

Full Length Research Paper

Significance of pelvic ultrasonographic examinations in girls with pubertal precocity and premature thelarche before and after GnRH-analogues treatment

Lorenza Driul¹, Ambrogio P Londero^{1*}, Christine Papadakis¹, Daniela Driul², Monica Della Martina¹, Laura Peressini¹, Serena Bertozzi³, Alfred Tenore², Diego Marchesoni¹

¹Clinic of Obstetrics and Gynecology, University Hospital of Udine, Italy.

²Clinic of Pediatrics, University Hospital of Udine, Italy.

³Department of Surgery, University Hospital of Udine, Italy.

Accepted 13 July, 2011

The aim of this study was to evaluate the ovarian volume and the number of follicles before and after GnRH analogues therapy in girls with previous diagnosis of precocious puberty or thelarche. We collected data from all the girls afferent to the Clinic of Obstetrics and Gynecology and the Clinic of Pediatrics in the University Hospital of Udine (Italy) between 2005 and 2009. 85 girls with a diagnosis of precocious puberty or thelarche were analyzed. We treated 29 of these with GnRH analogues (34.12%), and they were monitored by pelvic ultrasonography before and at a median of 14 months (IQR 5-29) after the beginning of the therapy. Pelvic ultrasonography was performed by two operators. The girls with precocious puberty have a median age of 8 years (IQR 7-9). In the 29 cases treated with GnRH analogues, they presented, after the treatment, a trend to decrease the ovarian volume, but this is not statistically significant (Wilcoxon test: p n.s.). If we consider the observed follicles in the right and left ovaries during the first and the second ultrasonography, we can observe a decreased number of follicles in the follow up, but this is not statistically significant (Chi-square test p n.s.). In our study, we observed a trend in ovarian volume reduction and a decreased number of follicles after GnRH analogues therapy but without statistical significance.

Keywords: Precocious puberty, pelvic ultrasonography, ovarian volume, GnRH analogues therapy

INTRODUCTION

Ultrasonography (US) is the technique of choice for studying the female pelvis in children. Transabdominal US in the evaluation of female genital internal organs is a rapid, non invasive and accurate diagnostic procedure. Pediatric pelvic US is performed using bladder fluid as an ultrasonic window. With this method, we are able to

visualize ovaries in all ages of human development. The volume of ovaries is progressively increasing starting by six year-old girls (Ziereisen et al., 2005). In order to diagnose and monitor girls affected by precocious thelarche, pubarche and puberty it is important to evaluate with US the volume of ovaries, the number of follicles and their vascular pattern. Several investigators have documented increases in uterine and ovarian volume during childhood, with an increase in the number and size of follicles in the years leading up to puberty (Badouraki et al., 2008; Buzi et al., 1998; Griffin et al., 1995; Razzaghy-Azar et al., 2011).

Isosexual precocious puberty in girls may be defined as the premature development of secondary sexual characteristics associated with uterine and ovarian maturation. Approximately 95% of isosexual precocity is gonadotropin-releasing hormone (GnRH) -dependent and

*Corresponding author E-mail: ambrogio.londero@gmail.com;
Tel +39 0432 559635, fax number: +39 0432 559641

Abbreviations

GnRH; gonadotropin releasing hormone, US, Ultrasonography, GnRHa; GnRH analogues, LH; luteinizing hormone, FSH; follicle stimulating hormone, IQR; interquartile range.

is due to idiopathic activation of the hypothalamic-pituitary-ovarian axis without evident underlying anatomical causes (Klein, 1999).

It is important to define the limits of an active hypothalamic-pituitary-ovarian axis in order to determine if true puberty has begun. To inhibit pubertal progression and improve adult height, standard treatment of GnRH-dependent precocious puberty involves the suppression of the hypothalamic-pituitary-ovarian axis with gonadotropin-releasing hormone analogues (GnRHa), whereas GnRH-independent precocious puberty requires the solution of the underlying problem (Sklar et al., 1991).

The aim of this study was to evaluate the ovarian volume and the number of follicles in girls with proved precocious puberty and in girls with proved thelarche before and after GnRHa therapy.

MATERIALS AND METHODS

We analyzed retrospectively all the records collected in the Clinic of Obstetrics and Gynecology and Pediatrics (University of Udine School of Medicine) between 2005 and 2009. We found to have 63 girls with documented precocious puberty and 22 with documented thelarche; 29 of all these have done a therapy with GnRHa. Girls with pelvic pain, chronic disease, endocrine disorders, urological and gynecological malformation were excluded from this study. Pubertal development was classified according to Tanner puberty stages. The presence of thelarche (onset of breast development) was the criterion used to distinguish pubertal girls from prepubertal girls. The pubertal development in all girls was staged by a single examiner in Pediatric Endocrine Clinic at the University of Udine (Tanner, 1962).

All the girls were further submitted to basal hormonal assay, GnRH stimulation test and ovarian and uterine gray-scale ultrasound. On the basis of the GnRH stimulation test, the patients were subdivided into girls presenting serum luteinizing hormone (LH) and follicle stimulating hormone (FSH) prepubertal response or pubertal response. Girls with pubertal response were submitted to hypothalamic-pituitary-ovarian axis suppression with GnRHa and periodical clinical, hormonal and ultrasound evaluations.

The pelvic US was performed by two experienced operators and the equipment used was an Acuson Sequoia ultrasonic imaging device with an abdominal probe of 3.5-MHz (6-C2). The ultrasound scans were performed transabdominally when the participants had a full bladder, obtained by voluntary urine retention and oral administration of fluids. The parameters evaluated were the following:

- the ovarian volume was obtained with the reported equation ($\text{length} \times \text{width} \times \text{depth} \times 0.5233$);
- ovarian morphology, the number of follicles, and the volume before and after the treatment with GnRHa.

Several preparations of GnRHa are currently available. These include Leuprorelin, Triptorelin and Goserelin, which are each available as monthly and 3-monthly depot preparations. The rationale of therapy for the suppression of pubertal development is that in girls treated with GnRHa the pituitary gonadotropins are subject to prolonged rather than intermittent exposure to the releasing hormone (pulsatile release is essential for pubertal development).

We usually start treatment with a monthly depot preparation given in a dose of 3.75 mg by i.m. injection. A withdrawal bleed may occur in some girls following the first GnRHa injection. The GnRH stimulation test is repeated after a few months to determine whether LH and FSH levels are suppressed. Later we often maintain adequate gonadotropin suppression using a 3-monthly long-acting preparation, using a dose of 11.25 mg. Triptorelin (Decapeptyl SR) is the only 3-monthly injection licensed.

We performed a search in Medline and Scholar to find all literature about ovarian ultrasound evaluation during prepubertal development to identify the reference values of girls not affected by precocious puberty. Moreover, we compared those values with our data.

The local ethics committee approved the research project. Informed consent was obtained from every parent or guardian. All statistical analyses were performed with R (version 2.9.1). The records were compared using t-tests and Wilcoxon tests for continuous data and chi-square tests of significance for categorical data. All the variables were tested for normality with Kolmogorov-Smirnoff test. We considered significant p values <0.05 . Moreover, we performed a retrospective analyses of the literature about the variables that we have considered in our study. Particularly, we compared the volume and the correlation between volume and age of the girls in studies considering a normal population and in our data (a subset of girls affected by precocious puberty).

RESULTS

The girls diagnosed with precocious puberty considered in our study had a median age of 8 years (IQR 7-9). All these girls developed one or more secondary sexual characteristics before 8 years of age; they had a growth velocity compared with the same age controls greater than 2 years; and they had a bone age greater than 2 years if compared with chronological age. Besides, this was confirmed through the diagnosis with a GnRH-test.

Considering the 63 girls with precocious puberty and the 22 with thelarche just 29 of all were submitted to GnRHa therapy. In the group of 29 treated girls was performed a second follow up US. The median interval between the first and the second US was 14 months (IQR 5-29). The median volume of the right and the left ovaries

Table 1: Volume of ovary right and left in patients before and after Gn-RH therapy. In the table are represented median and interquartile range, it is used one-sided Wilcoxon test for significance.

	Before therapy	After therapy	P
Right ovary volume (cc)	1.11 (0.76-1.86)	0.94 (0.48-1.48)	0.600
Left ovary volume (cc)	1.16 (0.62-1.7)	1.07 (0.68-1.45)	0.630
Age stratification			
Until 6 years old (5 cases)			
Right ovary volume (cc)	0.89 (0.61-0.98)	0.61 (0.59-1.08)	0.410
Left ovary volume (cc)	1.11 (0.8-1.51)	0.74 (0.61-0.96)	0.130
More than 6 years old (24 cases)			
Right ovary volume (cc)	0.98 (0.42-1.71)	0.82 (0.4-1.46)	0.600
Left ovary volume (cc)	1.09 (0.55-1.59)	0.89 (0.48-1.21)	0.750
Follow up time stratification			
1 to 4 months follow up (5 cases)			
Right ovary volume (cc)	0.89 (0.42-0.93)	0.93 (0.42-1.21)	0.186
Left ovary volume (cc)	0.86 (0.48-1.24)	1.23 (1.11-1.27)	0.500
5 to 12 months follow up (8 cases)			
Right ovary volume (cc)	0.68 (0.36-1.76)	0.46 (0.36-1.09)	0.780
Left ovary volume (cc)	0.82 (0.56-1.04)	0.52 (0.49-1.09)	0.680
13 to 24 months follow up (5 cases)			
Right ovary volume (cc)	1.25 (0.88-1.35)	0.61 (0.59-2.03)	0.500
Left ovary volume (cc)	1.2 (0.79-1.87)	0.8 (0.72-2.15)	0.438
25 to 48 months follow up (11 cases)			
Right ovary volume (cc)	1.15 (0.68-1.75)	0.83 (0.62-1.44)	0.711
Left ovary volume (cc)	1.5 (0.61-1.61)	0.96 (0.51-1.17)	0.703

in these 29 cases after therapy with GnRHa had a trend to decrease but this was not statistically significant (right ovary $p=0.600$, and left ovary $p=0.630$ - one-sided Wilcoxon test) (Table 1) (Figure 1). The median volume of right and the left ovaries before and after therapy in girls until 6 years old (5 cases) and after 6 years old (24 cases) was decreased; however, these results were not statistically significant either (Table 1). In addition, we found the median volume of right and the left ovaries before and after therapy to decrease in every follow up time stratum except for the girls with a follow up of less than 4 months after therapy start (Table 1).

Considering the observed follicles in the right and left ovaries during the first and the second US, we had a decreased number of follicles in the follow up, but this was not statistically significant (Chi-square test $p =$

0.505).

In Table 2 we present our data compared to data published by Herter and colleagues, we found in our population a significant greater volume in the class of 7 years old girls ($p<0.05$), and generally a non-significant greater ovarian volume in our study than in data published by Herter and colleagues. In Figure 2 we reported all the studies we found to evaluate ovarian sonographic volume of normal girls in English literature and our data. We note that Razzaghy-Azar and colleagues found the greatest ovarian volumes in a normal population, and in most of the age classes the distribution was apparently non-parametric. For this second reason we decided not to compare this data directly to our population of girls affected by precocious puberty.

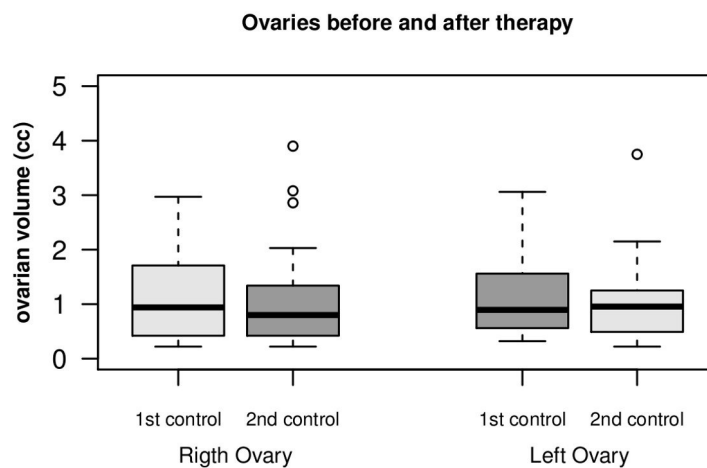


Figure 1: Right and left ovarian volume before and after therapy in all cases.

Table 2: The mean of ovary volume, DS and number of patients in different studies in literature and in our study

Age (y)	Herter 2002			Our data (*)			p(**)	Our data			p(**)
	mean	sd	num	mean	sd	num		mean	sd	num	
5	0.52	0.22	10	0.41	0.48	2	0.402	0.42	0.41	5	0.318
6	0.65	0.23	12	0.80	0.61	3	0.358	0.80	0.61	4	0.332
7	0.59	0.25	11	1.15	0.71	8	<0.05	1.11	0.67	18	<0.05
8	0.69	0.39	8	1.53	0.59	2	0.153	1.52	1.94	22	0.053
9	0.93	0.23	6	2.24	2.15	8	0.074	2.24	2.15	9	0.065
10	1.15	0.18	5	1.35	0.70	6	0.269	1.35	0.70	10	0.222
11	1.12	0.43	11	2.16	0.59	2	0.127	2.82	3.13	6	0.121

(*) This column refers to the 29 treated patients and the other column refers to the whole population.

(**) The p-value refers to one-sided t-test.

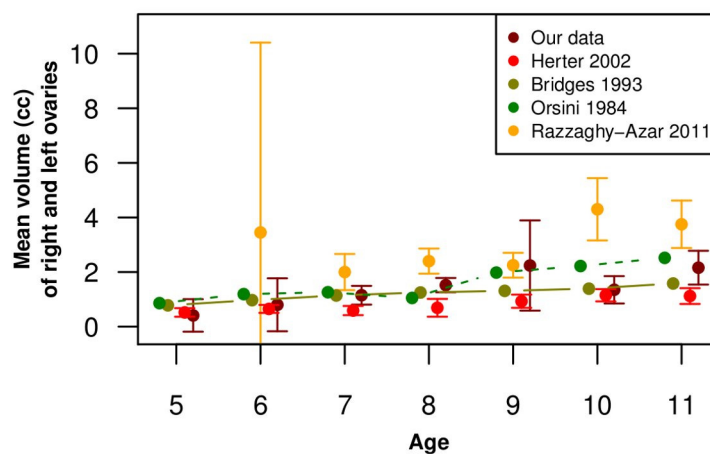


Figure 2: Right and left ovarian volume (mean value and 95% confidence interval) of our study and other studies from the literature. In case of Bridges 1993 and Orsini 1984 it was not possible to calculate confidence interval of the mean value.

DISCUSSION

In our study, we found the ovarian volume and the number of follicles in girls with proved precocious puberty or proved thelarche to have a non-significant decrease after GnRHa therapy. In addition, we presented a review of the studies that considered ultrasound ovarian volume in prepubertal girls.

Transabdominal pelvic US is routinely performed to evaluate uterus and ovaries in girls with premature pubertal status, but it cannot alone be used to diagnose pubertal anomalies (Andolf, 1999; Badouraki *et al.*, 2008). Several studies have assessed the growth of the uterus and ovaries during childhood and adolescence; however, they have shown wide variation in their results (Badouraki *et al.*, 2008; Herter *et al.*, 2002; Holm *et al.*, 1995; Orbak *et al.*, 1998; Razzaghy-Azar *et al.*, 2011) (Figure 2).

The wide variation in ultrasound measurements is due partly to biological variation and partly to accuracy of US. The ultrasound is user-dependent and in many cases it is not clear what to look for. The ovary is an almond structure, less echogenic than the bowel and around 1-3 cm, with or without echo-free spaces. The ultrasound is also patient-dependent and visualization is hampered by failure to fill the urinary bladder and obesity. The ultrasound appearance differs significantly between different pathologic conditions and normal girls of the same age. It is therefore not surprising that the ultrasound appearance of the internal genitalia in patients with pubertal anomalies varies. Moreover in non-pathologic girls uterine volume and body length presented the best correlation with age and stage of puberty (Razzaghy-Azar *et al.*, 2011), but ovarian volume was the best parameter for identifying patients with precocious puberty (Badouraki *et al.*, 2008).

Haber *et al.* (Haber *et al.*, 1995) reported cut-off points with a 100% sensitivity and specificity in discriminating patients with central precocious puberty from patients with premature thelarche. Later studies failed to establish adequate cut-off points owing to overlapping values in ultrasound parameters (Battaglia *et al.*, 2003; de Vries *et al.*, 2006). These variable results could be attributed to differences in the populations studied, in the methodology and statistical analysis being employed in the different studies.

Significant impairment of final height in untreated precocious puberty has dominated the rationale for intervening with GnRH treatment (Sigurjonsdottir and Hayles, 1968). It has been calculated that height loss is of the order of 20 and 12 cm in boys and girls respectively (Carel *et al.*, 2004). Few studies have evaluated psychosocial outcome following early or precocious puberty, but a long-term Swedish study reported more antisocial behavior in adolescence and lower academic achievement in adulthood (Johansson and Ritzén, 2005).

The correlation between endocrinological and ultrasound changes is very important for the diagnostic value.

One of the main questions that we can find in the literature about the usefulness of US in the evaluation of the internal female genital organs in the pediatric ages concerns the accuracy of this method and above all concerns the role of this method in monitoring the efficacy of the therapy. Our question is about the existence of an ovarian volume response in the patients treated with GnRHa therapy (this response was observed by US).

In previous literature, when we compare the cases with the controls in the same age group, we can find an increase in the ovarian volume in the girls with precocious puberty (Bridges *et al.*, 1995; Ciotti *et al.*, 1995). Moreover, in the literature we find an increased prevalence of ovaries with a cystic pattern in the cases of precocious puberty over the normal controls (Salardi *et al.*, 1988).

In our study, we have not controls matched for age groups to compare the ovarian volumes of the girls affected by precocious puberty. For this reason, we have analyzed the normal series of ovarian volumes published in the literature to compare those with our data (Ambrosino *et al.*, 1994; Bridges *et al.*, 1993; Herter *et al.*, 2002; Orsini *et al.*, 1984). If we compare our data with values collected by Hertner and colleagues in a normal population, we can observe a trend to have a greater ovarian volume in our study of pathological girls, but this is not statistically significant except for the category of 7 years old (t-test $p < 0.05$) (Table 2).

Analyzing our data we can find a trend in decreasing the ovarian volume between the first US and the follow up US done after the GnRHa therapy; however, this is not statistically significant (Wilcoxon test). One reason to have not reached significance could be the variability in time interval between the first and the second US. This time interval varies between 1 month to a maximum of 48 (median of 14 months, IQR 5-29). In the literature we can find a decreased ovarian volume after GnRHa therapy after 6 months of follow up (Ambrosino *et al.*, 1994).

We have performed also an analysis stratifying for age and one stratifying for follow up time. Taking in consideration every single age strata we observed a non-significant decrease in ovarian volume before and after therapy. Furthermore, we observed a non-significant decrease in ovarian volume in every follow up time strata except for girls with less than 4 months follow up. This could suggest that it is necessary to wait at least 4 months to observe a decrease of ovarian volume after therapy start, but more data are needed to give an answer.

In our study we find also a greater number of observations of follicles in the ovaries before the GnRHa treatment if compared with the ultrasound findings after the treatment, but this is not statistically significant (Chi-

square test $p = 0.505$).

We have not considered the ultrasound morphology of the ovaries; however, it is normally done in the other studies published in the literature. Because of this difference, our results are not easily comparable with other findings in the previous published literature.

The literature presents data that remark the importance of US as a non-invasive method with a good sensitivity to evaluate the precocious puberty as a first step and as a second step to monitor the changes in the patients treated with GnRHa (Ambrosino *et al.*, 1994). However, more evidence and a better standardization of this technique through multicentric studies is needed to prove the usefulness of this method.

In conclusion, in our study we observed a trend in ovarian volume reduction after GnRHa therapy, and we cannot exclude that with a great number of observed subjects and more standardization in time interval between first and second US it would be possible to find an ovarian volume regression with therapy (we mean a regression measured by US), to easily monitor its efficacy.

REFERENCES

- Ambrosino MM, Hernanz-Schulman M, Genieser NB, Sklar CA, Fefferman NR, David R (1994). Monitoring of girls undergoing medical therapy for isosexual precocious puberty. *J. Ultrasound Med.* 13:501–508.
- Andolf E (1999). Pelvic ultrasonography cannot alone be used to diagnose pubertal anomalies. *Acta Paediatr.* 88:246–247.
- Badouraki M, Christoforidis A, Economou I, Dimitriadis AS, Katzos G (2008). Evaluation of pelvic ultrasonography in the diagnosis and differentiation of various forms of sexual precocity in girls. *Ultrasound Obstet Gynecol.* 32:819–827.
- Battaglia C, Mancini F, Regnani G, Persico N, Iughetti L, Aloysio DD (2003). Pelvic ultrasound and color doppler findings in different isosexual precocities. *Ultrasound Obstet. Gynecol.* 22:277–283.
- Bridges NA, Cooke A, Healy MJ, Hindmarsh PC, Brook CG (1993). Standards for ovarian volume in childhood and puberty. *Fertil. Steril.* 60:456–460.
- Bridges NA, Cooke A, Healy MJ, Hindmarsh PC, Brook CG (1995). Ovaries in sexual precocity. *Clin. Endocrinol. (Oxf)* 42:135–140.
- Buzi F, Pilotta A, Dordoni D, Lombardi A, Zaglio S, Adlard P (1998). Pelvic ultrasonography in normal girls and in girls with pubertal precocity. *Acta. Paediatr.* 87:1138–1145.
- Carel JC, Lahlou N, Roger M, Chaussain JL (2004). Precocious puberty and statural growth. *Hum Reprod. Update.* 10:135–147.
- Ciotti G, Gabrielli O, Carloni I, Gangale AM, Bevilacqua M, Principi F, Garzetti GG, Giorgi PL (1995). (echographic and sonographic study of ovaries in girls with precocious puberty). *Minerva Pediatr.* 47:107–110.
- de Vries L, Horev G, Schwartz M, Phillip M (2006). Ultrasonographic and clinical parameters for early differentiation between precocious puberty and premature thelarche. *Eur. J. Endocrinol.* 154:891–898.
- Griffin IJ, Cole TJ, Duncan KA, Hollman AS, Donaldson MD (1995). Pelvic ultrasound findings in different forms of sexual precocity. *Acta. Paediatr.* 84:544–549.
- Haber HP, Wollmann HA, Ranke MB (1995). Pelvic ultrasonography: early differentiation between isolated premature thelarche and central precocious puberty. *Eur. J. Pediatr.* 154:182–186.
- Herter LD, Golendziner E, Flores JAM, Becker E, Spritzer PM (2002). Ovarian and uterine sonography in healthy girls between 1 and 13 years old: correlation of findings with age and pubertal status. *AJR Am. J. Roentgenol.* 178:1531–1536.
- Holm K, Laursen EM, Brocks V, Müller J (1995). Pubertal maturation of the internal genitalia: an ultrasound evaluation of 166 healthy girls. *Ultrasound Obstet Gynecol.* 6:175–181.
- Johansson T, Ritzén EM (2005). Very long-term follow-up of girls with early and late menarche. *Endocr. Dev.* 8:126–136.
- Klein KO (1999). Precocious puberty: who has it? who should be treated? *J. Clin. Endocrinol. Metab.* 84:411–414.
- Orbak Z, Sagsoz N, Alp H, Tan H, Yildirim H, Kaya D (1998). Pelvic ultrasound measurements in normal girls: relation to puberty and sex hormone concentration. *J. Pediatr. Endocrinol. Metab.* 11:525–530.
- Orsini LF, Salardi S, Pilu G, Bovicelli L, Cacciari E (1984). Pelvic organs in premenarcheal girls: real-time ultrasonography. *Radiol.* 153:113–116.
- Razzaghy-Azar M, Ghasemi F, Hallaji F, Ghasemi A, Ghasemi M (2011). Sonographic measurement of uterus and ovaries in premenarcheal healthy girls between 6 and 13 years old: correlation with age and pubertal status. *J. Clin. Ultrasound* 39:64–73.
- Salardi S, Orsini LF, Cacciari E, Partesotti S, Brondelli L, Cicognani A, Frejaville E, Pluchinotta V, Tonioli S, Bovicelli L (1988). Pelvic ultrasonography in girls with precocious puberty, congenital adrenal hyperplasia, obesity, or hirsutism. *J. Pediatr.* 112:880–887.
- Sigurjonsdottir TJ, Hayles AB (1968). Precocious puberty. a report of 96 cases. *Am. J. Dis. Child.* 115:309–321.
- Sklar CA, Rothenberg S, Blumberg D, Oberfield SE, Levine LS, David R (1991). Suppression of the pituitary-gonadal axis in children with central precocious puberty: effects on growth, growth hormone, insulin-like growth factor-i, and prolactin secretion. *J. Clin. Endocrinol. Metab.* 73:734–738.
- Tanner J(1962). Growth at adolescence. Blackwell and Mott. Oxford.
- Ziereisen F, Guissard G, Damry N, Avni EF (2005). Sonographic imaging of the paediatric female pelvis. *Eur. Radiol.* 15:1296–1309.