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Decoding primary amenorrhea: insights from four contrasting cases and a practical diagnostic algorithm

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Case Report

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Abstract

Background

Primary amenorrhea is a complex clinical manifestation with diverse genetic, anatomical, and endocrinologic etiologies. This case series is reported to illustrate the novelty of contrasting four distinct pathophysiological nodes—outflow tract obstruction, receptor resistance, and gonadal failure—within a single clinical framework, emphasizing how a systematic diagnostic approach can navigate overlapping phenotypes to identify specific underlying pathologies.

Case Presentation:

This series describes four adolescent patients presenting with a failure of menarche. Patient 1 (46,XX) demonstrated normal secondary sexual characteristics but Müllerian agenesis with ectopic ovaries and absent uterus. Patient 2 (46,XY) presented with a female phenotype, well-developed breasts, and sparse pubic hair, diagnosed as complete androgen insensitivity syndrome (CAIS), with an MRI showing intraabdominal gonads. Patient 3 exhibited cyclic pelvic pain and a functional uterus with an obstructed outflow tract due to vaginal atresia, and a hypoplastic uterus. Patient 4 displayed short stature and sexual infantilism with elevated FSH, diagnosed as Turner syndrome (45,X). The diagnostic evaluation was carried out through a combination of karyotyping, hormonal profiling (FSH, LH, estradiol), and pelvic imaging (ultrasound/MRI) to localize the defects. Interventions included non-surgical oophoropexy with vaginoplasty for patient 1, hormonal therapy and laparoscopic gonadectomy for patient 2, surgical reconstruction to relieve the obstruction in patient 3, and pubertal induction with hormone replacement therapy (HRT) for patient 4. Outcomes centered on restoring anatomical function, achieving pain relief, and initiating long-term metabolic and psychological support.

Conclusions

This case series puts into evidence that a structured diagnostic approach based on the assessment of secondary sexual characteristics and pelvic anatomy is essential for accurate localization of the defect. Timely identification allows for etiology-specific interventions—such as HRT for bone protection in Turner syndrome or surgical drainage for obstructive defects—while addressing the profound psychosexual impact and future fertility options, including uterus transplantation or surrogacy, for these patients.

Background

Primary amenorrhea is defined as the failure of menarche to occur by age 15 in the presence of normal growth and secondary sexual characteristics, or by age 13 if these characteristics are absent (1). Modern clinical thresholds have shifted to reflect secular trends toward an earlier age of menarche, emphasizing that evaluation should also begin if menstruation has not occurred within three years of

thelarche (1). Normal menstruation is a coordinated event requiring an intact hypothalamic-pituitary-ovarian (HPO) axis and a patent genital outflow tract; a disruption at any functional node in this pathway results in the absence of menses (2).

The clinical presentation of primary amenorrhea is highly variable and depends on the underlying etiology (3). In cases of outflow tract obstructions (e.g., imperforate hymen or transverse vaginal septum), patients typically present with cyclic abdominopelvic pain and a palpable midline mass due to the accumulation of menstrual blood, such as hematocolpos or hematometra (3). Conversely, patients with Müllerian agenesis or complete androgen insensitivity syndrome (CAIS) usually present with a painless absence of menses despite normal-appearing female external genitalia and breast development (3). Those with hypergonadotropic hypogonadism, such as in the Turner syndrome, often display sexual infantilism and characteristic somatic stigmata, including short stature, a webbed neck, and widely spaced nipples (3).

The evaluation of primary amenorrhea is fraught with potential pitfalls that can lead to delayed diagnosis or inappropriate management (4). A fundamental pitfall is excluding pregnancy based solely on a patient's reported sexual or menstrual history. Because ovulation precedes the first menstruation, pregnancy is possible before menarche and must always be the first condition ruled out with a high sensitivity hCG test (3). Pelvic ultrasound is a primary diagnostic tool, but its accuracy depends on the operator's expertise (3). A common pitfall is the failure to detect a prepubertal or rudimentary uterus, leading to a false-positive diagnosis of Müllerian agenesis (5).

A structured diagnostic approach is essential to avoid delays in management (4). Clinical evaluation begins with a comprehensive history that includes pubertal developmental milestones, eating behavior, exercise habits, substance abuse, and the symptoms and signs of endocrinological disorders such as thyroid disorders, hyperprolactinemia, and developmental and growth disturbances, and physical assessment, focusing on Tanner staging to determine prior estrogen (breast development) and androgen (sexual hair) exposure and response (3), height, body mass index, presence of stigmata of Turner syndrome the presence of pelvic or hypogastric masses, the stage of development of the external genitalia, the condition of the hymen and, whenever possible, vaginal patency, vaginal length, and the presence or absence of the cervix. First-line diagnostic studies include a pregnancy test, serum FSH, LH, prolactin, and TSH levels, and pelvic imaging — ultrasound or magnetic resonance imaging — to confirm the presence or absence of a uterus (4). Karyotyping is indicated if FSH is elevated or if Müllerian structures are absent to differentiate between conditions like Turner syndrome, Mayer-Rokitansky-Küster-Hauser (MRKH) syndrome, and CAIS (1).

The etiologies of primary amenorrhea are diverse, ranging from chromosomal abnormalities like Turner syndrome (the most common cause of primary ovarian insufficiency) to congenital anatomical anomalies such as MRKH or vaginal midline defects (3). Additionally, disorders of sex development (DSD) like CAIS present unique diagnostic challenges where a 46,XY genotype results in a female phenotype (3). This article presents four clinical cases representing these distinct pathophysiological

nodes, to provide a comprehensive framework for clinical evaluation and management, and a proposed algorithm for diagnosis approach and evaluation of patients with primary amenorrhea.

Case presentation

Case 1

A 23-year-old phenotypic female presented for evaluation of failure of menarche. The patient reported a history of thelarche at age 14 and pubarche at age 13.

Physical examination revealed a normal female habitus and external genitalia; however, a blind-ending vaginal pouch was noted, with the absence of a vaginal introitus. Laboratory evaluation demonstrated a 46,XX karyotype. Hormonal profiling showed a serum follicle-stimulating hormone (FSH) level of 10.17 mIU/mL (normal levels in follicular phase from 2.50 to 10.20 mIU/mL), luteinizing hormone (LH) of 13.45 mIU/mL (normal levels in follicular phase from 2.50 to 13.5 mIU/mL), 17 alpha-hydroxyprogesterone of 4.29 ng/mL, (normal levels from 0.2 to 27.0 ng/mL and androstenedione of 1.03 ng/mL (normal levels in from 0.40 to 3.4 ng/mL). Thyroid function tests were within normal limits, with a free T4 of 1.12 ng/dL and TSH of 1.79 mIU/mL.

Pelvic magnetic resonance imaging (MRI) confirmed the congenital absence of the uterus and identified ectopic ovaries located in the superior third of the inguinal canal. Additionally, the MRI revealed a right pelvic kidney, identifying the case as Type II MRKH syndrome (or MURCS association), which is characterized by the presence of extragenital malformations, most frequently renal anomalies like ectopia or unilateral renal agenesis. Management involved surgical neovaginoplasty to restore anatomical function and oophoropexy to reposition the ectopic ovaries within the pelvic cavity.

Case 2

A 22-year-old phenotypic female with a known history of hypothyroidism presented with primary amenorrhea. She reported thelarche at age 15 but emphasized a complete absence of pubarche. Clinical examination revealed Tanner stage 4 breast development but absent axillary and pubic hair, a hallmark of androgen receptor insensitivity. Speculoscopy confirmed a blind-ending vagina with the absence of a cervix (Fig. 1). Genetic analysis identified a 46,XY karyotype, shifting diagnosis toward a disorder of sex development (DSD).

Biochemical studies revealed a total testosterone level of 580 ng/dL (male range), an estradiol level of 47 pg/mL, FSH of 5.88 mIU/mL, and a significantly elevated LH of 42.6 mIU/mL. The elevated LH is characteristic of CAIS due to the lack of negative androgenic feedback at the hypothalamic-pituitary level. Thyroid function was slightly altered, with a TSH of 7.86 mIU/mL and Free T4 of 1.38 mIU/mL.

Based on the phenotypic female appearance and the 46,XY genotype, a diagnosis of complete androgen insensitivity syndrome (CAIS) was established. Pelvic MRI demonstrated the absence of a uterus and ovaries, identifying nodular structures consistent with primitive gonads located in the external iliac chain near the psoas muscles (Fig. 2)

Due to the inherent risk of germ cell malignancy (e.g., gonadoblastoma or dysgerminoma) associated with retained Y-chromosome-bearing gonads, the patient underwent bilateral laparoscopic gonadectomy, documenting intraoperatively two gonad-like masses adjacent to external iliac vessels, as well as absent uterus (Fig. 3).

Histopathological analysis confirmed the presence of immature testicular structures with no evidence of spermatogenesis or maturation, nor of malignancy. Long-term management was initiated with estrogen replacement therapy to maintain secondary sexual characteristics, prevent vasomotor symptoms, and mitigate the risk of early-onset osteoporosis.

Case 3

A 27-year-old female was referred to the gynecological endocrinology service for the evaluation of primary amenorrhea. The patient's medical history was significant for a midline defect. Physical examination revealed Tanner stage 3 breast development and Tanner stage 1 pubic hair, suggesting a dissociation between estrogenic action and adrenarche. Genital examination demonstrated significant findings: genital atrophy, fusion of the labia majora and minora, atresia of the middle third of the vagina, and stenosis of the vaginal canal. Cytogenetic analysis confirmed a normal 46,XX karyotype. Laboratory investigations revealed profoundly suppressed gonadotropin levels, with a luteinizing hormone (LH) of < 0.216 mIU/mL and a follicle-stimulating hormone (FSH) of < 0.66 mIU/mL. These low values are indicative of hypogonadotropic hypogonadism, which can be associated with central nervous system or midline developmental anomalies.

Initial management focused on tissue priming using oral ethinylestradiol and dienogest, supplemented with topical conjugated equine estrogens to address vaginal atrophy. Pelvic MRI identified cervicouterine atrophy/hypoplasia consistent with a Type I Müllerian anomaly, hypotrophic ovaries, and a large hematocolpos with a volume of 235 cc. The patient underwent surgical intervention to relieve the obstruction, involving the dilation of the vaginal stenosis, permeabilization of the hymenal membrane, and drainage of the hematocolpos. Following an uneventful postoperative recovery and three months of continuous hormone replacement therapy (HRT), the patient achieved menarche.

At a six-month follow-up, she reported regular menstrual periods, confirming a patent and functional outflow tract.

Case 4

A 21-year-old female with a history of arterial hypertension under pharmacological management, presented for the initiation of hormone replacement therapy (HRT). She received a diagnosis of Turner syndrome at age 16 following an evaluation for primary amenorrhea, supported by a 45,X karyotype. Clinical examination revealed classic somatic stigmata, including short stature, lymphedema, and pterygium colli (webbed neck). The patient exhibited sexual infantilism, characterized by a complete absence of pubertal development (Tanner stage 1 for both breast and pubic hair), reflecting a profound deficiency of endogenous estrogen. To induce secondary sexual characteristics and provide essential bone protection against early-onset osteoporosis, HRT was initiated. The therapeutic regimen consisted of estradiol hemihydrate for pubertal induction and 100 mg of micronized progesterone to ensure endometrial protection and establish a cyclic withdrawal bleeding pattern.

Discussion

Primary amenorrhea is a complex clinical manifestation with a wide range of genetic, anatomical, and endocrinologic etiologies. The four cases presented in this series represent the primary diagnostic categories encountered in clinical practice: Müllerian agenesis, androgen resistance, obstructive anatomical defects, and gonadal dysgenesis. These scenarios underscore the necessity of a systematic evaluation centered on the presence of secondary sexual characteristics and pelvic anatomy.

Mayer-Rokitansky-Küster-Hauser (MRKH) syndrome (patient 1), or Müllerian agenesis, is characterized by the congenital absence of the uterus and the upper two-thirds of the vagina in 46,XX females (6). It is the second most common cause of primary amenorrhea, affecting approximately 1 in 5,000 live female births (6). Patients have normal reproductive endocrine function and reach puberty with normal breast and pubic hair development because their ovaries are functional and independent of Müllerian duct development (7). Type II MRKH (or MURCS association) involves extragenital malformations, most frequently renal anomalies like unilateral renal agenesis (30–40%) and skeletal defects such as scoliosis or Klippel-Feil anomaly (7). Recent genetic research has identified recurrent copy number variations at the 17q12 locus and mutations in genes such as *GREB1L* and *LHX1* as significant contributors to the syndrome's pathogenesis (7). Particularly, in our case the patient did not have any skeletal defects but had a kidney in ectopic (pelvic) location.

Complete androgen insensitivity syndrome (CAIS) (patient 2) represents a unique diagnostic challenge where a 46,XY genotype results in a female phenotype (8). It is caused by loss-of-function mutations in the androgen receptor (AR) gene (8). Although testes develop and secrete testosterone and anti-Müllerian hormone (AMH), the body is completely resistant to androgens (9). AMH causes the regression of Müllerian structures (no uterus or fallopian tubes), while testosterone is peripherally aromatized to estrogen, leading to normal breast development. However, pubic and axillary hair is typically sparse or absent because its growth is androgen-dependent—a key clinical differentiator from MRKH (9). These patients usually have intraabdominal male gonads, that do not descend during development, which is concordant with our patient's case.

Midline defects and vaginal atresia (patient 3) result from a failure of the vaginal plate to canalize during embryonic development (10). Unlike MRKH, these patients often have a functional uterus, but because the outflow tract is obstructed, they typically present with cyclic lower abdominal pain and a palpable pelvic mass caused by hematocolpos or hematometra (10). This case was also associated with hypogonadotropic hypogonadism (suppressed LH and FSH), likely linked to the patient's underlying midline defect, which can affect the hypothalamic GnRH pulse generator (10).

Turner syndrome (patient 4) which is due to X monosomy, isochromosome or X ring or to various chromosomal mosaicisms in which one line is 45,X, is the most common cause of primary ovarian insufficiency and hypergonadotropic hypogonadism (11). The hallmark is accelerated oocyte atresia, leaving non-functional "streak gonads" that cannot produce estrogen (11).

This leads to sexual infantilism (Tanner 1) and primary amenorrhea; characteristic somatic features include short stature, a webbed neck (pterygium colli), and widely spaced nipples (11). Diagnosis of Turner syndrome usually occurs during the prepuberal period, and management focuses on hormonal replacement therapy, growth hormone, and a comprehensive investigation to study hearing and vision disorders, associated autoimmune endocrinopathies, cardiovascular abnormalities and kidney alterations, among others.

The diagnostic workup for primary amenorrhea must be systematic to avoid delays. Assessing Tanner staging is vital, because the presence of breasts indicates prior estrogen exposure, either endogenous or through peripheral conversion of androgens, while sexual hair reflects androgen action (3). Pelvic examination or transabdominal ultrasound is essential to confirm the presence of a uterus (3).

Regarding laboratory testing, all patients must have first-line tests that include a pregnancy test (to rule out ovulation and pregnancy prior to menarche), FSH, LH, estradiol, prolactin, and TSH, to ensure a complete evaluation of the hypothalamic-hypophysis-gonadal axis (4). High FSH levels (> 40 mIU/mL) point to gonadal failure, while low/normal FSH levels suggest hypothalamic or pituitary issues, and absent uterus with normal FSH allow to differentiate MRKH (46,XX, normal testosterone) from CAIS (46,XY, male-range testosterone) (12). Pelvic MRI is considered the gold standard for visualizing complex Müllerian anatomy and identifying rudimentary uterine remnants that may contain active endometrium (12). Figure 4 shows a proposed diagnostic algorithm for the evaluation of primary amenorrhea.

Management is directed at specific etiology and requires a multidisciplinary approach involving gynecologists, endocrinologists, and psychologists (3). Patients with hypoestrogenism (Turner syndrome or hypogonadotropic hypogonadism) require HRT to induce secondary sexual characteristics and support uterine growth (8). Early initiation is critical for bone protection to prevent osteoporosis (8). In Turner syndrome, HRT typically begins with low-dose transdermal estradiol, adding a progestogen once breakthrough bleeding occurs to prevent endometrial hyperplasia (11).

Anatomical obstructions, such as the one occurred in the third patient, require timely surgical correction to relieve pain and prevent complications like endometriosis due to menstrual reflux (8). In CAIS,

prophylactic gonadectomy is recommended due to the risk of germ cell tumors (gonadoblastomas); however, current guidelines favor delaying this until early adulthood to allow for natural, estrogen-driven pubertal development (8). Gonadectomy is also indicated when there is a mosaicism with the presence of the Y chromosome or part of the Y chromosome.

For MRKH and CAIS, non-surgical vaginal dilation (Frank's method) is the gold standard first-line therapy, achieving success rates of 90–96% (7). Surgical neovaginoplasty is reserved for cases where dilation fails (7). Comprehensive care must address the profound psychosexual impact of these diagnoses, and every clinician must take into consideration gender identity to determine the correct surgical or hormonal treatment. Future fertility options are expanding, with gestational surrogacy and emerging uterus transplantation providing new hope for patients with MRKH to achieve biological motherhood (13).

Conclusion

Primary amenorrhea is a multifaceted clinical entity that necessitates a structured and organized clinical approach, with the goal of evaluating differential diagnosis and determining an integral approach. The cases of MRKH, CAIS, vaginal atresia, and Turner syndrome presented here highlight the diversity of underlying pathologies, from chromosomal anomalies to anatomical obstructions. Early identification through a complete medical history, systematic physical assessment and focused imaging is crucial for initiating etiology-specific therapy, ensuring skeletal health, and providing essential psychosexual support throughout the patient's lifespan. A multidisciplinary approach is also necessary to address, not only clinical, but also the psychosocial impact of these diagnosis and gender dysphoria symptoms that may emerge across the patient's lifespan.

Declarations

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Availability of data and material: The data used in the present study is available upon request to the corresponding author.

Ethical Standards

Ethical compliance with the Helsinki Declaration, current legislation on research Res. 008430-1993 and Res. 2378-2008 (Colombia) and the International Committee of Medical Journal Editors (ICMJE) were

ensured under our Ethics and Research Institutional Committee (IRB) approval, prior signing of informed consent by the patient.

The protocol for this article was previously approved by the Ethics Committee of our institution (Subred Integrada de Servicios de Salud Norte).

Consent to Publish

All the patients subject of this report previously signed the written informed consent form in the presence of two witnesses, authorizing the anonymous use of all the information from their medical history and corresponding images in any publications with scientific or academic interest.

References

1. Munro MG, Balen AH, Cho S, Critchley HOD, Díaz I, Ferriani R, Henry L, Mocanu E, van der Spuy ZM, FIGO Committee on Menstrual Disorders and Related Health Impacts, FIGO Committee on Reproductive. Medicine, Endocrinology, and Infertility. The FIGO ovulatory disorders classification system. Int J Gynaecol Obstet. 2022;159(1):1–20. 10.1002/ijgo.14331. . Epub 2022 Aug 19.
2. Creatsas GK, Creatsas M. Pathophysiology and Clinical Assessment of Primary Amenorrhea. In: Genazzani AR, Brincat M, editors. Frontiers in Gynecological Endocrinology. ISGE Series. Cham: Springer; 2014. https://doi.org/10.1007/978-3-319-03494-2_1.
3. Gaspari L, Paris F. Primary Amenorrhea in Adolescents: Approach to Diagnosis and Management. Endocrines. 2023;4(3):536–47. https://doi.org/10.3390/endocrines40300.
4. Klein DA, Paradise SL, Reeder RM. Amenorrhea: A Systematic Approach to Diagnosis and Management. Am Fam Physician. 2019;100(1):39–48. PMID: 31259490.
5. Practice Committee of the American Society for Reproductive Medicine. Electronic address: asrm@asrm.org. Current evaluation of amenorrhea: a committee opinion. Fertil Steril. 2024;122(1):52–61. 10.1016/j.fertnstert.2024.02.001. . Epub 2024 Mar 6.
6. Pai A, Shakir M. Mayer-Rokitansky-Kuster-Hauser syndrome type II: A rare case. Indian J Hum Genet. 2013;19(1):113–5. 10.4103/0971-6866.112928. . PMID: 23901207; PMCID: PMC3722622.
7. Schiau C, Csutak C, Ciurea AI, Pintican R, Dulgheriu IT, Manole S. Mayer-Rokitansky-Kuster-Hauser Syndrome: From Radiological Diagnosis to Further Challenges-Review and Update. Diagnostics (Basel). 2026;16(1):138. 10.3390/diagnostics16010138. . PMID: 41515635; PMCID: PMC12785829.
8. Witchel SF. Disorders of sex development. Best Pract Res Clin Obstet Gynaecol. 2018;48:90–102. 10.1016/j.bpobgyn.2017.11.005. . Epub 2017 Nov 22. PMID: 29503125; PMCID: PMC5866176.
9. Gallardo-Durán KG, De la Fuente-Cortez BE, Villarreal-Benavides TC, García-Vielma C. Síndrome de insensibilidad completa a los andrógenos: diagnóstico y manejo multidisciplinario [Complete androgen insensitivity syndrome: diagnosis and multidisciplinary management]. Rev Med Inst Mex Seguro Soc. 2023;61(1):117–22.

10. Deligeoroglou E, Athanasopoulos N, Tsimaris P, Dimopoulos KD, Vrachnis N, Creatsas G. Evaluation and management of adolescent amenorrhea. Ann N Y Acad Sci. 2010;1205:23–32. 10.1111/j.1749-6632.2010.05669.x. PMID: 20840249.
11. Gravholt CH, Andersen NH, Christin-Maitre S, Davis SM, Duijnhouwer A, Gawlik A, Maciel-Guerra AT, Gutmark-Little I, Fleischer K, Hong D, Klein KO, Prakash SK, Shankar RK, Sandberg DE, Sas TCJ, Skakkebæk A, Stochholm K, van der Velden JA, International Turner Syndrome Consensus Group, Backeljauw PF. Clinical practice guidelines for the care of girls and women with Turner syndrome. Eur J Endocrinol. 2024;190(6):G53–151. 10.1093/ejendo/lvae050. . PMID: 38748847; PMCID: PMC11759048.
12. Seppä S, Kuiri-Hänninen T, Holopainen E, Voutilainen R. MANAGEMENT OF ENDOCRINE DISEASE: Diagnosis and management of primary amenorrhea and female delayed puberty. Eur J Endocrinol. 2021;184(6):R225–42. 10.1530/EJE-20-1487.
13. Testa G, McKenna GJ, Wall A, Bayer J, Gregg AR, Warren AM, Lee SHS, Martinez E, Gupta A, Gunby R, Johannesson L. Uterus Transplant in Women With Absolute Uterine-Factor Infertility. JAMA. 2024;332(10):817–24. PMID: 39145955; PMCID: PMC11327905.

Figures



Figure 1

Speculoscopy of the second patient with CAIS, showing the absence of cervix.

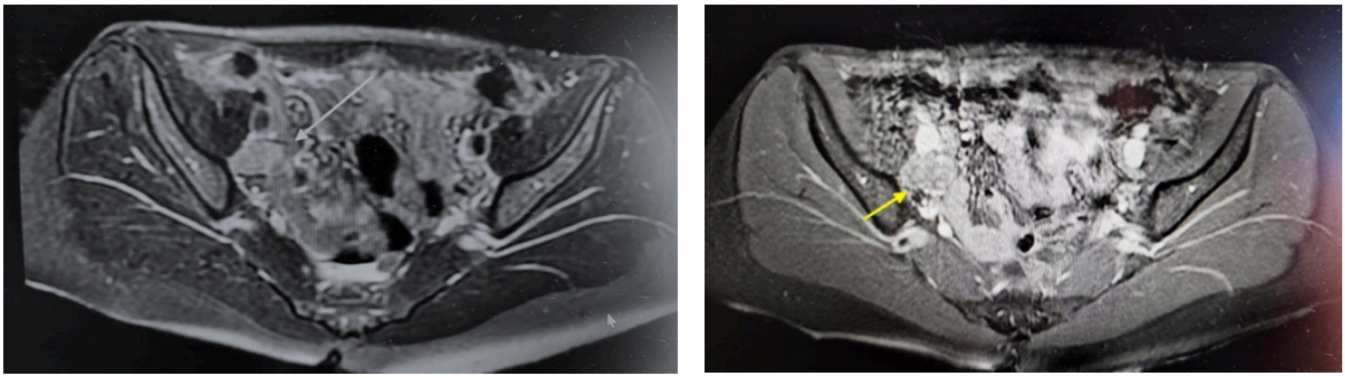


Figure 2

Pelvic MRI images showing bilateral primitive gonads located in the external iliac chain near psoas muscles (arrows).

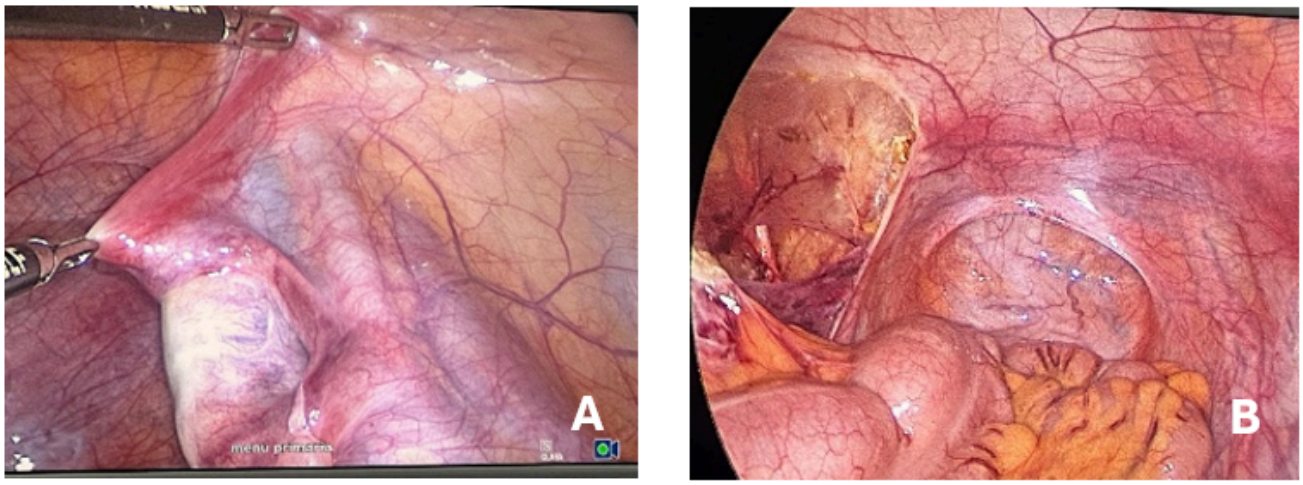
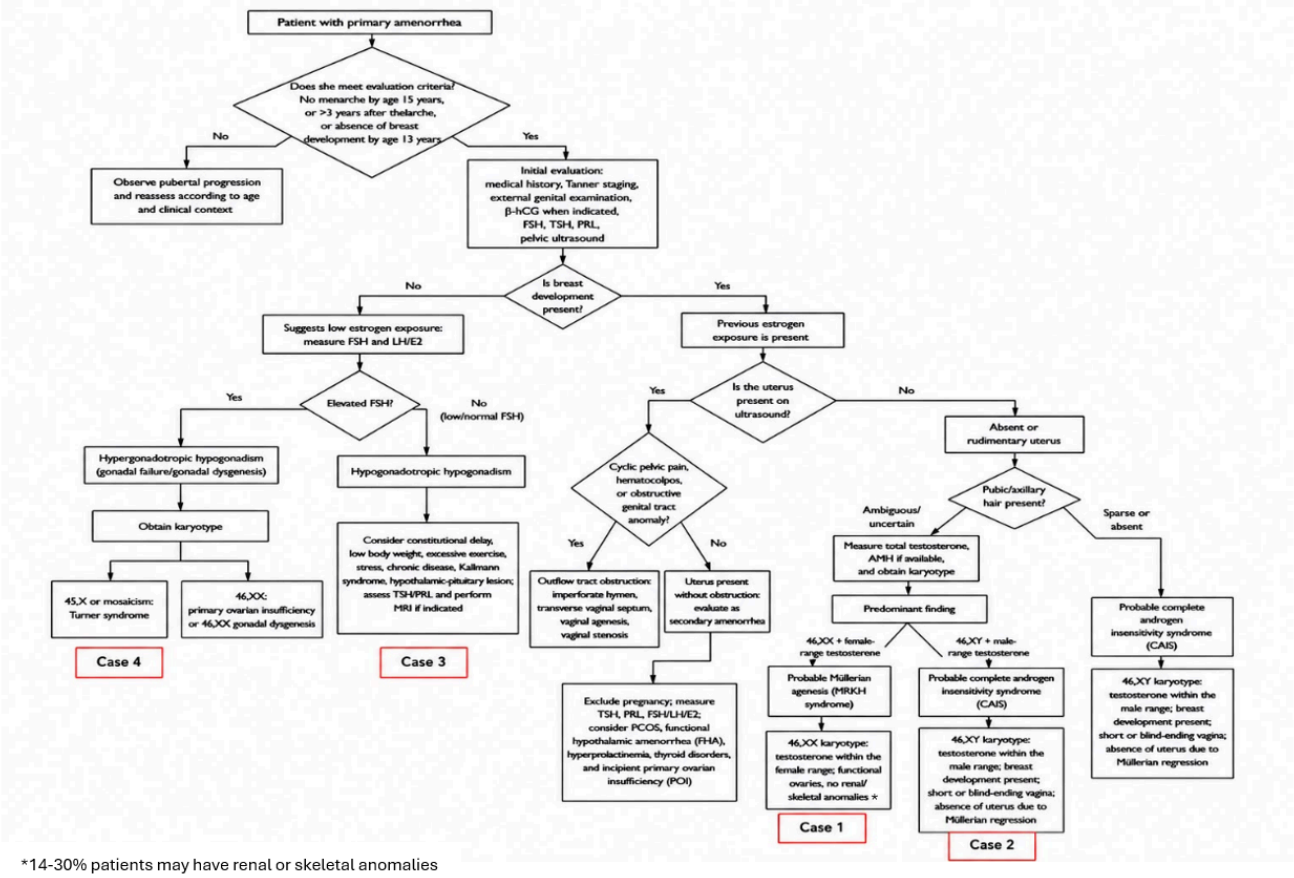


Figure 3

Laparoscopic intraoperative findings show (A) gonad-like structures near external iliac vessels and (B) absent uterus in the pelvic cavity.



*14-30% patients may have renal or skeletal anomalies

Figure 4

Comprehensive diagnostic algorithm for primary amenorrhea.