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CASE REPORT

Prenatal diagnosis of female pseudohermaphroditism associated with bilateral luteoma of pregnancy

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Female pseudohermaphroditism associated with luteoma of pregnancy (LP) is a rare condition characterized by varying degrees of masculinization of a female fetus. We describe a case, diagnosed at 13 weeks gestation. Transvaginal ultrasound at 5 weeks of gestation revealed a normal intrauterine gestational sac and an enlarged maternal right ovary. Re-examination at 13 weeks showed a fetus with male external genitalia. Cytogenetic investigation on amniotic fluid revealed a normal female karyotype 46,XX. Follow-up sonography confirmed the previous assignment of male external genitalia and a second amniocentesis was negative for the *SRY* gene. High levels of androgens were found in the maternal blood. A diagnosis of female pseudohermaphroditism associated with bilateral LP was made. A healthy girl was born by Caesarean section with complete masculinization of external genitalia (Prader V). Histology confirmed a bilateral LP. To the best of our knowledge this represents the first case of prenatal diagnosis of female pseudohermaphroditism associated with LP and demonstrates the feasibility of diagnosis by sonography from 13 weeks gestation. This is also the first case described of Prader V masculinization associated with LP.

Key words: Female pseudohermaphroditism/fetal gender/pregnancy luteoma/prenatal diagnosis/ultrasonography

Introduction

Luteoma of pregnancy was first described by Sternberg (Sternberg, 1963). This is a non-neoplastic hormone-dependent tumour-like lesion of the ovary that occurs during pregnancy and regresses after delivery (Joshi and Dunaif, 1995). Malinak and Miller first documented masculinization of a female infant associated with luteoma of pregnancy (Malinak and Miller, 1965).

To date, fewer than 200 cases of luteoma in pregnancy have been reported in the literature (Cronjè, 1984; Baxi *et al*., 1988; Manganiello *et al.*, 1995; Choi *et al*., 2000). In 25% of cases luteomas are hormonally active, with secretion of androgens, 10–50% of mothers with elevated androgen levels are masculinized (Verhoeven *et al*., 1973; Cohen *et al*., 1982; Cronjè, 1984) and 60–70% of female infants born to masculinized mothers are themselves masculinized to varying degrees (Verhoeven *et al.*, 1973; Garcia-Bunuel *et al*., 1975; Hensleigh and Woodruff, 1978; Cohen *et al.*, 1982; Nagamani *et al.*, 1982; Cronjè, 1984; Joshi and Dunaif, 1995; Manganiello *et al.*, 1995; Choi *et al.*, 2000).

All reported cases of female pseudohermaphroditism associated with luteoma of pregnancy have been identified postnatally. We present the first case in which a prenatal diagnosis of male phenotype was made at 13 weeks of gestation. This is also the first case described of Prader V masculinization associated with luteoma of pregnancy.

Case report

A 34 year old white primigravida woman was referred to our ultrasound diagnostic unit for abdominal pain at 5 weeks gestation. The first sonogram revealed a normal intrauterine gestational sac. It also demonstrated an enlarged maternal right ovary of 5 cm diameter containing small hypo-echoic and hyperechoic areas (Figure 1).

The patient had no important medical history and the current pregnancy had been uncomplicated so far. No medication was used except vitamins and iron. Her menarche occurred at 15 years of age. Menses were generally regular and her past medical history was uneventful, without any sign of elevated androgen production or infertility. An ultrasonographic evaluation performed three months before her last menstrual period (LMP) had revealed a normal uterus and adnexa.

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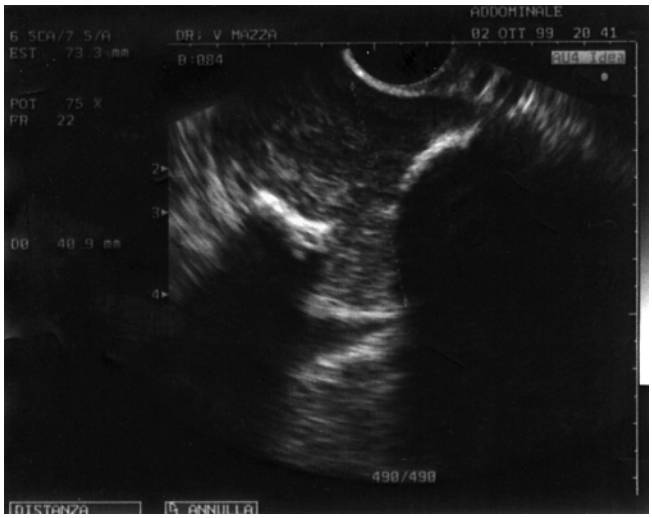

Figure 1. Sonography of the enlarged right maternal ovary at 5 weeks gestation (mean diameter 5 cm).

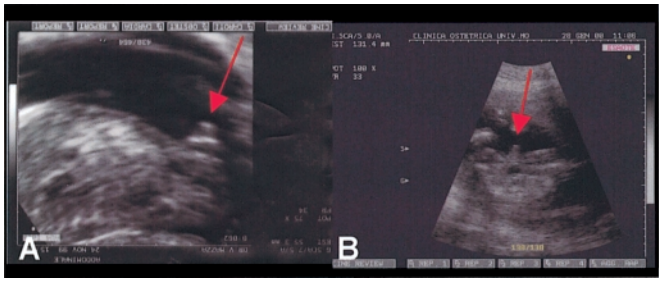

Figure 2. Sonography for the fetal gender assignment. (**A**) Sagittal section of external genitalia at 13 weeks gestation and 23 mm of BPD. The red arrow points to the bulge/penis—male gender assignment. (**B**) Coronal section of external genitalia at 22 weeks gestation. The red arrow points to the penis and the scrotum-like structures—male gender assignment.

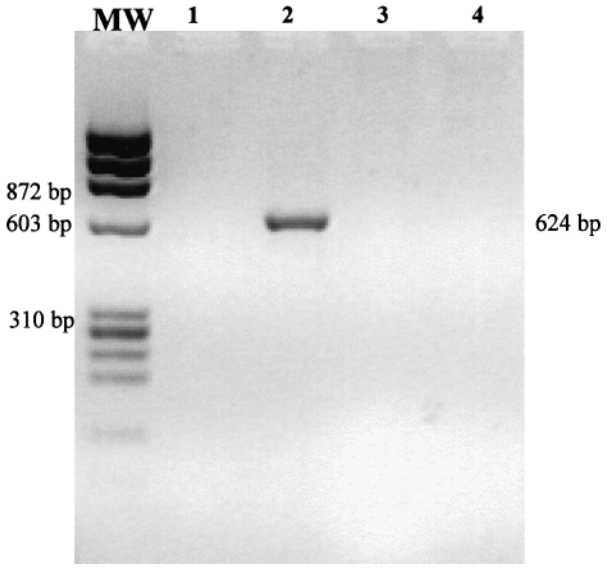

Figure 3. PCR analysis of the *SRY* gene with primers that amplify the entire coding region. Normal female (lane 1); normal male (lane 2); patient (lane 3); blank (lane 4).

Figure 4. Uterus and adnexa during caesarian section—both ovaries were enlarged (mean diameter 8 cm).

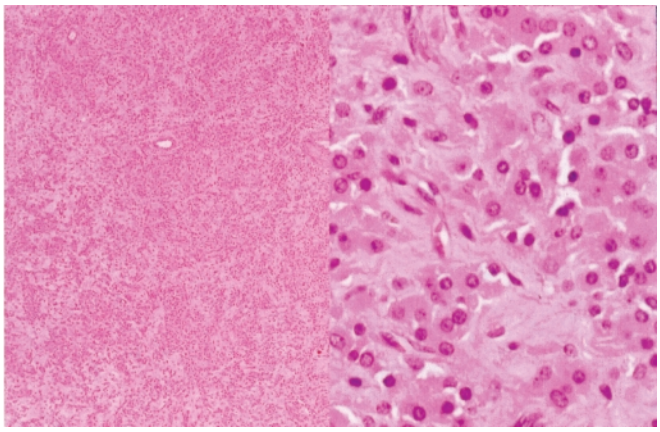

Figure 5. Histological findings of the ovary. **Left:** cells showed an abundant granular cytoplasm—only few mitotic figures were present. **Right:** round cells with abundant cytoplasm were arranged in sheets and lobules and separated by thin fibrovascular septa.

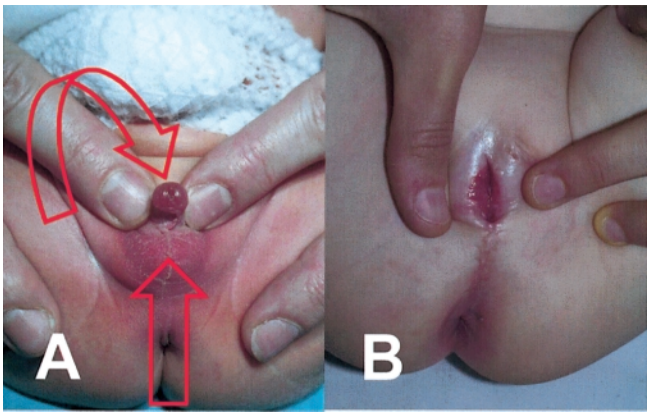

Figure 6. (**A**) External genitalia of the girl at birth with a complete masculinization (Prader V). The straight arrow indicates the completely fused labiascrotal-swellings, the curved arrow indicates the urethral meatus opening at the apex of the penis. (**B**) External genitalia after feminizing genitoplasty.

A follow-up sonogram was performed at 13 weeks gestation to evaluate the risk of trisomy 21. It revealed a nuchal translucency of 1 mm and a biparietal diameter (BPD) of 23 mm. The patient asked to know the fetal gender. Using a method validated by our group (Mazza *et al.*, 1999, 2001) a cranial notch was seen indicating male fetal gender (Figure 2A).

The patient came back at 20 weeks gestation with abdominal pain, and at the ultrasonographic evaluation both of the maternal ovaries were enlarged (6 cm diameter). Cytogenetic investigation on amniotic fluid revealed a normal female karyotype 46,XX in 20 cells of 10 independent colonies. The patient complained that an error had been made in the ultrasonographic fetal gender assignment, but a further sonogram confirmed the previous gender assignment, with a phallus and scrotum-like structure (Figure 2B).

SRY gene analysis was negative (Figure 3) after a second amniocentesis was performed to provide a rapid fetal sex determination and also to exclude a mistake in the fetal karyotype, maternal cell contamination (MCC) and XX male.

Androgen levels were measured in maternal blood and found to be high. Serum testosterone was 2000 ng/dl (normal value 50–300) and androstenedione 6500 ng/dl (normal value 100–250). A diagnosis of female pseudohermaphroditism with bilateral luteoma of pregnancy was made. The parents were made aware of the implications of the diagnosis and extensively counselled by their primary physician, obstetrician, paediatric surgeon, geneticist and endocrinologist. Both parents made a fully informed decision to continue the pregnancy.

During the last three months of pregnancy, the mother developed increased abdominal and lower extremity hair, deepening of the voice and an enlarged clitoris.

At 39 weeks gestation, the patient was admitted with ruptured membranes and fetal distress. She then delivered by caesarean section. Laparotomy revealed bilaterally enlarged maternal ovaries (Figure 4) about 8 cm in diameter, totally replaced by a single mass with a smooth surface. Bilateral resection was performed. On the cut surface, they appeared solid, fleshy and focally haemorrhagic. On microscopic examination, they were entirely composed of round to polygonal cells, with abundant, finely granular, eosinophilic cytoplasm and small nuclei. The cells were arranged in lobules or, occasionally, in pseudoacinar spaces and were separated by thin fibrous septa. No necrotic areas or typical Reinke's crystalloids were identified and, only occasionally, mitotic figures were found (Figure 5). The diagnosis of luteomas of pregnancy was confirmed.

The patient was observed closely using serial ultrasonography at 1, 2, 3, 4, 5 and 8 months post-partum. The ovarian masses slowly decreased in size over this interval of time. Clinically, hirsutism also slowly decreased and resolved by 5 months post-partum.

At birth, the healthy baby weighed 3250 g, but showed complete masculinization of the external genitalia with the external urethral meatus opening at the apex of the penis and the labioscrotal swellings totally fused to form a scrotum (Figure 6A).

The karyotype was 46,XX in blood lymphocytes. The testosterone level in the umbilical cord blood sample was 160 ng/dl (normal value $\leqslant$45) and androstenedione was 320 ng/dl (normal value 30–150).

Forty days after delivery, feminising genitoplasty was performed and completed at five months of age in the newborn (Figure 6B).

Discussion

We have previously shown, using high resolution ultrasound, that fetal gender assignment is possible at the biometrical threshold of 23 mm of BPD corresponding to the 50th percentile of 12 weeks of gestation (Mazza *et al.*, 1999). In pregnancies arising from IVF, absolute accuracy in fetal gender prediction was achieved at 69 days from fertilization corresponding to 11+6 weeks from the last menstrual period (Mazza *et al.*, 2001).

Under normal circumstances, chromosomal sex agrees with phenotypic sex. Occasionally, however, it does not. When the sonographic and cytogenetic findings show that sex is discordant, one should not assume that the cytogenetic sex is more accurate than the sonographic gender assignment.

MCC of amniotic fluid cell cultures is a potential source of error in prenatal diagnosis. The overall frequency of MCC is 0.24%; because MCC would generally not be recognized when the fetal sex is female, the true incidence of MCC is therefore 0.5%. Considerable variability in terms of observed frequency of MCC exists between laboratories. In the majority of cases in which MCC was not detected, only one culture and/or fewer than 20 cells had been examined (Hsu, 1998). In the current report the absence of the *SRY* gene at second amniocentesis excluded MCC.

Several genital malformations have been diagnosed by prenatal sonography (Mandell *et al.*, 1995). There are several well-recognized disorders that interfere with normal genital development, leading to discrepancies between the anatomy of the external genitalia and the underlying chromosomal constitution. Sex reversal can occur in humans. The incidence of 46,XX karyotype in phenotypic men is approximately one in 20 000–24 000 births (De la Chapelle, 1981). Most 46,XX male patients with normal genitalia are *SRY* positive; in contrast the great majority of the XX males with genital ambiguity are *SRY* negative (Ferguson-Smith *et al.*, 1990). Neverthless, exceptions do occur and Y-negative XX males may exhibit complete masculinization (Vergnaud *et al.*, 1986; Ramos *et al.*, 1996; Zenteno *et al.*, 1997). Recently, 46,XY monozygotic twins with discordant sex phenotype have been described (Somkuti *et al.*, 2000). Male and female development occurred despite the presence of a common karyotype and despite the presence of the intact *SRY* conservative motif.

Masculinization of external genitalia by androgens in a 46,XX fetus is another possible explanation for discordant phenotypic and cytogenetic sex. Female embryos have the same androgen receptor system in the urogenital tract as male embryos (George and Noble, 1984); therefore, administration of androgens at the appropriate time during embryogenesis causes profound masculinization of the female offspring (Schultz and Wilson, 1974). In humans, clinical findings are classified as Prader I–V (Prader, 1986) and for Prader V the external genitalia look completely

male. The internal genitalia are, however, not masculinized and Wolffian duct remnants are normal.

Although congenital adrenal hyperplasia is the most common cause of masculinization in a female fetus, masculinization as a consequence of a maternal hormone-producing tumour is becoming a more frequently recognized clinical entity (Novak *et al.*, 1970; Vehoeven *et al.*, 1973).

Luteoma of pregnancy must be presumed in pregnant women with ovarian mass and discrepancy between fetal karyotype and external genitalia. Luteoma of pregnancy represents a specific puzzling clinicopathological entity. Recognition of this entity is important so that unnecessary oophorectomy, with concomitant risk to both the patient and the fetus, is avoided. The sonographic features of luteoma of pregnancy have been described as that of a solid mass, with either single or multiple nodes and can be unilateral or bilateral (Choi *et al.*, 2000). Because of the solid nature of the mass, it is impossible to differentiate luteomas from other solid ovarian neoplasms, such as luteinized thecoma, granulosa cell tumour or Leydig cell tumour, based on imaging characteristics alone. Bilaterality and multinodularity are more common in luteomas than in these other tumours.

Only 65% of masculinized mothers, however, deliver masculinized female infants (Rice *et al.*, 1969; Joshi and Dunaif, 1995). Even when concentrations of free testosterone are increased in a masculinized mother, some protective mechanisms appear to prevent fetal masculinization. Placental aromatization of androgens and the protective buffering effect of the high concentrations of estrogens found in the fetal blood might be responsible for the protection of the fetus against masculinization (Hensleigh *et al.*, 1975).

Variables including the duration and timing of embryo-fetal androgen exposure, a deficit of protective factors and fetal organ sensitivity can also influence the degree of fetal masculinization. A case with a mutation in the *P-450* aromatase gene led to female pseudohermaphroditism that reached Prader V stage (Ludwig *et al.*, 1998). In the present case the early onset androgenic stimulation may have been responsible for the complete fetal masculinization.

In conclusion, to the best of our knowledge, all cases of female pseudohermaphroditism associated with luteoma of pregnancy reported so far have been Prader types I–III; the current case is the first with a total masculinization of external genitalia in a girl (Prader type V) and it is the first case in which prenatal diagnosis of the male phenotype was done at 13 weeks of gestation.

Families can benefit from prenatal identification of this pathology and several aspects, such as post-natal medical care, birth registration, gender assignment and timing of surgery, can be planned.

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