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Case Report

Congenital Imperforate Hymen with Hydrocolpos and Hydronephrosis associated with Severe Hydramnios and Increase of Maternal Ovarian Steroidogenic Enzymes

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Abstract. *Study Objective:* To study a clinical features of patient presented with severe hydramnios, associated with hydronephrosis, that was antenatally diagnosed and has been successfully treated immediately after birth. At a molecular level, we investigated the gene expression of key steroidogenic enzymes from the maternal ovary.

Design: Ultrasound scan, MRI, semi-quantitative RT-PCR

Setting: The patient was admitted to the University Hospital, University of Crete, Medical School, Greece, where all clinical data has been obtained. Gene expression studies took place at Biosciences, Brunel University, UK.

Results: Semi-quantitative RT-PCR analyses revealed that there is upregulation of key steroidogenic genes in the maternal ovary, including steroidogenic acute regulatory protein, and the cytochrome P450 heme-containing proteins CYP11A, CYP17 and CYP19. From a clinical perspective, the prenatal ultrasound scan and MRI findings showed a multicystic pelvic mass, bilateral hydronephrosis and prior to delivery severe polyhydramnios.

Conclusion: This clinical case is the only one that we have found in the current literature where congenital imperforate hymen accompanied with hematocolpos is associated with renal obstruction in combination with polyhydramnios and increase in maternal steroidogenic enzymes.

Key Words. Ovarian steroidogenic enzymes—Hydrocolpos—Polyhydramnios

Introduction

Congenital imperforate hymen is the most frequent congenital malformation of the female genital track, where mucus and blood from endometrial sloughing accumulate in the vagina.[1] When presented in adults, there is a distension of the vaginal canal that can lead to cervical dilation and formation of a hematometra and hematosalpinx. Symptoms in adults include cyclic and abdominal pain, amenorrhea, and difficulty with urination.[2] Retrograde menstruation, i.e., backward menstruation into the peritoneal cavity, can lead to the onset of endometriosis.

Endometriosis is a clinical and pathological entity that is characterized by the presence of tissue that resembles functioning endometrial glands and stroma outside the uterine cavity. Symptoms include infertility and chronic pelvic pain.[3] It has been hypothesized that vaginal secretions accumulate in response to circulating maternal estrogens.[4] Interestingly, recent clinical and laboratory studies support the concept that endometriosis is an estrogen-dependent condition. Indeed, high estradiol concentrations have been identified as a requisite for proliferation of endometriotic lesions, and inducement of a hypoestrogenic state is a current therapeutic approach.[5]

To the best of our knowledge, hydrocolpos (cystic dilatation of the vagina) as an isolated prenatal finding has only been reported twice previously.[6,7] Vaginal obstruction may be characterized by the sole presence of an imperforate hymen, a transverse vaginal septum, or by the presence of a urogenital sinus or cloacal malformation.[8] Imperforate hymen is the result of the failure of canalization of the vaginal plate,[1] which is formed in part of the Müllerian ducts and from the urogenital sinus.[8] A rare condition of congenital imperforate hymen has been presented in which obstruction of the vagina was associated with accumulation of uterovaginal secretions produced under circulating maternal estrogen stimulation, and the development of a hydrometrocolpos. Large hematocolpos may lead

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to secondary oligoamniosis, due to renal obstruction and deteriorating renal function. Interestingly, an adult case of hematocolpos was a consequence of radiotherapy for cervix carcinoma.[9] Moreover, in a recent study of a 15-year-old girl with imperforate hymen with hematocolpometra, the serum tumor marker CA125 was elevated.[10]

This particular clinical case is the only that we have found in the current literature in which congenital imperforate hymen accompanied with hydrocolpos is associated with renal obstruction in combination with polyhydramnios and increase in key steroidogenic components of the maternal ovary.

Methods

Ovarian Samples

The sample used in this clinical case was obtained during cesarean section surgery, due to the presence of a cyst. Therefore, apart from the cyst, an ovarian biopsy was also obtained from surface to clarify the nature of the cyst. The ovarian biopsy was taken with scissors and there was no significant bleeding. All control women were age matched, undergoing cesarean section, and there was a presence of ovarian cysts. For this study, maternal informed consent was obtained after the procedure was fully explained. All procedures followed were in accordance with the ethical standards of the responsible institutional committee on human experimentation. Ovarian samples (n = 4; 1 from the clinical case and 3 pooled controls) were placed in RNAlater® (Sigma Aldrich, UK) until further use.

RNA Isolation and cDNA Synthesis

Each sample was lysed in 600 μl of RNA lysis buffer using the TissueLyser (Qiagen, UK). Total RNA was extracted from these specimens using an RNA extraction kit (Sigma, UK), according to manufacturer's instructions. RNA concentration was determined by spectrophotometric analysis (NanoDrop, Thermo Scientific, UK). RNA (200ng) was reverse-transcribed into cDNA using 5 IU/μl RNase H reverse transcriptase (Invitrogen, UK).

Semiquantitative RT-PCR

PCR amplification was carried out using Taq polymerase (Invitrogen). The primers used for this study were: steroidogenic acute regulatory protein (StAR, 181 bp): 5'-CGTGACTTTGTGAGCG-3' and 5'-GCCACGTAAGTTTGGT-3'; CYP11A (202 bp): 5'-AGAGTTGAAATCCAACA CC-3' and 5'-TGGG ACAGACGACTGA-3'; CYP17 (205 bp): 5'-GTGA CCGTAA CCGTCT-3' and 5'- ATGAACTGATCC GGCT-3'; HSD3B2 (298 bp): 5'-CCATACCCGTA-CAGCA-3'and 5'-AT TGACCTCGGACACT-3'; CYP19 (242 bp) 5'-CAGAGGCCAAGAGTTTGA GG-3' and 5'-ACACTAGCAGGTGGGTTTGG-3'; β-actin (216 bp): 5'-AAGAGAGGCATCCTCACC CT-3' and 5'-TACATGGCTGG GGTGTTGAA-3'. After an initial denaturation step of 94°C for 4 min, 28 cycles were performed consisting of an initial denaturing step at 94°C for 30s, followed by extension at 60°C for 30 s and elongation at 72°C for 1 min. Densitometric analysis of resulted PCR products resolved on a 2% agarose gel were quantified using the AlphaEase FC software.

Results

Molecular Findings—Changes in the Gene Expression of Steroidogenic Components in the Maternal Ovary

As mentioned previously, it has been hypothesized that vaginal secretions accumulate in response to circulating maternal estrogens.[4] In this study we also sought to investigate this hypothesis by assessing the gene expression of key steroidogenic enzymes from the maternal ovary compared to age-matched pooled controls. Semi-quantitative RT-PCR revealed significant upregulation of the following genes when compared to an age-matched normal ovary: StAR by 1.55, CYP11A by 1.67, CYP17 by 7.1 and CYP19 by 5.2 fold (Fig 1). The most profound increase was that of CYP17 and CYP19 when compared to the normal ovary, whereas the levels of 3βHSD2 remained unaltered (data not shown). This is the first study that we have found to describe a fetus with hydrocolpos exhibiting changes in the expression of maternal steroidogenic enzymes at the ovarian level.

Clinical Findings—Identification of Cystic Mass and Hydronephrosis Using Ultrasound Scan and Surgical Procedures

An abdominal cystic mass with maximum diameter 43.5 × 29.4 mm was discovered on a prenatal sonogram in a female fetus at 29 weeks gestation in a 32-year-old woman para 1, gravida 3. Fetal biometry was within the normal range for gestational age. The patient's prenatal course, which included amnioparacentesis (because of patient's family history for trisomy 21 in a maternal aunt) had been uncomplicated and previous ultrasound scan examinations as well as 21 weeks ultrasound scan were reported normal. Cytogenetic analysis revealed a normal female karyotype (46 XX), and no chromosomal abnormalities were detected. There was no history of familial imperforate hymen. All serial prenatal ultrasound scans were performed at a specialist fetal medicine unit. On follow-up scans every 5−10 days, the appearance of the pelvic cystic mass showed a gradual enlargement (Fig 2, panels a, b) and mild bilateral

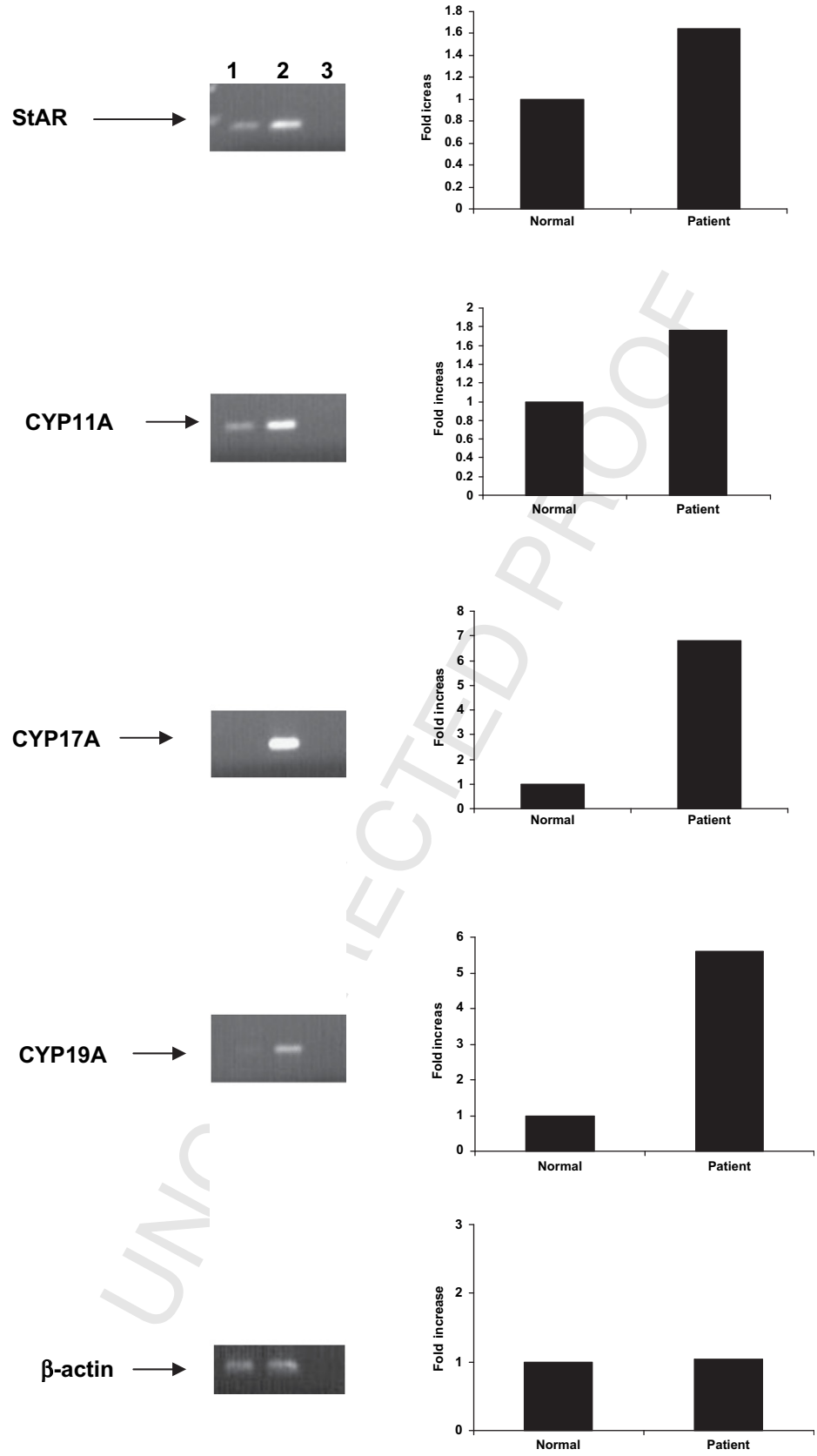

Fig. 1. Changes in the gene expression of steroidogenic components in the maternal ovary. Lane 1: cDNA from normal ovaries; Lane 2: cDNA from patient; Lane 3: negative control.

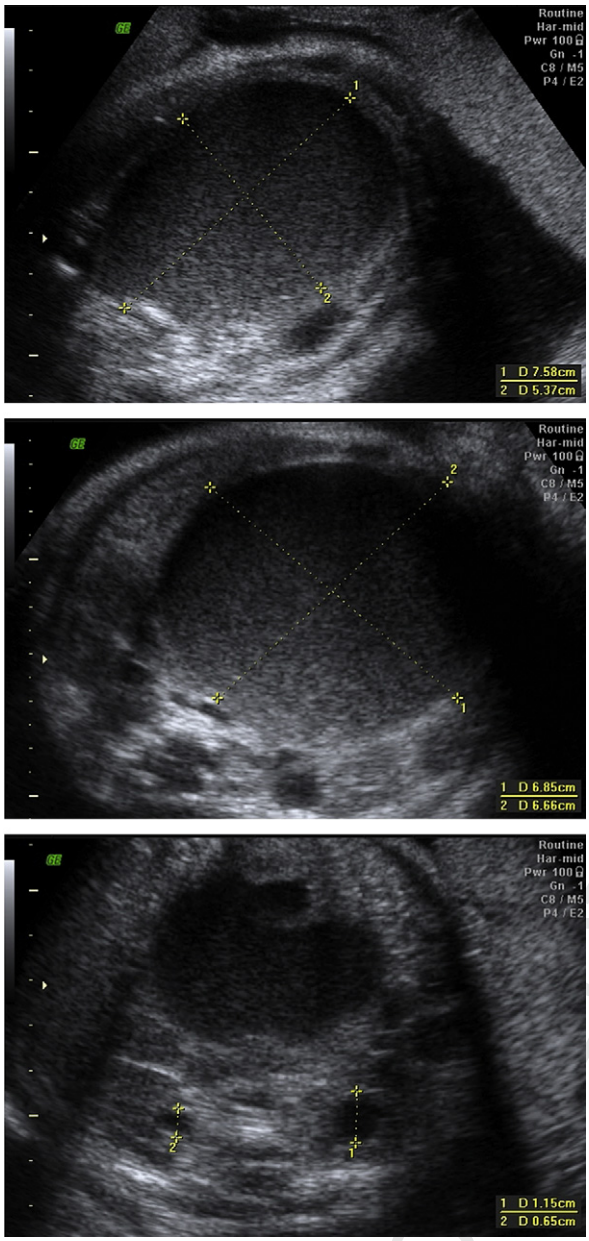

Fig. 2. Panel a: Longitudinal view of the large cystic abdominal mass with gravity dependent echoes, extending up to the diaphragm at 33 ± 5 weeks gestation. Panel b: Transverse view at 33 ± 5 weeks gestation reveals a large cystic abdominal mass. Panel c: Sagittal view of bilateral hydronephrosis, at 33 ± 5 weeks gestation

hydronephrosis (Fig 2, panel c), but there were no changes in amniotic fluid volume.

The patient was admitted to the hospital at 32 weeks because of premature uterine contractions and corticosteroids were administered to prepare for eventual preterm birth as well as tocolytic therapy intravenously (IV) to control contractions. At 35 weeks of gestation the size of cystic mass increased, measuring 86×75 mm maximum and hydronephorsis worsened.

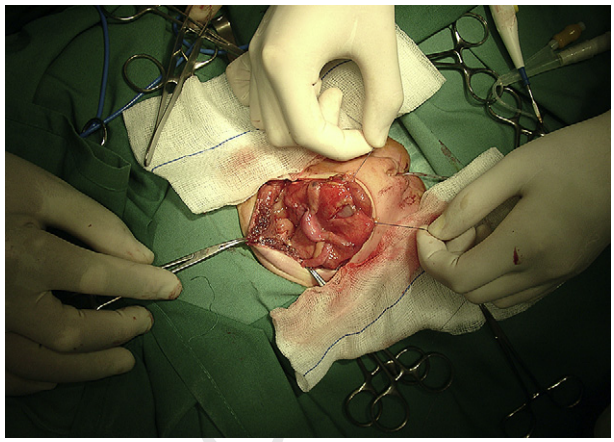

Fig. 3. A view of surgical hymenotomy performed by pediatric surgeons.

Ureteronephrosis was noticed while amniotic fluid volume significantly increased (amniotic fluid index (AFI) was 25−27 cm) followed by hydramnios development. The estimated fetal weight was 3150 g.

Due to premature contractions and a history of previous cesarean section, the patient underwent an emergency cesarean section and a female neonate weighting 3250 g, at 35 weeks of pregnancy, with Apgar scores of 8 and 9 after 1 and 5 minutes, respectively, was delivered. The neonate was admitted to the neonatal intensive care unit for ventilatory support and further investigations. Physical examination showed soft but distended mass above the umbilicus abdomen measuring 8×7 cm.

Bladder catheterization was performed with difficulty and clear urine was expressed. Renal function tests were normal at birth, with a creatinine of 0.7 μmol/L, and urea at 24 mmol/L. Abdominal ultrasound examination showed a cystic mass posterior to the bladder, bilateral hydronephrosis (1.3 cm) and hydroureter. MRI revealed an abdominal cystic mass with maximal diameter 90×70 mm, which was felt to be a hydrometrocolpos due to an imperforate hymen.

Additional investigation of the urinary system demonstrated normal kidneys, renal calyces, ureter, and a small bladder displaced to the anterior abdominal wall due to the mass effect of the hydrocolpos. Neither reflux nor ureteroceles were observed. The patient was referred to pediatric surgery. Consent was obtained from the baby's parents, and a hymenectomy was performed where approximately 200 mL of clear serous and mucoid fluid was drained (Figs 3 and 4).

Discussion

Congenital imperforate hymen is the most frequent obstructive anomaly of the female genital tract. This generally occurs sporadically, with a reported

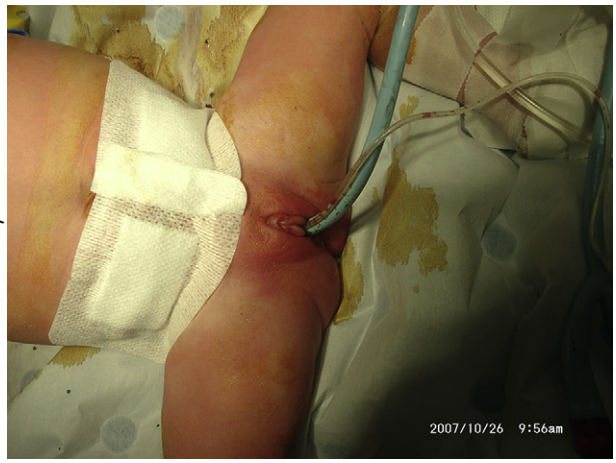

Fig. 4. View of tubing for drainage of retained secretions trough vagina.

incidence at term[11] of 0.014−1% and gives rise to hydrometrocolpos in less than 1/16000 female births.[12] Failure of this membrane to rupture results in congenital imperforate hymen, which in combination with accumulation of uterovaginal secretions, (produced as a consequence of intrauterine stimulation of cervical mucous glands by maternal estrogen hormones), may lead to the development of an internal hydrometrocolpos.[13] This can be presented as a pelvic mass in combination or not with urinary track obstruction and oligohydramnios.

In this clinical case the cystic mass presented for the first time during the 3rd trimester (29 weeks of gestation), having hypoechoic content with a maximum diameter of 43.5 × 29.4 mm. At 35 weeks the cystic mass had a maximum diameter of 86 × 75 mm causing bilateral urinary obstruction and hydronephrosis. What is interesting and unique in our case is the fact that although there was a lack of a normal urinary outlet, oligohydramnios was not found. On the contrary, an evident increase of the amniotic fluid volume (AFI, 25−27 cm) was observed. Cianciosi et al suggested the following mechanisms for accompanied hydramnios in abdominal cystic masses: (a) the compression of the adjacent bowel by the expanding cyst causing a hypoperistaltic intestine, or reduction of the absorptive capacity of the stomach and bowel by modifying the gastrointestinal vascularization, and (b) the depression or an incorrect swallowing process, as suggested by tongue protrusion, reducing the fluid removal from the amniotic cavity.[14]

The differential diagnosis of imperforate hymen is from labial adhesions, vaginal atresia, vaginal agenesis, and transverse vaginal septum. However, the abdominal mass has to be diagnosed differentially from distental urinary bladder, ovarian tumors and neoplasms, mesenteric cysts, anterior meningoceles, reduplication of sigmoid and sacral tumors. Prenatal ultrasonographic diagnosis of hydrocolpos has only been reported in three cases,[7,15,16] none of which had hydramnios detected.

As mentioned previously, it has been hypothesized that vaginal secretions accumulate in response to circulating maternal estrogens.[4] Therefore, based on our observations from the gene expression studies, it is attractive to speculate that, in this particular clinical case of hydrocolpos, the overall steroidogenic activity in the maternal ovary would have been enhanced. Indeed, when compared to control patients, the largest increase in gene expression was that of CYP17 and CYP19. CYP17 is expressed in all classic steroidogenic organs and in the human ovary it is selectively

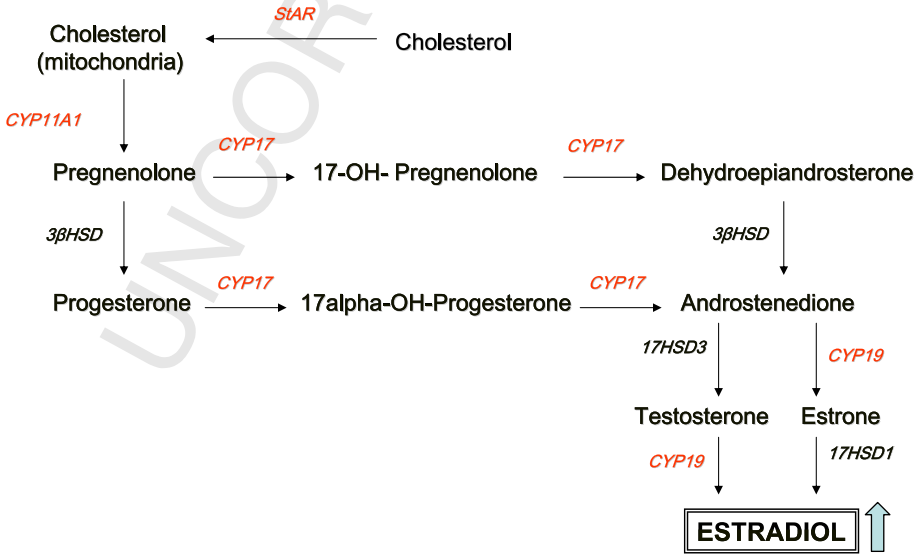

Fig. 5. Diagrammatic representation of steroid hormone biosynthesis steps in the human ovary. In red are the key components that have been upregulated in the maternal ovary of the fetus with hydrocolpos.

expressed in thecal cells.[17] CYP19, on the other hand, has a far wider distribution and in the human ovary is primarily expressed in the corpus luteum. This is of particular importance to this case because CYP19 catalyzes reactions leading to estrogen biosynthesis. Depending on its expression, CYP19 (P450arom) catalyzes the conversion of the C19 androgens, androstenedione and testosterone, to the C18 estrogens, estrone and estradiol, respectively.[17]

It is well known that steroids such as maternal estrogens as well as progesterone rise exponentially during the later stages in pregnancy. What is interesting in this clinical case is that the control ovarian samples were also taken from age-matched women, undergoing cesarean section. As a result, it is the possible dysregulation of the maternal steroidogenic pathway in this clinical case that might contribute to the upregulation of certain enzymes. In addition, with regard to a link between types of circulating estrogens and polyhydramnios, very little is known. However, a study by Phocas et al showed that there was a discrepancy in the estrogen levels regarding their increase. This was due to the type polyhydramnios, the pregnancy outcome, and the condition of fetus at birth.[18]

In conclusion, this is a novel clinical case where there is clear evidence of a maternal steroidogenic over-drive associated with fetal hydrocolpos, thus potentially linking maternal ovarian steroidal stimulation with this pathophysiological condition.

Uncited Figure

Fig 5

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