-being. This unrealistic genital image is far from the diversity of existing physiologic vulvar appearances.

Therefore, a detailed comprehension and description of morphological vulvar changes throughout puberty is necessary for both HCP and patients, to better describe physiological development, providing reassurance regarding physiologic variants and enabling better identification of any potentially pathological conditions.

The objective of this systematic review is to provide an overview of the current scientific knowledge regarding vulvar changes and development during puberty, including morphological, histological, and physiological changes.

Methods

We conducted a systematic review using the search strategies described in appendix 1 (12). Medline (through PubMed), Embase, Web of Science, and Cochrane were searched in June 2023, using language filters for English, French, Italian and Spanish, and without any time restriction. The protocol was registered in PROSPERO (study ID: CRD42023443266).

Study eligibility was determined using the PICO framework (13). Observational studies describing the vulva of individuals between the ages of 8 and 16 years old were included. This age range was chosen because puberty onset is defined by thelarche, which usually appears between the ages of 8 and 13 (14). Menarche is the last stage of puberty and starts on average 2 to 3 years after thelarche (14), therefore marking the end of the included age range at 16 years. Studies including participants outside the age range but providing distinct data for the age category 8-16 years were included. The outcomes of interest were quantitative and/or qualitative descriptions of the vulva, including anatomical, physiological and/or histological changes to puberty. Articles were excluded if they only described abnormal vulvar conditions or traumatisms, or if the population included individuals with precocious or delayed puberty, variations in sex development or exposure to sexual abuse. Articles reporting non original research and book chapters were excluded.

Duplicate records were eliminated using the Bramer method with EndNote (15), and using Rayyan's deduplication software. Titles and abstracts were then screened by two

independent reviewers (FC and CB) using Rayyan software, and any disagreements were resolved between the two authors. The full texts of selected studies were screened by FC, and a second independent reviewer (DM or VC). Any discrepancies between the reviewers were resolved by consensus or a third reviewer (MY) if no consensus was reached. Excluded articles were labelled with a reason from the following list: wrong study design, wrong population, wrong outcome, wrong exposure.

Data including author, year published, study design, country, recruitment period, number of subjects, age range and outcomes were extracted by FC and included in a summary diagram (Table 1). The methodological quality of included studies was assessed independently by two authors (FC and DM) using the Johanna Briggs Institute (JBI) Critical Appraisal Tools (16), and disagreements were resolved by consensus. A raw score was calculated for each of the selected studies by dividing the number of 'Yes' responses by the total number of applicable statements in the JBI critical appraisal tools. High risk of bias was defined as a raw score of 49% or lower, moderate risk of bias was defined as raw score between 50% and 69%, and low risk of bias was defined as a raw score of 70% or above (17).

Due to important differences in methodologies and outcomes for the included articles, results were synthesized using a narrative approach.

Results

Study selection

A total of 1658 studies were identified for screening after removal of duplicate articles. After abstract review, 105 reports were sought for full-text retrieval. Following full text review and resolution of disagreements, 10 articles met the inclusion criteria (Figure 1), of which 4 cohort studies (18-21), 4 cross-sectional studies (22-25), and 2 case series (11, 26), involving a total of 349 subjects.

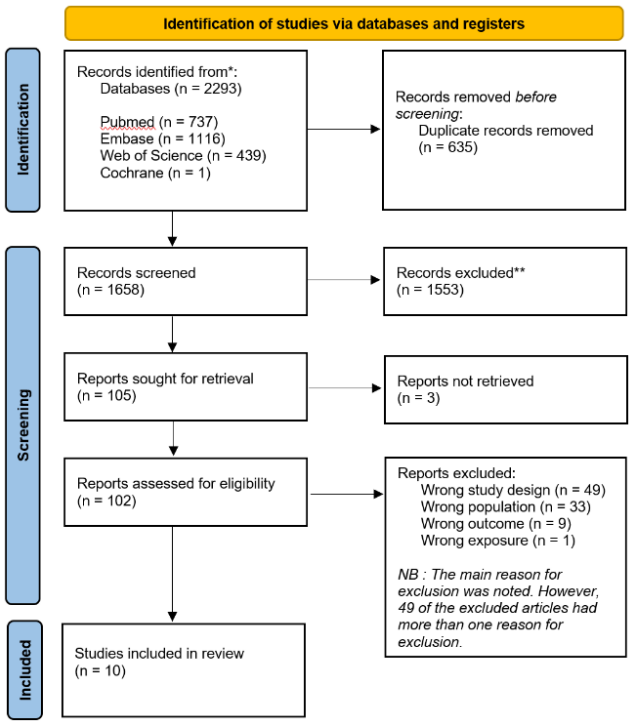

Figure 1 : Flow chart for included articles, following the PRISMA 2020 flow diagram guidelines (27)

Table 1

Summary of Characteristics and Key Results of Included Studies

Author	Year	Study Design	Study Country	Recruitment period	No. of subjects	Age of subjects (years)	Ethnicity (and % of participants in each category)	Measuring position	Primary outcomes	Key results
Berenson et al (18)	2002	Cohort	USA	Not described	61	9	Black, non-Hispanic (51%), White, non-Hispanic (26%), Hispanic (23%)	Supine with labial traction and knee-chest	Hymen	90% of participants had a crescentic hymenal configuration. Mean horizontal and vertical trans-hymenal diameter increase as the sample aged ($p < 0.01$). Hymenal features, such as periurethral band and longitudinal intravaginal ridges were very common, with a 100% and 92% prevalence respectively.
Biro et al (19)	2003	Cohort	USA	Not described	152	9-10	White (100%)	Not described	Pubic hair maturation	Mean age (± SD) for onset of puberty among participants in adrenarche pathway = 10.7 ± 0.9. In the year after puberty onset, 78% of subjects in adrenarche pathway advanced in areolar maturation and 37% advanced in pubic hair maturation ($p < 0.001$)
Boulos et al (26)	2018	Case series	USA	Not described	4	11-13	Not described	Not described	Inner labia	Hypertrophy of inner labia is a variant of normal anatomy. Typical histological features of inner labial hypertrophy include papillomatosis, sebaceous gland hyperplasia, dilated and increased lymphatic vessels, a fibrous stroma, and features of lichen simplex chronicus.
Brodie et al (22)	2016	Cross-sectional	USA	Sept. 2014 – May 2015	22	9-16	White non-hispanic, White Hispanic, African American, Mixed Hispanic, Native American (% of each category not available)	Lithotomy	Glans, inner labia, overall vulvar structure	All genitalia measurements increased with age, but with large variation between subjects. Clitoral hood to urethral orifice distance, inner labia length and width showed steep growth during puberty. The tent shape is the most common clitoral hood shape.
Gardner J (23)	1992	Cross-sectional	Australia	April 1988 – March 1989	9	10-11	White (100%)	Not described	Hymen	Posterior rim (or crescentic) hymen was the most common configuration.
Michala et al (11)	2011	Case series	Greece	June 2009-December 2010	12	10-16	Not described	Not described	Inner labia	Average inner labia width (mm) = 35.6 (range 20-55mm). 9 out of 12 participants had asymmetrical inner labia, with an average difference of 15.22 mm between right and left inner labia.
Myhre et al (20)	2010	Cohort	Norway	April-September 2005	31	10-13	White (100%)	Supine	Hymen	Crescentic hymen was the most common configuration. Increase in hymenal thickness and redundancy at follow-up examination ($p = 0.000$), with a tendency of folding out and developing hymenal groove. Increase in horizontal hymenal diameter.
Onderoglu et al (24)	1992	Cross-sectional	Turkey	Not described	29	10-16	Not described	Lithotomy	Hymen	A fimbriated hymenal configuration is the most common, and its rate increases at puberty compared to prepubertal years.
Sane et al (25)	1991	Cross-sectional	USA	Not described	18	8-13	Not described	Frog-leg	Glans	Average clitoral index (mm2 ± SEM) = 16.7 ± 0.9. Clitoral index increased during puberty, and is correlated with breast Tanner stage ($p = 0.001$), pubic hair Tanner stage ($p < 0.002$), and body surface area ($p < 0.001$)
Qin et al (21)	2023	Cohort	China	January 2016- May 2022	11	9-16	Not described	Not described	Inner labia	8 out of 11 participants had asymmetrical inner labia.

Characteristics of Studies and Participants

Included studies were published between 1991 and 2023. Five studies were conducted in North America, and one in each of the following countries : Australia, Greece, Turkey, China, and Norway. The number of participants varied from 4 patients in Boulos et al. case series (26), to 152 in Biro et al. cohort study (19).

Descriptions of ethnicity were limited. Five articles did not specify the ethnicity of included subjects, (11, 21, 24-26), three articles included only White participants (19, 20, 23), one article included Black non-Hispanic, White non-Hispanic, and Hispanic participants (18) and one article included White non-Hispanic, White Hispanic, African-American, Mixed Hispanic, and Native American participants (22).

Studies were divided according to which vulvar structure they analyzed. Four articles discussed changes in hymenal morphology (18, 20, 23, 24), four focused on the inner labia (11, 21, 22, 26), two on the clitoris (22, 25), one on pubic hair maturation (19), and one on the overall vulvar measurements (22).

For clarity purposes, Figure 2 provides an outline of the key anatomical vulvar structures which will be referred to throughout this article.

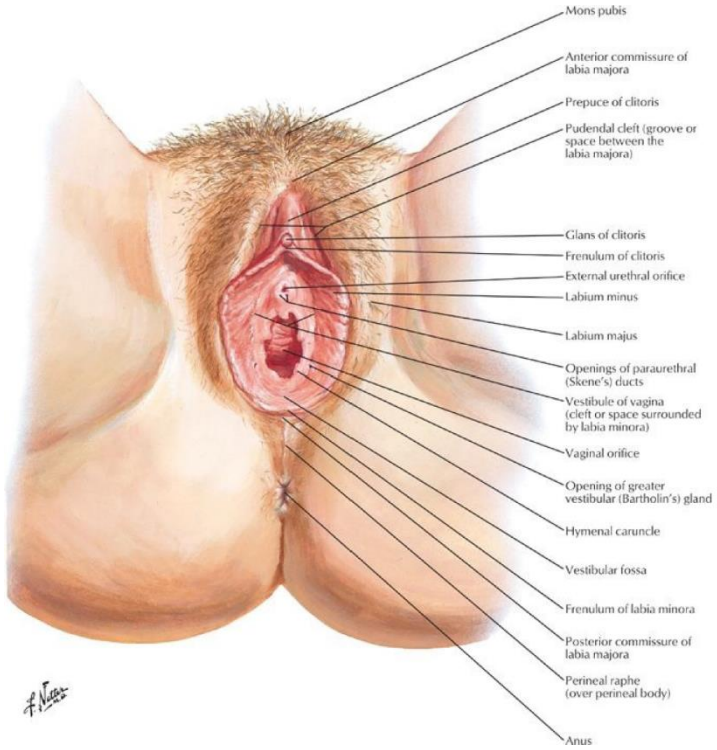

Figure 2 : Anatomical structures of the female perineum and external genitalia, adapted from Frank H. Netter Atlas of Human Anatomy (28)

Pubic hair maturation

A longitudinal study evaluating the initial manifestations of puberty recruited 443 subjects at 9 or 10 years of age, and followed them annually for 10 years (19). Participants were classified as either following the thelarche or adrenarche pathway, depending on whether the first manifestation of puberty was areolar maturation or pubic hair, respectively. A third of the participants (152/443, 34.3%) had adrenarche as the initial manifestation of puberty, and the mean age of onset was 10.7 ± 0.9 years. In the year following onset of puberty, 78% of participants in the adrenarche pathway had advanced in areolar maturation and 37% advanced in pubic hair maturation.

Clitoris glans and clitoral hood

Brodie et al. report an increase in mean length of clitoral hood (Figure 3a), length of sides of clitoral hood (Figure 3b), and mean clitoral diameter (Figure 3c) between groups of subjects aged 9-12 and 13-16, in a cross-sectional study (22). Mean clitoral diameter, and mean length of clitoral hood increased by an average of 0.6 and 4.0 mm respectively. They also reported four shapes of clitoral hoods (horseshoe, trumpet, coffee bean and tent; see Figure 4). The tent shape, with the clitoral hood dividing into right and left leaflets to provide a central opening for the glans, was the most common in both groups. As age increased, the clitoral hood exhibited greater retractility and rugosity. Across both age categories, the clitoral hood and inner labia generally formed two distinct structures, with the upper extremities of the inner labia connecting under the glans to form the frenulum of the clitoris.

Another study reported the *clitoral index* (25), or glans' surface area, calculated as a product of the longest sagittal and transverse lengths of the glans, in mm^2. The authors found an average of 16.7 +/- 0.9 mm^2 for subjects in the 8-13 years category. This index was increased compared to the prepubertal age groups, and was also correlated with breast Tanner stage ($p = 0.001$), pubic hair Tanner stage ($p < 0.002$), and body surface area ($p < 0.001$) (25).

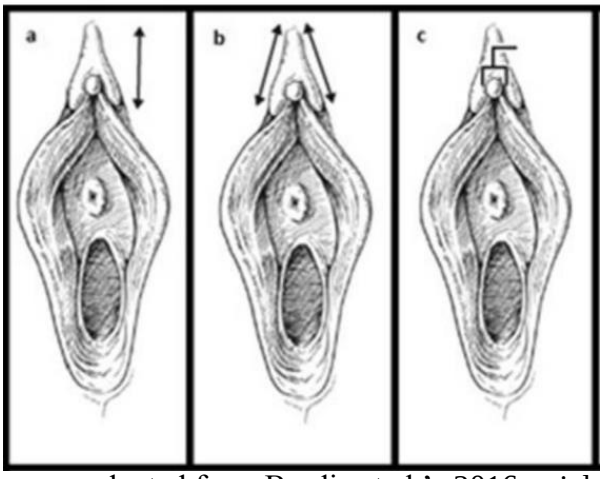

Figure 3 : Diagram adapted from Brodie et al.'s 2016 article (22), with diagrams indicated measurements taken in mm : a) Length of clitoral hood; b) Length of sides of clitoral hood; c) Clitoral diameter

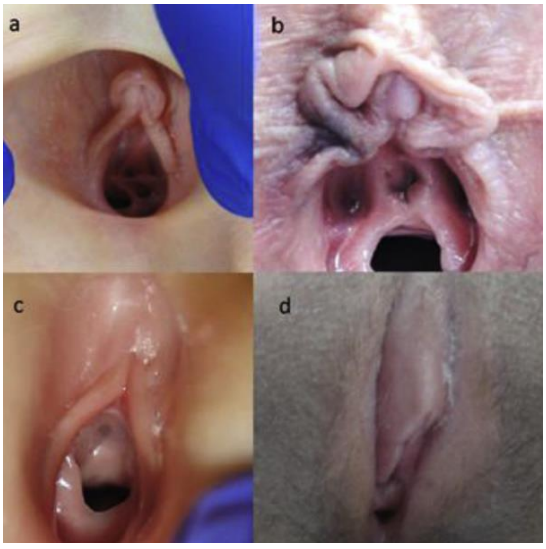

Figure 4 : Figure adapted from Brodie et al.'s 2016 article (22), with observed clitoral hood shapes : a) horseshoe; b) trumpet; c) coffee bean; d) tent.

Inner labia

Similarly to clitoral measurements, Brodie et al. report an increase in inner labia length and width with age (22) in their cross-sectional study (see Figure 5). Between the age groups of 9-12 and 13-16, there was an average of 163% increase in length, and a 212% increase in the width of inner labia. There was some variability of measurements, even amongst the same age categories. For example, in patients 9-12 years old, the range of inner labia length was from 6-90mm (average = 15.2mm), and their width ranged from 1-15mm (average = 3.9mm). In subjects from 13-16 years old, the range of length as 20-80mm (average = 40.0mm), and the range of width was 3-17mm (average = 12.2mm), indicating substantial overlap between age groups (22). Michala et al. measured labial width from the hymenal ring to the edge of the labium in traction, in patients aged 10-16, and reported a range of width between 20-55mm (average = 35.6mm) (11).

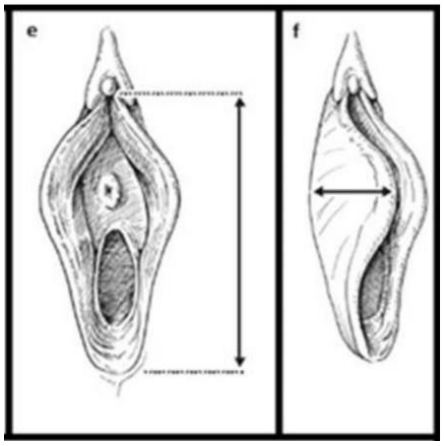

Figure 5 : Diagram adapted from Brodie et al.'s 2016 article (22), with diagrams indicating measurements taken in mm : e) length of inner labia, measured from the frenulum to the posterior fourchette; f) width of inner labia, measured from the inter-labial sulcus to the unstretched edges.

Asymmetry in labial size was noted in several studies. In eight of out eleven patients aged 9-16 undergoing labiaplasty, Qin et al. found asymmetrical inner labia before surgery (21). Similarly, Michala et al observed asymmetry in 9 out of 12 patients seeking assessment of their genitalia, with asymmetry ranging from 6mm to 35mm (11).

Histological features of inner labia in four patients aged 11-13 presenting with unilateral inner labia hypertrophy were presented in Boulos et al's 2018 article, revealing papillomatosis, sebaceous gland hyperplasia, dilated and increased lymphatic vessels, a fibrous stroma and features of lichen simplex chronicus (26).

Hymen

Four of the included articles analyzed hymenal configuration and descriptions. Figure 6 shows some of the variations in hymenal configuration, and their corresponding definitions. A cross sectional study conducted in 9 girls between the ages of 10-11 years old, reports hymenal configuration, texture, irregularities and vascularity (23). The most frequent hymenal configuration found was a crescentic type (6/9, 67%), with annular (1/9, 11%), fimbriate (1/9, 11%) and remnant (1/9, 11%) configurations also observed. Hymenal irregularities (such as bumps and notches) were not described, although one case of asymmetric hymen was noted.

Hymenal vascularity was mostly classified as either “lacy”, with delicate capillary beds, or increased if thicker capillaries were noted. Features of the posterior fourchette also demonstrated significant variability, including an increased vascularity, a ragged surface (if the epithelial surface seemed irregular), notches, midline sparing, and others were featureless (if the epithelium was intact and smooth) (23).

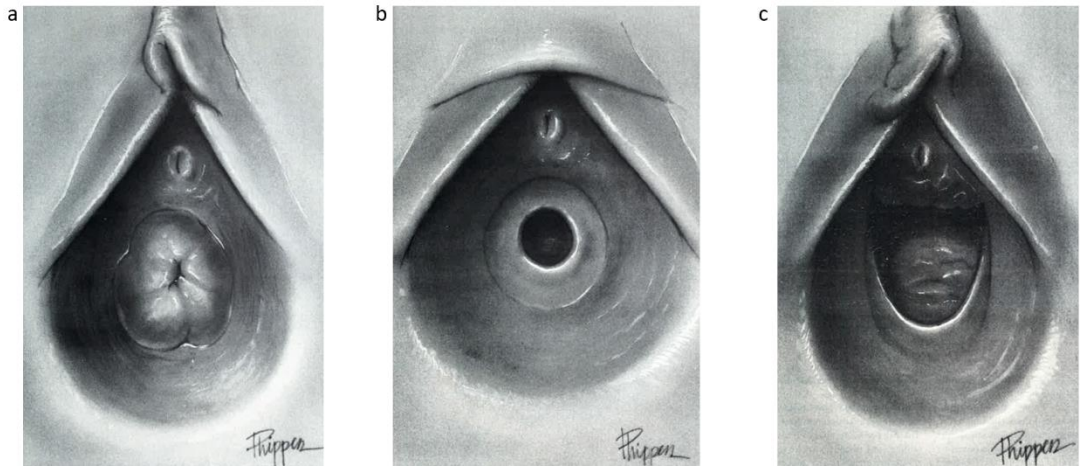

Figure 6 : Diagram adapted from Pokorny et al.’s article (29), showing different hymenal configurations: a) fimbriate hymen, with an abundance of tissue surrounding the vaginal introitus; b) circumferential (or annular) hymen, with a smooth and annular opening; c) crescentic (or posterior rim) hymen, with an abundance of tissue anteriorly.

A longitudinal study analysing hymenal development from ages 3 to 9 showed similar results (18). Hymenal crescentic configuration was by far the most common at 9 years of age (54/60, 90%), and the proportion of crescentic configuration seemed to increase with age, whilst the annular hymen became less common with age ($p < 0.01$). Most subjects had at least one longitudinal intravaginal ridge (56/61, 92%), and this proportion was seen most frequently at the age of 9 ($p < 0.01$). The presence of at least one mound also tended to increase with age ($p < 0.01$). The number of notches did not vary significantly over the different examinations. An illustration of these hymenal characteristics can be seen in Figure 7. Mean horizontal and vertical trans-hymenal diameters also increased with age ($p < 0.01$). In patients with an annular hymen, the mean vertical diameter was 10.9 mm, and the average horizontal diameter was 6.1mm, in the supine position. Both measurements showed variability, with ranges from 4.25 to 17.25mm for vertical diameter and 1.75 to 12.25mm for horizontal diameter. Participants were divided into three ethnic groups (Black non-Hispanic, White non-Hispanic, and Hispanic). Mounds were more frequently observed in Black non-Hispanic participants, while notches were more common in Black non-Hispanic and Hispanic patients (18).

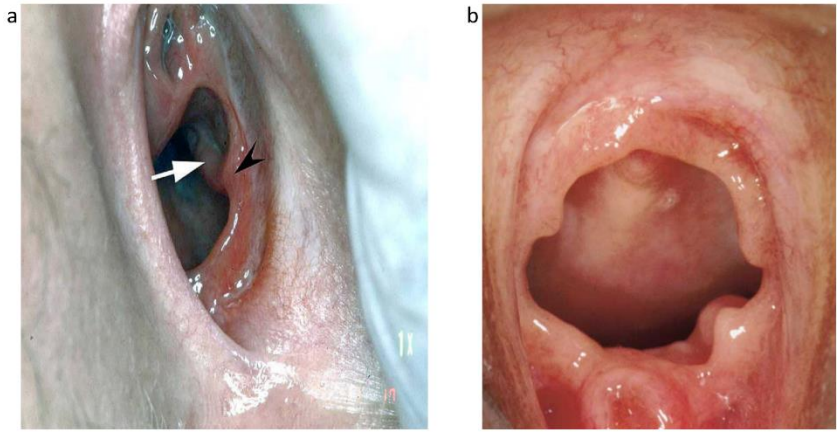

Figure 7 : Diagram illustrating hymenal features; a) oblique external view showing a longitudinal intravaginal ridge (white arrow) which reaches the hymen and forms a mound (black arrowhead) (30) ; b) multiple mounds and superficial notches (indentation in the edge of hymenal tissue) (18)

In contrast to Gardner et al's conclusion that fimbriated hymens were associated with infancy (23), a cross-sectional study of 29 participants aged 10-16 reported that the fimbriate hymen was the most common configuration (24). Specifically, 41% had a fimbriate hymen, whilst the majority of other subjects had annular or semilunar configurations (24).

In Myhre et al. cohort study, hymenal configuration and features were compared in subjects of 5-7 years old and 11-13 years old (20). During the follow up visits they found the hymen to thicken ($p = 0.000$) and an outward folding of the hymenal membrane to be more common. The presence of a fossa groove (see Figure 8), an indentation in the mucosal membrane of the fossa navicularis (see Figure 2), was only present at follow-up ($p = 0.02$). An increase in the horizontal hymenal diameter ($p = 0.011$) was noted, with an average of 5.13 mm for subjects between 11-13 years. When comparing prepubertal (Tanner stages B1/P1, B2/P1, or B1P2) and pubertal (Tanner stages B2/P2 or above) subjects, they found the crescentic configuration to be more common amongst prepubertal girls ($p = 0.063$) (20).

Figure 8 : Hymen with fossa groove (arrow), seen at follow-up examination (20)

Overall vulvar measurements

Brodie et al.'s study analyzed changes in overall vulvar dimensions through puberty. They measured the distance from clitoral hood to urethral orifice (Figure 9a), clitoral hood to lower border of pubic symphysis (Figure 9b), and clitoral hood to outer labia (Figure 9c). All

three measurements increased between the ages 9-12 and 13-16, with clitoral hood to urethral orifice distance showing the steepest growth, from an average of 24.8 mm to 39.4 mm (22).

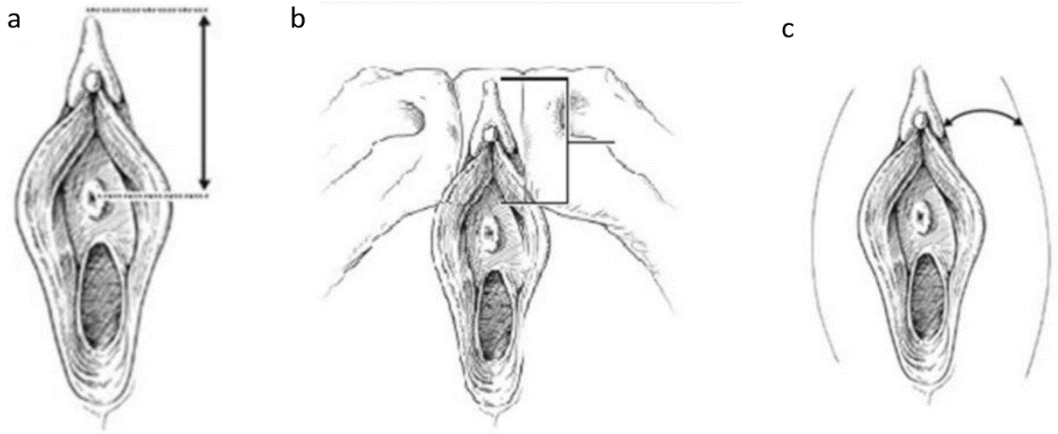

Figure 9 : Diagram adapted from Brodie et al.'s 2016 article (22), with diagrams indicating measurements taken : a) apex of clitoral hood to urethral orifice (mm) ; b) apex of clitoral hood to lower border of pubic symphysis (mm) ; c) clitoral hood to outer labia (mm)

Critical Appraisal of Methodology

Of the 10 studies included in this review, two were categorized as having low risk of bias, two as having moderate risk of bias and six as having high risk of bias. The details and raw scores of these studies are shown in Appendix 2.

Discussion

To our current knowledge, this is the first systematic review aimed at exploring detailed morphological vulvar changes during puberty. Despite limited information, we know that sexual hormones promote dermal deposition of adipose tissue in the outer labia and mons pubis, and can change pigmentation and rugosity, whilst increasing the size of other vulvar structures, such as the glans, inner labia and vestibule, (3, 31).

In this systematic review, we found that the glans of the clitoris and the length of the clitoral hood increase in size throughout puberty. Additionally, the clitoral hood became more retractile and developed more superficial rugae (22).

Studies regarding inner labia showed that inner labia length and width increased in size between the ages of 9-16 (22). The studies also highlight the wide range of measurements that exist, and some report asymmetrical inner labia widths (11, 21), although there was no attempt to quantify the prevalence of asymmetric labia within a pubertal population. Nonetheless, one cross-sectional study with 244 adult women showed that 54% of them had protruding inner labia (32), and although we know that asymmetrical inner labia are common, the exact prevalence of asymmetry remains unknown. This information could be helpful when counseling patients who present with aesthetic concerns regarding an asymmetry. Moreover, no studies were found on changes that occur in the outer labia, vestibule and mons pubis using our search criteria.

Changes in the hymenal configuration have also been described. In three out of four articles discussing hymenal morphology (18, 20, 23), the crescentic configuration was the most common amongst subjects in early puberty, and increased proportionally with age, probably due to effects of oestrogen leading to dynamic changes such as increased thickness, with an

outward folding of the hymenal membrane (20). In prepubertal girls, the hymen is thinner, dry and smooth-edged due to the suppression of the hypothalamic-pituitary-gonadal axis (33). During puberty, the surrounding epithelial tissue increases in thickness and often appears less erythematous than before estrogenization (34). The hymen itself can develop fimbriations, deep notches, tags, ridges and rugae, which are normal anatomic variants (31), as discussed in the included articles.

Despite these overall trends, one of the main findings of this systematic review is the high variability between subjects and the wide range of appearances and sizes of the vulva. This variability may be partially attributed to differences in study design and measuring standards, but also to the inherent diversity of female external genital organs. This is important in clinical practice for HCP and patients themselves. The vulva is often neglected during pre and post graduate medical training and most general practitioners, gynecologists and pediatricians are not familiar in differentiating physiological and pathological findings (35). In an Australian survey of 443 general practitioners, 97% of respondents had been asked by patients regarding genital "normality", and 65% had consulted with patients requesting referral for female genital cosmetic surgery. However, only 76% of GPs expressed confidence in assessing female genital anatomy (36). Misconceptions and misunderstandings can lead to unjustified procedures such as labiaplasty and hymenoplasty, a controversial surgical procedure aimed at reducing the hymenal opening after vaginal penetration (33, 37). Despite the lack of scientific evidence to supporting the indications, standardization and outcomes of these procedures, growing numbers of labiaplasty and hymenoplasty have been reported in industrialized countries (5, 6, 38).

Reliable resources are important for HCP in their daily practice, and to provide reliable evidence based information to their patients. In an analysis of 78 anatomical textbooks, Hayes et al did not find any references in textbooks published between 1848 and 2021 to vulvar diversity, asymmetry or measurements (39), despite available photographs, and publications which include normative measurements. In another study by Andrikopoulou et al's, only one out of 59 textbooks cited measurements for adult inner labia (range of 2-6 mm) which is largely inferior to those reported in recent studies (40).

Evidence-based acknowledgment and communication on vulvar diversity in terms of appearance and ethnic background may support and empower adolescents, who tend to compare their own body to unrealistic media images. Non-medicalized initiatives such as the "Labia Library" (41), the "Vulva Gallery" (42), or the "Vulvaversity" project (43), aim to show the diversity of female genital organs, and are easily accessible educational tools. These resources may also be useful in clinical practice to inform individuals seeking cosmetic genital surgery based on aesthetic dissatisfaction. It has been shown that a holistic sexual education, including conversations about genital anatomy and body image, can also improve protection against sexual abuse, and other unhealthy sexual activity such as unintended pregnancy and sexually transmitted infections (44, 45).

Limitations

Several limitations were identified while conducting this systematic review. Firstly, there was a lack of description regarding changes in the outer labia, vestibule, and mons pubis. Secondly, amongst studies analyzing specific vulvar structures, measurement methods varied due to differences in measuring positions and reference points used. For each vulvar structure, the articles also focused on slightly different outcomes; some focused only on hymenal

configuration whilst others included descriptions of hymenal features. Some authors used more qualitative approaches describing hymenal configurations or labial symmetry, and others more quantitative approaches with measurements of length and width, which limited the comparability of studies and limited our synthesis to a narrative approach. Thirdly, there was largely insufficient detail regarding potential variations in vulvar morphologies across different ethnicities.

Finally, the quality of included studies was mixed, with limited identification of confounding factors and a lack of detail regarding study subjects and setting often introducing a high risk of bias. The number of participants was also often quite limited, which reduced the reliable interpretation and comparison of results.

Conclusion

In conclusion, this systematic review highlights some of the morphological changes of the vulva during puberty, including an increase in clitoral diameter, length and width of the inner labia, and thickening of the hymenal membrane. There is a high variability in vulvar morphology and appearance between individuals. Currently, data is insufficient to categorize vulvar pubertal development into stages, as has been done for other secondary sexual characteristics. This reinforces the need for a new, high-quality longitudinal study analysing the changes of each structure and the correlation and chronology between them. This would provide patients with reliable information regarding how vulvas develop, and would help HCP to differentiate between physiological and pathological variants. Moreover, acknowledgement of the diversity of vulvas may be of importance for all individuals, in terms of empowerment and improvement of self-esteem.

References

1. J.M. T. Growth at Adolescence Springfield, Illinois, USA: Charles C. Thomas; 1955.
2. Tanner JM. Growth at Adolescence 2nd ed. ed. Oxford: Blackwell Scientific Publications; 1962.
3. Cohen Sacher B. The Normal Vulva, Vulvar Examination, and Evaluation Tools. Clin Obstet Gynecol. 2015;58(3):442-52.
4. Lloyd J, Crouch NS, Minto CL, Liao LM, Creighton SM. Female genital appearance: "normality" unfolds. BJOG. 2005;112(5):643-6.
5. Crouch NS, Deans R, Michala L, Liao LM, Creighton SM. Clinical characteristics of well women seeking labial reduction surgery: a prospective study. BJOG. 2011;118(12):1507-10.
6. Deans R, Liao LM, Crouch NS, Creighton SM. Why are women referred for female genital cosmetic surgery? Med J Aust. 2011;195(2):99.
7. Sharp G, Tiggemann M. Educating women about normal female genital appearance variation. Body Image. 2016;16:70-8.
8. Michala L. The Adolescent and Genital Dissatisfaction. Clin Obstet Gynecol. 2020;63(3):528-35.
9. Sharp G, Tiggemann M, Mattiske J. Predictors of Consideration of Labiaplasty. Psychology of Women Quarterly. 2014;39(2):182-93.
10. Howarth C, Hayes J, Simonis M, Temple-Smith M. 'Everything's neatly tucked away': young women's views on desirable vulval anatomy. Cult Health Sex. 2016;18(12):1363-78.
11. Michala L, Koliantzaki S, Antsaklis A. Protruding labia minora: abnormal or just uncool? J Psychosom Obstet Gynaecol. 2011;32(3):154-6.
12. Muka T, Glisic M, Milic J, Verhoog S, Bohlius J, Bramer W, et al. A 24-step guide on how to design, conduct, and successfully publish a systematic review and meta-analysis in medical research. Eur J Epidemiol. 2020;35(1):49-60.
13. Schardt C, Adams MB, Owens T, Keitz S, Fontelo P. Utilization of the PICO framework to improve searching PubMed for clinical questions. BMC Med Inform Decis Mak. 2007;7:16.
14. Abreu AP, Kaiser UB. Pubertal development and regulation. Lancet Diabetes Endocrinol. 2016;4(3):254-64.
15. Bramer WM, Giustini D, de Jonge GB, Holland L, Bekhuis T. De-duplication of database search results for systematic reviews in EndNote. J Med Libr Assoc. 2016;104(3):240-3.
16. Institute JB. Critical appraisal tools Adelaide, Australia: Johanna Briggs Institute; 2020 [Available from: https://jbi.global/critical-appraisal-tools.
17. Xiang L, Yoon S, Low AHL, Leung YY, Fong W, Lau TC, et al. Approaches to improving symptom appraisal: a systematic literature review. BMJ Open. 2022;12(8):e064521.
18. Berenson AB, Grady JJ. A longitudinal study of hymenal development from 3 to 9 years of age. J Pediatr. 2002;140(5):600-7.
19. Biro FM, Lucky AW, Simbartl LA, Barton BA, Daniels SR, Striegel-Moore R, et al. Pubertal maturation in girls and the relationship to anthropometric changes: pathways through puberty. J Pediatr. 2003;142(6):643-6.
20. Myhre AK, Myklestad K, Adams JA. Changes in genital anatomy and microbiology in girls between age 6 and age 12 years: a longitudinal study. J Pediatr Adolesc Gynecol. 2010;23(2):77-85.
21. Qin F, Xia Z, Yang Y, Kang Y, Zhang M, Shan M, et al. Labiaplasty in Adolescents: Indications and Treatment Protocol. Aesthet Surg J. 2023;43(7):NP516-NP27.
22. Brodie KE, Grantham EC, Huguelet PS, Caldwell BT, Westfall NJ, Wilcox DT. Study of clitoral hood anatomy in the pediatric population. J Pediatr Urol. 2016;12(3):177 e1-5.

23. Gardner JJ. Descriptive study of genital variation in healthy, nonabused premenarchal girls. J Pediatr. 1992;120(2 Pt 1):251-7.
24. Onderoglu S, Aksit D, Onderoglu LS. The configuration of prepubertal hymen in Turkish population and its clinical aspect. Okajimas Folia Anat Jpn. 1993;70(1):35-9.
25. Sane K, Pescovitz OH. The clitoral index: a determination of clitoral size in normal girls and in girls with abnormal sexual development. J Pediatr. 1992;120(2 Pt 1):264-6.
26. Boulos S, Rubin AI, Yan AC, McMahon PJ, Perman MJ. Unilateral hypertrophy of the labia minora: A case series. Pediatr Dermatol. 2018;35(3):e198-e9.
27. 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.
28. Netter FH. Atlas of Human Anatomy. 5th edition ed. Philadelphia: Saunders/Elsevier; 2011.
29. Pokorny SF. Configuration of the prepubertal hymen. Am J Obstet Gynecol. 1987;157(4 Pt 1):950-6.
30. Altchek A, Wasserman B, Deligdisch L. Prepubertal distal longitudinal vaginal folds. J Pediatr Adolesc Gynecol. 2008;21(6):351-4.
31. Sugar NF, Graham EA. Common gynecologic problems in prepubertal girls. Pediatr Rev. 2006;27(6):213-23.
32. Lykkebo AW, Drue HC, Lam JUH, Guldberg R. The Size of Labia Minora and Perception of Genital Appearance: A Cross-Sectional Study. J Low Genit Tract Dis. 2017;21(3):198-203.
33. Moussaoui D, Abdulcadir J, Yaron M. Hymen and virginity: What every paediatrician should know. J Paediatr Child Health. 2022;58(3):382-7.
34. Mancuso AC, Ryan GL. Normal Vulvovaginal Health in Adolescents. J Pediatr Adolesc Gynecol. 2015;28(3):132-5.
35. Dubow SR, Giardino AP, Christian CW, Johnson CF. Do pediatric chief residents recognize details of prepubertal female genital anatomy: a national survey. Child Abuse Negl. 2005;29(2):195-205.
36. Simonis M, Manocha R, Ong JJ. Female genital cosmetic surgery: a cross-sectional survey exploring knowledge, attitude and practice of general practitioners. BMJ Open. 2016;6(9):e013010.
37. Ahmadi A. Recreating Virginity in Iran: Hymenoplasty as a Form of Resistance. Med Anthropol Q. 2016;30(2):222-37.
38. Moaddab A, McCullough LB, Chervenak FA, Stark L, Schulkin J, Dildy GA, et al. A survey of honor-related practices among US obstetricians and gynecologists. Int J Gynaecol Obstet. 2017;139(2):164-9.
39. Hayes JA, Temple-Smith MJ. New context, new content-Rethinking genital anatomy in textbooks. Anat Sci Educ. 2022;15(5):943-56.
40. Andrikopoulou M, Michala L, Creighton SM, Liao LM. The normal vulva in medical textbooks. J Obstet Gynaecol. 2013;33(7):648-50.
41. Labia Library Melbourne (Australia): Women's Health Victoria [Available from: https://www.labialibrary.org.au/.
42. The Vulva Gallery Amsterdam (Netherlands): The Vulva Gallery; [Available from: https://www.thevulvagallery.com/.
43. Vulvaversity Berlin (Germany): Vulvaversity; [Available from: https://www.vulvaversity.de/index.html.

44. Breuner CC, Mattson G, Committee On A, Committee On Psychosocial Aspects Of C, Family H. Sexuality Education for Children and Adolescents. Pediatrics. 2016;138(2).
45. Yaron Michal SC, Narring Francoise, Brockmann Céline, Merglen Arnaud. Adolescence and sexuality : a risky businness. How best to inform parents? Rev Med Suisse. 2018.

Appendix

Appendix 1 : search strategies

For PubMed : ("Vulva/anatomy and histology"[Mesh] OR "Vulva/growth and development"[Mesh] OR "Vulva/physiology"[Mesh] OR "Genitalia, Female/anatomy and histology"[Mesh:NoExp] OR "Genitalia, Female/growth and development"[Mesh:NoExp] OR "Genitalia, Female/physiology"[Mesh:NoExp] OR "Vulva"[Title/Abstract] OR "female external genital*"[Title/Abstract] OR "Mons pubis"[Title/Abstract] OR "Labia majora"[Title/Abstract] OR "Labia minora"[Title/Abstract] OR "Clitoris"[Title/Abstract] OR "Vulval vestibule"[Title/Abstract] OR "Vaginal introitus"[Title/Abstract] OR "Urinary meatus"[Title/Abstract] OR "bartholin gland*"[Title/Abstract] OR "skene gland*"[Title/Abstract] OR "pudendum femininum"[Title/Abstract] OR "vestibule of the vagina"[Title/Abstract] OR "vulvar vestibule"[Title/Abstract] OR "vaginal orifice"[Title/Abstract] OR "fourchette"[Title/Abstract] OR "hymen"[Title/Abstract]) AND ("Anatomy"[MeSH Terms:noexp] OR "Organ Size"[MeSH Terms:noexp] OR "Sexual Maturation"[MeSH Terms] OR "Sexual Development"[MeSH Terms:noexp] OR "histology"[MeSH Terms:noexp] OR "anatom*"[Title/Abstract] OR "Morphology"[Title/Abstract] OR "organ Size"[Title/Abstract] OR "measurement*"[Title/Abstract] OR "Morphometric"[Title/Abstract] OR "Developmental change*"[Title/Abstract] OR "growth and development"[Title/Abstract] OR "Size"[Title/Abstract] OR "Change*"[Title/Abstract] OR "appearance"[Title/Abstract] OR "dimension*"[Title/Abstract] OR "length"[Title/Abstract] OR "width"[Title/Abstract] OR "Distance"[Title/Abstract]) AND ("Puberty"[MeSH Terms] OR "Adolescent"[MeSH Terms] OR "pubert*"[Title/Abstract] OR "adolescen*"[Title/Abstract] OR "teen*"[Title/Abstract] OR "preteen*"[Title/Abstract] OR "pre teen*"[Title/Abstract] OR "girl*"[Title/Abstract] OR "prepubert*"[Title/Abstract] OR "youth*"[Title/Abstract] OR "girl"[Title/Abstract] OR "girls"[Title/Abstract]) NOT ("Animals"[MeSH Terms] NOT "Humans"[MeSH Terms]).
A language filter was applied for English, French, Italian and Spanish.

For Embase : ('vulva'/exp OR vulva:ab,ti,kw OR 'female external genital*':ab,ti,kw OR 'mons pubis':ab,ti,kw OR 'labia majora':ab,ti,kw OR 'labia minora':ab,ti,kw OR 'clitoris':ab,ti,kw OR 'vulval vestibule':ab,ti,kw OR 'vaginal introitus':ab,ti,kw OR 'urinary meatus':ab,ti,kw OR 'bartholin gland*':ab,ti,kw OR 'skene gland*':ab,ti,kw OR 'pudendum femininum':ab,ti,kw OR (('vestibule' NEAR/2 'vagina'):ab,ti,kw) OR 'vulva* vestibule':ab,ti,kw OR 'vaginal orifice':ab,ti,kw OR 'fourchette':ab,ti,kw OR 'hymen':ab,ti,kw) AND ('anatomy'/de OR 'organ size'/de OR 'histology'/de OR 'sexual maturation'/de OR 'anatom*':ab,ti,kw OR 'organ size':ab,ti,kw OR 'histology':ab,ti,kw OR 'morphology':ab,ti,kw OR 'sexual maturation':ab,ti,kw OR 'sexual development':ab,ti,kw OR 'measurement*':ab,ti,kw OR 'morpho*':ab,ti,kw OR 'developmental change*':ab,ti,kw OR 'growth':ab,ti,kw OR 'development':ab,ti,kw OR 'size':ab,ti,kw OR 'change':ab,ti,kw OR 'appearance':ab,ti,kw OR 'dimension':ab,ti,kw OR 'length':ab,ti,kw OR 'width':ab,ti,kw OR 'distance':ab,ti,kw) AND ('adolescence'/de OR 'puberty'/de OR 'pubert*':ab,ti,kw OR 'adolescen*':ab,ti,kw

OR 'teen*':ab,ti,kw OR 'preteen*':ab,ti,kw OR 'pre-teen*':ab,ti,kw OR 'prepubert*':ab,ti,kw OR 'youth*':ab,ti,kw OR 'girl*':ab,ti,kw OR "p$ediatric*":ab,ti,kw OR "p$ediatrician*":ab,ti,kw) NOT ([animals]/lim NOT [humans]/lim) AND ([english]/lim OR [french]/lim OR [italian]/lim OR [spanish]/lim)

For Web of Science : (TS=(vulva OR "female external genital*" OR "mons pubis" OR "labia majora" OR "labia minora" OR "clitoris" OR "vulval vestibule" OR "vaginal introitus" OR "urinary meatus" OR "bartholin gland*" OR "skene gland*" OR "pudendum femininum" OR "vestibule of the vagina" OR "vulvar vestibule":ab,ti,kw OR "vaginal orifice" OR "fourchette" OR "hymen") AND TS=("anatomy" OR "organ size" OR "histology" OR "anatom*" OR "morphology" OR "organ size" OR "sexual maturation" OR "sexual development" OR "size" OR "change*" OR "appearance" OR "dimension" OR "length" OR "width" OR "distance" OR "measurement*" OR "morphometric" OR "developmental change*" OR "growth and development") AND TS=("pubert*" OR "adolescen*" OR "teen*" OR "preteen*" OR "pre-teen*" OR "prepubert*" OR "youth*" OR "girl*" OR "pediatric*" OR "pediatrician*" OR "paediatric*" OR "paediatrician*"))
A language filter for English, French and Spanish was also added.

For Cochrane : ("vulva":ab,ti,kw OR "female external genital*":ab,ti,kw OR "mons pubis":ab,ti,kw OR "labia majora":ab,ti,kw OR "labia minora":ab,ti,kw OR "clitoris":ab,ti,kw OR "vulva* vestibule":ab,ti,kw OR "vaginal introitus":ab,ti,kw OR "urinary meatus":ab,ti,kw OR "bartholin gland*":ab,ti,kw OR "pudendum femininum":ab,ti,kw OR "vulvar vestibule":ab,ti,kw OR "vaginal orifice":ab,ti,kw OR "hymen":ab,ti,kw) AND ("anatom*":ab,ti,kw OR "organ size":ab,ti,kw OR "sexual maturation":ab,ti,kw OR "Sexual Development":ab,ti,kw) AND ("pubert*":ab,ti,kw OR "adolescen*":ab,ti,kw OR "teen*":ab,ti,kw OR "preteen*":ab,ti,kw OR "pre-teen*":ab,ti,kw OR "prepubert*":ab,ti,kw OR "girl*":ab,ti,kw OR "pediatric*":ab,ti,kw OR "paediatric*":ab,ti,kw OR "pediatrician*":ab,ti,kw OR "paediatrician*":ab,ti,kw)

Appendix 2 : questions used for Johanna Briggs Quality Assessment

Quality assessment conducted using Johanna Briggs Institute (JBI) Critical Appraisal Tools

Study	Q1	Q2	Q3	Q4	Q5	Q6	Q7	Q8	Q9	Q10	Q11	Raw score
Cohort												
Berenson et al	NA	NA	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No	Yes	88.9%
Biro et al	No	Yes	Yes	Yes	Unclear	Yes	Yes	Yes	Unclear	Unclear	Yes	63.6%
Myhre et al	NA	NA	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No	Yes	88.9%
Qin et al.	NA	NA	Yes	Unclear	No	Yes	Unclear	Yes	Unclear	Unclear	NA	37.5%
Case series												
Boulos et al	Unclear	Unclear	Yes	Unclear	Unclear	No	No	Unclear	No	NA		11.1%
Michala et al	Unclear	Yes	Yes	Yes	Unclear	No	No	Yes	No	NA		44.4%
Cross-sectional												
Brodie et al.	Yes	Unclear	Yes	Yes	Unclear	No	Yes	NA				57.1%
Gardner J	Unclear	No	Yes	Unclear	Yes	No	Yes	NA				42.9%
Onderoglu et al.	No	No	Unclear	No	No	No	Yes	NA				14.3%
Sane et al	Unclear	No	Yes	Yes	Unclear	No	Yes	NA				42.9%

Checklist for cross-sectional studies
Q1. Were the criteria for inclusion in the sample clearly defined?
Q2. Were the study subjects and the setting described in detail?
Q3. Was the exposure measured in a valid and reliable way?
Q4. Were objective, standard criteria used for measurement of the condition?
Q5. Were confounding factors identified?
Q6. Were strategies to deal with confounding factors stated?

Q7. Were the outcomes measured in a valid and reliable way?
Q8. Was appropriate statistical analysis used?

Checklist for cohort studies
Q1. Were the two groups similar and recruited from the same population?
Q2. Were the exposures measured similarly to assign people to both exposed and unexposed groups?
Q3. Was the exposure measured in a valid and reliable way?
Q4. Were confounding factors identified?
Q5. Were strategies to deal with confounding factors stated?
Q6. Were the groups/participants free of the outcome at the start of the study (or at the moment of exposure)?
Q7. Were the outcomes measured in a valid and reliable way?
Q8. Was the follow-up time reported and sufficient to be long enough for outcomes to occur?
Q9. Was follow-up complete and, if not, were the reasons to loss to follow-up described and explored?
Q10. Were strategies to address incomplete followup utilized?
Q11. Was appropriate statistical analysis used?

Checklist for case series
Q1. Was there clear criteria for inclusion in the case series?
Q2. Was the condiiton measured in a standard, reliable way for all participants included in the case series?
Q3. Were valid methods used for identification of the coniditon for all participants included in the case series?
Q4. Did the cas e series have consecutive inclusion of participants?
Q5. Did the case series have complete inclusion of participants?
Q6. Was there clear reporting of the demographics of the participants in the study?
Q7. Was there clear reporting of clinical information of the participants?
Q8. Were the outcomes or follow up results of cases clearly reported?
Q9. Was there clear reporting of the presenting site(s)/clinic(s) demographic information?
Q10. Was statistical analysis appropriate?