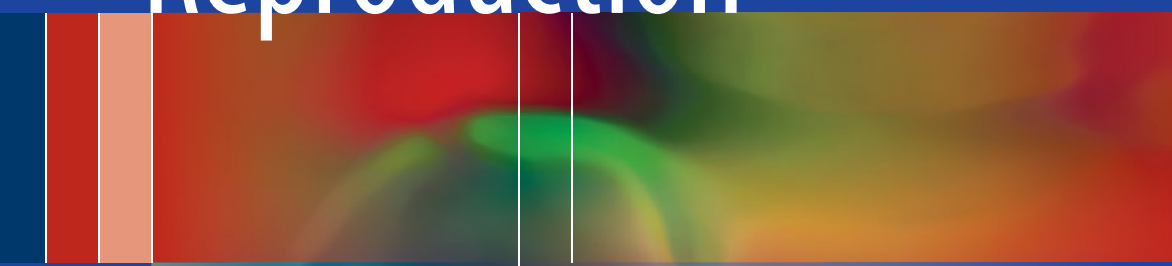
Managing Ultrasonography in Human Reproduction



A Practical Handbook

Stefano Guerriero
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Preface

This book was motivated by the desire to offer a critical appraisal on the use of ultrasound in daily reproductive medicine practice. Ultrasonography has developed remarkably and has become an indispensable tool in reproductive medicine. Several new techniques were developed in the recent years: they are useful for the diagnosis of important fertility-related conditions, to predict the success rate and the risk of complications, for planning and monitoring the treatment, for guiding the invasive procedures, and also to evaluate the initial pregnancy.

Keeping up to date and identifying what is really useful among all the available information can be really challenging. Although there are a few books in the area, we felt the need of one focused on practical aspects and that would be useful during daily routine for those that use ultrasound in reproductive centers.

With this in mind, we have requested help of several top experts worldwide: they were asked to summarize how they use ultrasound in their centers and also to give valuable tips on how to achieve the best images and more reliable conclusions. The experience was amazing and we are delighted we met such excellent contributors; we are really grateful for their valuable time and effort directed to write these beautiful chapters. Thanks to them, we are sure the readers will enjoy this book.

Cagliari, Italy — Stefano Guerriero
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Electronic supplementary material is available in the online version of the related chapter on SpringerLink: http://link.springer.com/

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Part I

Ultrasound Evaluation of Women with Subfertility

Assessment of the Functional Ovarian Reserve

1

Wellington P. Martins and Ligita Jokubkiene

1.1 Introduction

1.1.1 Functional Ovarian Reserve

The ovarian reserve is composed of primordial and resting follicles. The number of primordial follicles is determined during early fetal life and declines until menopause. The number of antral follicles reflects the size of primordial follicle pool [1]. Follicles destined to ovulate grow from primordial follicles through the accumulation of fluid in the antral cavity and the proliferation of granulosa and theca interna cells until it reaches the diameter of 2 mm. After the antral follicles reach 2 mm in diameter, they become highly sensitive and responsive to follicle-stimulating hormone (FSH). Follicles in diameter of 2–5 mm constitute a pool of follicles of which one follicle destined to ovulate is selected and finally undergoes ovulation [2, 3]. Those follicles constitute the functional ovarian reserve [4].

The number of ovarian follicles decreases with age as most follicles undergo degeneration and apoptosis, while some others ovulate during the fertile age [5]. The chance of achieving pregnancy decreases with age, especially after 40 years, and it is reflected not only by fall in the functional ovarian reserve but also in worsening in oocyte quality [4]. However, age by itself is a poor predictor factor of

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pregnancy outcome because the age-related fall of the functional ovarian reserve can differ between women.

A variety of tests to study functional ovarian reserve have been suggested and investigated. The role of these tests to predict natural fertility is still uncertain; however, they have been extensively evaluated among subfertile women undergoing assisted reproductive technology (ART) [6, 7].

The most used hormonal tests are the follicle-stimulating hormone (FSH) and the anti-Müllerian hormone (AMH). FSH can be quantified during the early follicular phase of a menstrual cycle, a period when its secretion increases to stimulate a new cohort of growing follicles. An exaggerated increase in FSH reflects a poor ovarian reserve and is a specific marker of low response to controlled ovarian stimulation (COS). Anti-Müllerian hormone (AMH) is produced by the granulosa cells of pre-antral and antral follicles and therefore reflects the functional ovarian reserve [8], having a small variation during menstrual cycle, being highest in the follicular phase [9]. Despite some technical issues that undermine its reliability [7], AMH can aid in predicting age of menopause [10] and is a good tool to predict poor and high response to COS in women undergoing ART [2, 8, 11], comparable to that observed by follicle count and better than FSH [12, 13].

Antral follicle can be easily identified by transvaginal ultrasound; but the size of the follicles to be counted is a debated issue: there are reports including 2–5, 2–6, 2–9, 2–10, and <10 mm diameter of the follicles [14–16]. Although follicles <2 mm in size might be identified at a transvaginal ultrasound, we prefer not including them to avoid confusion with other small anechoic structures, as vessels or artifacts. Regarding the upper limit of the follicle size, although the number of smaller follicles (2–5 mm) is thought to better represent the functional ovarian reserve [16], counting only these follicles is more difficult and time-consuming: in order to exclude follicles of 5–7 mm, one will need to measure several follicles in almost all exams, and many will be of borderline size, probably worsening the reproducibility. Moreover, to the best of our knowledge, there are no studies showing that counting only small antral follicles better predicts ovarian response, hyperandrogenism, or menopause than counting all follicles of 2–9 or 2–10 mm in diameter, and most of the published studies consider counting follicles 2–9 or 2–10 mm [15]. The difference between these two techniques (2–9 mm and 2–10 mm) is irrelevant, since almost all ovaries will have no follicles or a maximum of one follicle sized between 9 and 10 mm when assessed in the early follicular phase. We prefer counting all follicles sized 2.0–10.0 mm in order to reduce the scan time and to improve standardization [17]. When the follicles are larger than 10 mm, they are considered to be dominant: in a natural cycle, such follicles continue to grow and ovulate, while others regress [3]. There is no standardization on which measurement of the follicle should be used – the largest diameter, mean of three measurements, or mean of two measurements.

We suggest using the following terminology when counting ovarian follicles: the follicle count in one ovary is called follicle number per ovary (FNPO), while the sum of follicles from both ovaries is called the total antral follicle count (AFC). The FNPO is more used in general gynecological practice when assessing ovarian

Table 1.1 Guideline for the interpretation of follicle count to be used when assessing ovarian morphology in regular gynecology practice and when assessing the functional ovarian reserve in fertility centers

Interpretation of FNPO to be used in regular gynecologic practice		
FNPO	**Ovarian morphology**	**Interpretation**
1–3	Oligofollicular	Menopause is more likely to occur in less than a decade
5–24	Normofollicular	Normal follicle count
≥25	Multifollicular	High risk of hyperandrogenic anovulation
Interpretation of total AFC to be used in fertility centers		
Total AFC	**Functional ovarian reserve**	**Interpretation**
0–4	Very low	Very high risk of poor response to COS; reduced chance of achieving pregnancy through assisted reproduction
5–8	Low	High risk of poor response to COS
9–19	Normal	Expected normal response
≥20	High	High risk of excessive ovarian response and OHSS

Adapted from Martins et al. [18]

Notes: Follicles should be counted preferentially at the early follicular phase and in the absence of hormonal contraception. For follicle number per ovary (FNPO), consider the highest value observed in the right and left ovary; for the total antral follicle count (AFC), sum the number of follicles of both ovaries

COS controlled ovarian stimulation, *OHSS* ovarian

morphology, while the total AFC is more used in fertility centers to predict the ovarian response to stimulation [18]. A high FNPO is related to hyperandrogenism [15], and low FNPO is frequently associated with a higher risk of menopause in a few years [19, 20]. In fertility centers, a low AFC suggests poor ovarian response and worse reproductive outcomes [21, 22], while a high AFC indicates a high risk of ovarian hyperstimulation syndrome (OHSS) [12, 23]. It is important to emphasize that the ability to detect follicles, particularly the small ones, dramatically increased during the last decades with evolution of the ultrasound equipment quality. Therefore, the interpretation of the follicle count should take this into account. For example, the suggested threshold for association with hyperandrogenic anovulation (or polycystic ovary syndrome) increased from a FNPO ≥12 in 2003 to a FNPO ≥25 in 2014 [15, 24]. A simple guide for the interpretation of FNPO and AFC to be used in both clinical practice and fertility centers when using new ultrasound machines is presented in Table 1.1 [18].

1.2 How We Do It

Follicles should be counted preferentially between days 2 and 5 of a spontaneous menstrual cycle. The standardization is suggested because the number of antral follicles can vary with approximately 9% during menstrual cycle, and there is an

average reduction of 30 % in women using hormonal contraceptives [25]. In clinical practice, large reductions in follicle count can be seen with only 1 month of hormonal contraception. Follicles should be counted using transvaginal ultrasound examination using one of the three methods: real-time 2D ultrasound, cine-loop 2D ultrasound, or 3D ultrasonography.

1.2.1 Real-Time 2D Ultrasound

The follicles are counted during "live" ultrasound examination by sweeping the transducer along the entire ovary.

How to perform:

- Scan the ovaries in both planes (sagittal and coronal) and decide which plane provides a better view of the ovary.
- Choose the best plane and use it to count all follicles starting from one margin until the other.
  - Don't measure the follicles during this step.
  - Consider all round anechoic structures as follicles
  - Don't consider elongated structures as follicles
- Scan again measuring only the follicles that could be <2.0 or >10.0 mm. The number of these follicles should be subtracted from the total follicle count.
- Repeat the same procedure in another ovary.

When using this technique, it is possible to use different machine settings and apply different probe pressures to improve the image quality. Additionally, it permits to better evaluate whether the structure is ovarian (follicle) or extra-ovarian (e.g., hydrosalpinx, para-ovarian cysts) by assessing the mobility in relation to the ovary. The scan, however, takes longer time and might be uncomfortable for a patient. Another limitation of this technique is that it does not allow future analysis of the whole ovary if necessary as only one image is available.

1.2.2 Cine-Loop 2D Ultrasound

A 3–5 s cine loop can be saved by an ultrasound examiner when sweeping the transducer through the entire ovary for counting the follicles later "off-line" (Video 1.1). This technique is available in almost all modern ultrasound machines.

How to perform:

- Scan the ovaries in both planes (sagittal and coronal) and decide which plane provides a better view of the ovary.
- Use the best plane, place the transducer in one of the margins of the ovary, and freeze the image.

- Unfreeze the image and scan quickly from one side to another, and then freeze again.
- Check whether the entire sweep will be stored, i.e., if the scan time needed for the entire ovary is less than the maximum allowed to save time (this time can be adjusted in the machine settings). If not, repeat the process; if yes, store the cine loop.
- Repeat the same procedure with another ovary.
- Perform follicle count on the ultrasound machine as described above for real-time 2D ultrasound.

The main advantage of this technique is reducing the scan time and patient discomfort. Additionally, the saved videos can be analyzed in the future by the examiner or other persons, which might be useful for training and auditing purposes. However, some extra training is required both for learning how to scan the whole ovary in a short time and also for learning how to use cine loops. Another limitation of this method is that only saved videos can be analyzed afterward, not being possible to retrieve any missing information. Compared to automatic acquisition of 3D ultrasound volume, the manual sweep required for the cine loop is frequently more uncomfortable for the patient because the probe moves inside the vagina when using a considerable pressure.

1.2.3 3D Ultrasound

Using 3D ultrasound the aim of the acquisition is to acquire a 3D ultrasound dataset of the whole ovarian volume to be analyzed after the scan.

How to perform:

- Scan the ovaries in both planes (sagittal and coronal) and decide which plane provides a better view of the ovary or the largest ovary diameter.
- Using this plane, place the probe in the center of the ovary.
- Select the maximum image quality (or the largest scan time) and the maximum acquisition angle (e.g., 120°). This will increase the acquisition time, but will improve image quality and reduce the risk of missing part of the ovary during acquisition
- Press the button that starts the automatic acquisition of the ovarian volume and check whether it included the entire ovary. If not, repeat the process; if yes, save the 3D dataset.
- Repeat the same process with the other ovary.
- Release the patient.
- Count the follicles either directly on the ultrasound machine or on a personal computer.

The 3D ultrasound allows data manipulation with the use of exclusive techniques as sonography-based automated volume calculation (SonoAVC), inversion mode, or

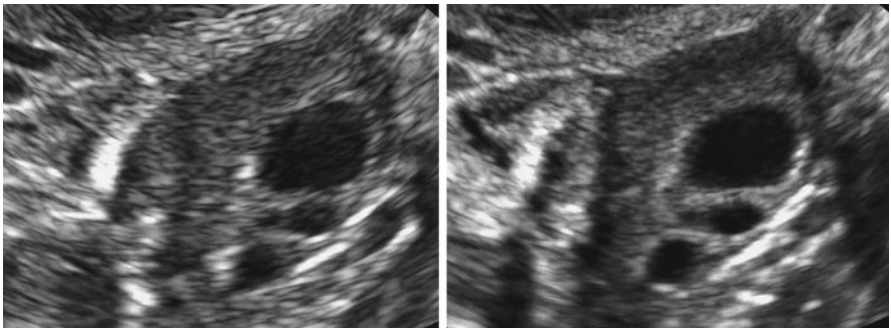

Fig. 1.1 Effect of the harmonics on image quality when using 2D ultrasound: same ovary without (*left*) and with harmonics (*right*)

volume contrast imaging (VCI). SonoAVC enables a semiautomatic calculating of both follicle size and number by automatically detecting low-echogenicity areas in a selected volume. The average diameter of the follicle is determined and all follicles are listed according to their size. Inversion mode permits identification the follicles when all hypoechoic areas are rendered. VCI enhances the contrast between the follicles and the parenchyma, and multiplanar images of the volume are presented.

The acquisition of a 3D dataset is a technique that causes less discomfort for woman and takes less time. It allows analysis of the whole ovarian volume and evaluating each follicle in the three orthogonal planes simultaneously. This technique, however, is restricted to more expensive ultrasound machines and requires examiner training for dataset acquisition and volume analysis.

1.3 Important Technical Tips

- Optimize the image; apply adequate probe pressure to reduce the distance between the ovary and the transducer; reduce pressure if the ovary is too close and there is reverberation; place the focal zone at the level of ovary; use the smallest depth possible, if the ovaries are not close to the ultrasound probe (e.g., cranial to the myometrium); and magnify the image until the ovary occupies at least 50 % of the screen on its largest plane; true zoom is recommended.
- When counting follicle using 2D ultrasound, prefer using harmonics. The harmonics imaging reduces the reverberation which frequently impairs the identification of follicles that are very close to the transducer (Fig. 1.1).
- When using 3D ultrasound, harmonics increases the acquisition time and impairs SonoAVC, and, although the basis is completely different, VCI results in similar or frequently better improvement on image quality (Fig. 1.2). Therefore, if you are using 3D ultrasound, only use harmonics if there is a large improvement on 2D image quality.

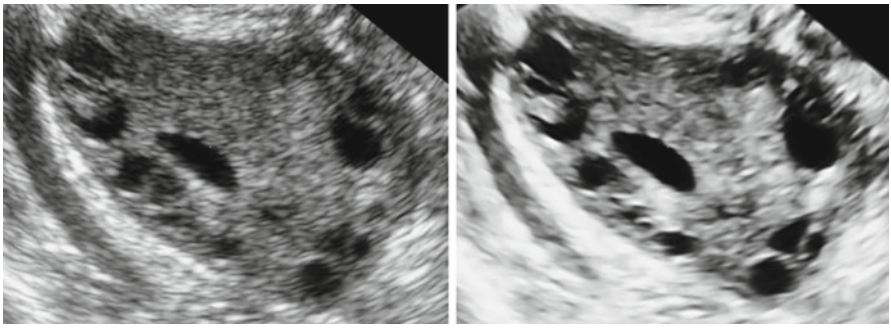

Fig. 1.2 Effect of the volume contrast imaging (VCI) on image quality when using 3D ultrasound: same ovary without (*left*) and with VCI (*right*)

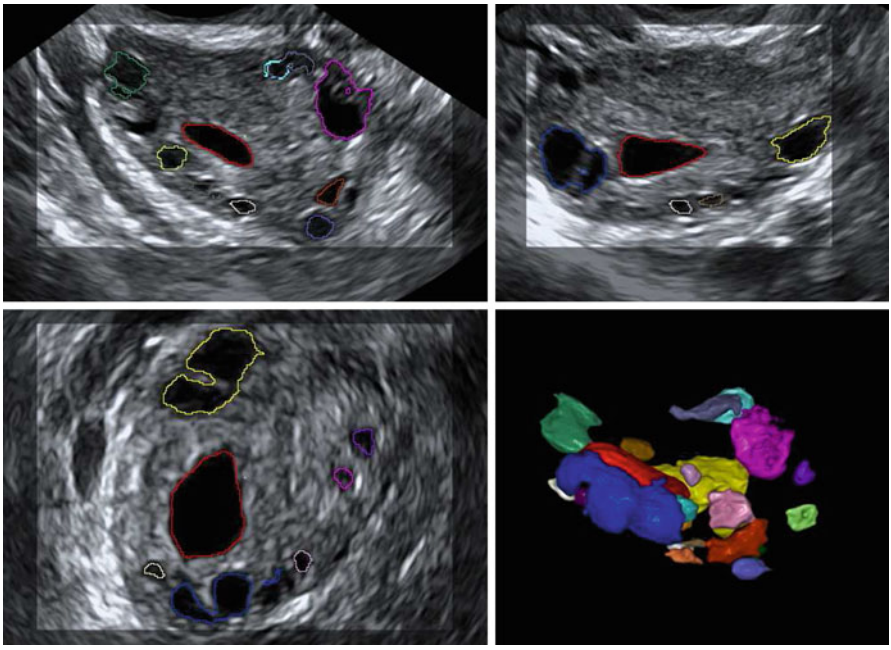

Fig. 1.3 Antral follicle count using SonoAVC: the automatic mode is making some obvious mistakes: two follicles are being counted as being only a larger one in *yellow*, and three follicles are being counted as being only a larger one in *blue*. Such mistakes need to be manually corrected

- We don't suggest using either SonoAVC (Fig. 1.3) or inversion mode (Fig. 1.4). SonoAVC may miss or incorrectly identify the outer contour of the follicle; therefore, the operator always needs to check the entire volume manually and to correct wrong information, which is frequently more time-consuming than counting follicles manually. Inversion mode provides a completely different

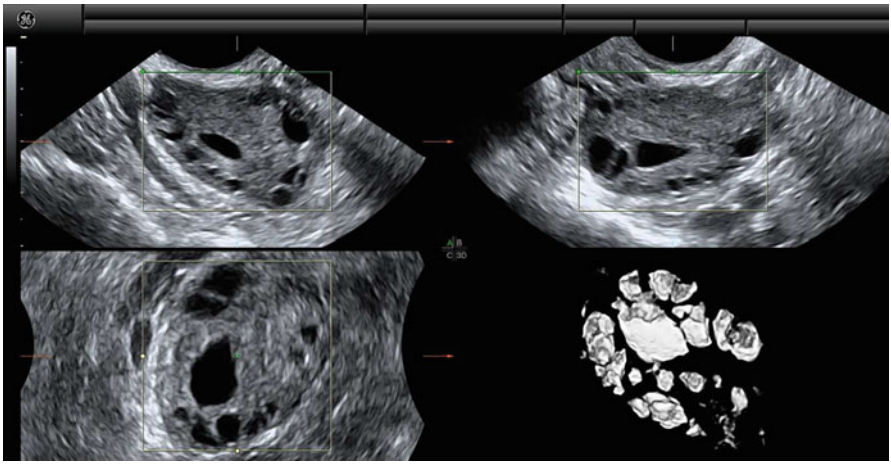

Fig. 1.4 Image of one ovary using the inversion mode

method for counting follicles, measuring follicles is more difficult, and the method is more dependent on image quality [26].

- Be aware of the influence of functional ovarian cysts and endometriomas. The presence of ovarian lesions may undermine the validity of follicle count assessment: it is probably underestimated in these situations [27, 28]. For example, the surgical removal of an endometrioma causes a decrease in AMH [29–32] but not in the AFC of the impaired ovary [33]. Such inconsistency probably happens because the presence of endometriosis in the ovary leads to inflammation and fibrosis and subsequent distortion of the pelvic anatomy with increase in the distance between the ovary and the probe, resulting in increased attenuation and reduced ability to identify small follicles by transvaginal ultrasound [27].

1.4 Future Perspectives

- We hope that semiautomatic follicle count technology will improve in the near future, reducing the need for training, improving standardization, and reducing the operator dependency. However, currently, manual counting should be used as the standard.
- We still need more studies assessing learning curve, patient satisfaction, reproducibility, and correlation with functional ovarian reserve comparing the different methods for counting ovarian follicles. Until all these points have been completely cleared, no method should be considered better than the others.
- Although there are some attempts, there is no universally accepted standardization for this technique, and results across different institutions might vary resulting in different cutoff points for the same condition.

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Anovulatory Disorders

2

Juan Luis Alcázar and Manuela Moya

2.1 Background

Anovulation is one of the main causes of infertility affecting about 30 % of women with infertility [1]. The World Health Organization (WHO) has classified anovulatory disorders into three categories [2, 3]:

1. WHO type I anovulation: In this type of disorder, women have low levels of LH and FSH; there is no follicle development stimulation and, therefore, oestradiol levels are low. Because of this fact, endometrial lining development is inadequate. This disorder may be caused by primary pituitary disease or a marked reduction in the frequency and amplitude of LH pulses secondary to severe weight loss (anorexia nervosa) or negative energy balance (e.g. athletes).
2. WHO type II anovulation: This is the most frequent type of anovulation. In this type of anovulation, women have normal levels of FSH, LH and oestradiol. However, a detailed analysis reveals subtle anomalies in LH secretion such as frequent high-amplitude pulses or failure or inadequate LH surge. Follicular development occurs, but it is arrested before full maturation of an ovulatory follicle and ovulation fails. The most frequent cause of this type of anovulation is polycystic ovarian syndrome (PCOS).

   Another cause of anovulation included in this group is the luteinised unruptured follicle (LUF) syndrome.

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3. WHO type III anovulation: In this type of anovulation, LH and FSH levels are high and oestradiol levels are low. This is caused by the absence or severely reduced number of primordial follicles, either by a primary cause (antenatal depletion of follicles, as Turner syndrome) or by a secondary cause that destroys ovarian tissue or follicles (e.g. radiotherapy, chemotherapy, surgery). In some instances, the cause is not known (primary idiopathic ovarian insufficiency).

A fourth, distinct group of anovulatory women includes women with hyperprolactinemia.

Table 2.1 summarises this classification.

In this chapter, we shall review how ultrasound may be used for assessing the ovary in women with anovulation.

2.2 How We Do It?

2.2.1 Technique

Transvaginal ultrasound (TVS) is the best approach for assessing the ovary and the endometrium. In the case of women in whom TVS cannot be performed (e.g. women with virgo intacta), transrectal ultrasound is a very good alternative since it provides quite similar images to TVS. Transabdominal ultrasound may be also an option, but the resolution of the ultrasound image uses to be worse.

Transvaginal ultrasound does not require any preparation before the procedure is done, except the mandatory use of a condom or ultrasound sheath for covering the ultrasound probe and the cleaning by any kind of disinfectant of the probe prior to be used in a new patient.

For transrectal ultrasound, rectal cleansing is recommended before ultrasound is performed and the same measures for probe covering than TVS are mandatory.

For transabdominal ultrasound, full bladder is required.

After inserting the endovaginal probe into the vagina, a thorough scanning of the pelvis is always advised including, obviously, the uterus and ovaries, for ruling out the presence of any uterine or adnexal pathology, such as congenital uterine anomalies, fibroids, adenomyosis or adnexal masses.

Once ruled out uterine and adnexal pathology attention should be paid to the ovaries and the endometrium.

The ovaries should be assessed completely by slight movement of the vaginal probe (Video 2.1). If a three-dimensional (3D) volume is acquired, the 3D bow should be adjusted to the ovary, usually as small-angle sweep can be used (35–40°) (Video 2.2).

The uterus should be also assessed by scanning side to side in the sagittal plane and from the cervix to the fundus in the transverse plane (Video 2.3). If a 3D volume of the uterus is acquired, the 3D box should be adjusted for including the whole uterus or at least the uterine corpus, and a wider 3D sweep angle is needed (90–120°) (Video 2.4).

Table 2.1 Summary of clinical features of anovulatory disorders

Anovulatory group	Type	Hormone levels	Prevalence	Causes	Clinical features
WHO I	Hypogonadotropic	FH↓↓	5–10 %	Pituitary destruction	Abnormal BMI (<20, 30)
	Hypogonadism	LH↓↓		Anorexia nervosa	Amenorrhoea
		E_2↓↓↓		Excessive exercise	Progesterone test negative
		AMH↓			Do not response to CC or AI
					Response to gonadotropins
WHO II	Normogonadotropic	FSH N	75–85 %	PCOS	BMI normal or >25
	Normogonadism	LH N or ↑↑		LUF	Oligomenorrhoea
		E_2N			Progesterone test positive
		AMH N			Response to CC or Al
		Androgens↑↑ (in PCOS)			Response to metformin (in case of PCOS)
WHO III	Hypergonadotropic	LH↑ or N	10–20 %	Primary ovarian insufficiency	Normal BMI
	Hypogonadism	FSH↑↑↑		CMT, RT, surgery	Amenorrhoea
		E_2↓			Progesterone test negative
		AMH↓			No response to CC, Al or gonadotropins
Hyperprolactinaemia		PRL↑↑	5 %	Pituitary adenoma	Normal BMI
		LH N		Pituitary dysfunction	Oligomenorrhoea
		FSH N		Dopamine insufficiency	Progesterone test positive
		E_2N			Response to PRL normalisation
		AMH N			

CC clomiphen citrate, *AI* aromatase inhibitor, *BMI* body mass index

2.2.2 Assessment of the Ovary

2.2.2.1 Biometry/Volume Calculation

The basic assessment of the ovary is measuring its size. The three orthogonal planes should be measured (Fig. 2.1). Using the prolate ellipsoid formula (height × width × length × 0.5233), the ovarian volume can be calculated and expressed in mL.

Ovarian volume may be also calculated using a 3D volume. Current evidence shows that the best method for calculating ovarian volume is the virtual organ computer-aided analysis (VOCAL™) method. This method consists of estimating the volume of the ovary by outlining the ovarian borders using a rotational method, visualising different ovarian planes at given rotational degrees over the axis *X*, *Y* or *Z* (Fig. 2.2).

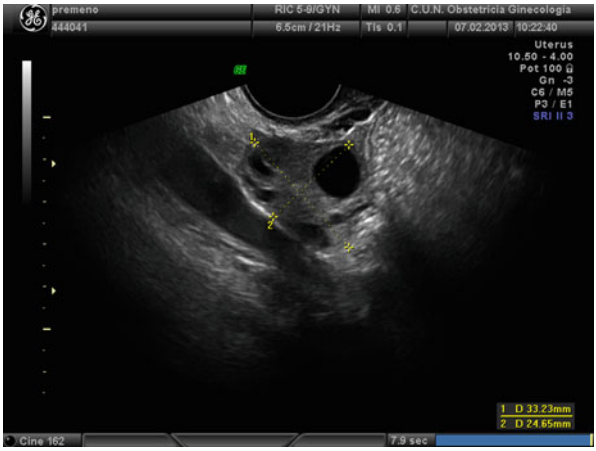

Fig. 2.1 Measuring the ovary using 2D transvaginal ultrasound

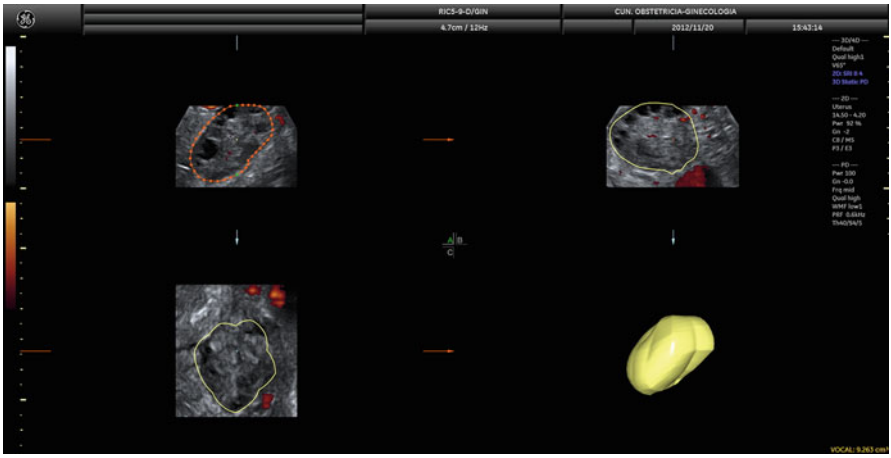

Fig. 2.2 Ovarian volume estimation using 3D ultrasound with VOCAL software

It has been shown that this method is reproducible and more accurate than 2D calculation [4, 5].

2.2.2.2 Antral Follicle Count

Antral follicle count (AFC) consists of counting all antral follicles visible within the ovary.

This issue is explained in Chap. 1. Please read this chapter for further information.

2.2.2.3 Doppler Assessment

The use of Doppler ultrasound allows the assessment of ovarian vascularisation. During 2D real-time ultrasound, the amount of flow in ovarian stroma, dominant/preovulatory follicle and corpus luteum can be subjectively assessed. Additionally, pulsed Doppler may be used for assessing vascular impedance of ovarian stroma, dominant/preovulatory follicle and corpus luteum vessels.

For assessing ovarian vascularisation, the colour/power Doppler window is activated over the ovary to identify stromal, preovulatory follicle (Fig. 2.3) or corpus luteum (Fig. 2.4) vessels. Then, pulsed Doppler gate is activated, and the flow velocity waveform (FVW) is obtained for calculating peak systolic velocity (PSV), resistance index (RI) and pulsatility index (PI).

In normal cycles the vascularisation increases after ovulation being maximum at 7 days post-ovulation. This is called "luteal conversion" of intraovarian FVW [6] (Figs. 2.5 and 2.6). The main problem for this assessment is its reproducibility between observers [7]. Three-dimensional ultrasound has been also used for assessing ovarian vascularisation. Using VOCAL™ software, we can calculate three vascular indices, namely, vascularisation index (VI), flow index (FI) and vascularisation-flow index (VFI). These indices represent, theoretically, the amount of vessels (VI), the amount of flow (FI) and perfusion (VFI) [12]. However, this concept has been challenged [8]. Additionally, some machine settings and depth affect significantly the estimation of these indices [9].

Favourably, the reproducibility of this approach has been shown to be good [10].

A more recent method, based on spatio-temporal correlation image, has been proposed [11]. This method allows the calculation of the so-called volumetric pulsatility index [12]. Theoretically this approach overcomes some of the problems released to static 3D-PD assessment. However, this approach is still in investigation and it has not been translated into clinical practice.

2.2.3 Assessment of the Endometrium

The basic assessment of the endometrium consists of measuring the endometrial thickness. This should be done in the sagittal plane of the uterus, including both endometrial layers (if an intrauterine collection is present, it must be excluded from measurement). The measurement should be done at the level of the maximum endometrial thickness (Fig. 2.7). This measurement is highly reproducible

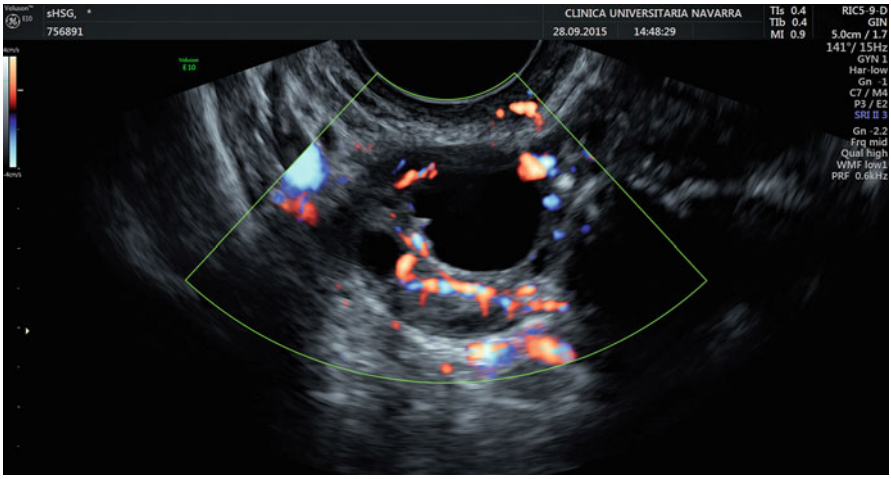

Fig. 2.3 Transvaginal colour Doppler ultrasound showing perifollicular flow

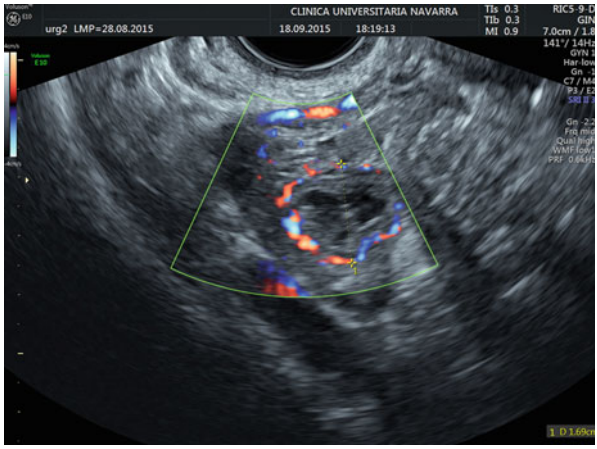

Fig. 2.4 Transvaginal colour Doppler ultrasound showing the characteristic "ring of fire" of vessels surrounding the corpus luteum

[13]. An additional important issue is the assessment of endometrial echogenicity. This refers to the subjective analysis of echogenic texture of the endometrium. 3D ultrasound allows the estimation of the endometrial volume. The best way for estimating endometrial volume is the use of VOCAL™ software. The use of 3D ultrasound for estimating endometrial volume has been validated [14] and it is reproducible [15].

2.2.4 Interpreting Findings

The use of ultrasound in combination with laboratory analysis has become essential for assessing anovulatory disorders.

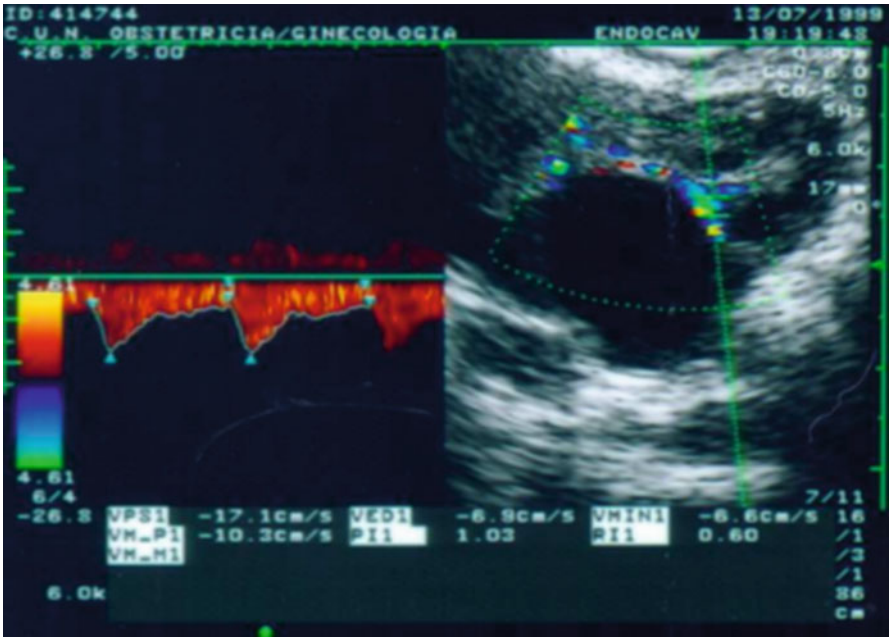

Fig. 2.5 Transvaginal pulsed Doppler ultrasound showing the flow velocity waveform from perifollicular vessels from a periovulatory follicle. Note high-resistance flow (RI: 0.60)

2.2.4.1 Normal Findings

For interpreting properly the findings of ultrasound evaluation, it is important to know what are the normal findings in both ovarian and endometrial cycles. Ideally, the assessment of the ovary and endometrium by ultrasound should be done throughout a menstrual cycle, starting on the 4th–7th day of the cycle and going on performing an ultrasound scan every 48 hours until confirming ovulation; a final ultrasound scan should be performed 7 days after ovulation. This approach will allow the assessment of the follicle and endometrium dynamics.

In the normal cycle, the ovaries at 4th–7th day of the cycle appear as oval structures containing antral follicles (Fig. 2.8). AFC should be done in this scan, as well as ovarian volume estimation. Normal AFC is 6–10 follicles per ovary and ovarian volume is 3–10 mL.

During follow-up scans, in normal cycles a dominant follicle (a follicle >10 mm) should appear, and this follicle will grow up to reach a diameter of 18–22 mm, the size when ovulation will occur (Fig. 2.9). After ovulation, the follicle shrinks and corpus luteum forms (Fig. 2.10).

At Doppler examination, we can observe ovarian stromal vascularisation in early follicular phase, but attention should be paid to dominant follicle vascularisation since the amount of flow around the follicle is related to oocyte quality [16]. After ovulation, the corpus luteum suffers important angiogenic phenomena and vascular mapping increases significantly as assessed by colour/power Doppler.

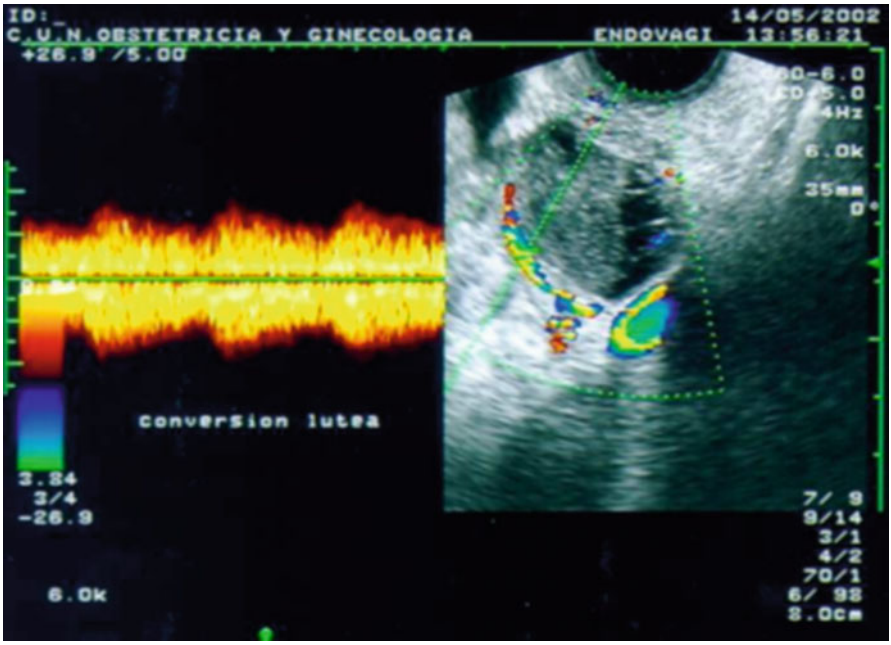

Fig. 2.6 Transvaginal pulsed Doppler ultrasound showing the flow velocity waveform from a corpus luteum. Note the increase in telediastolic velocity

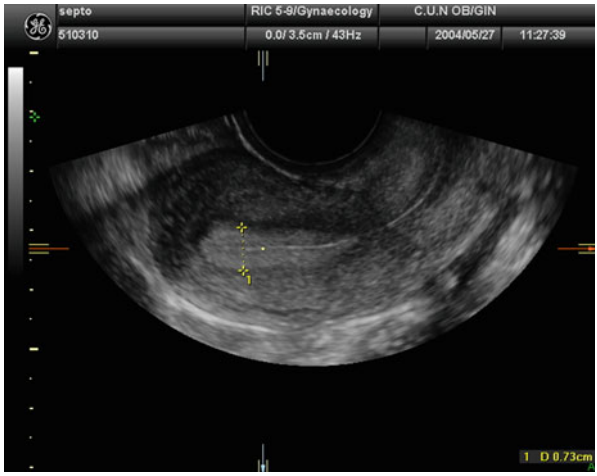

Fig. 2.7 Transvaginal ultrasound showing endometrial thickness measurement

Using pulsed Doppler, PSV increases in the dominant follicle's vessels from mid follicular phase to preovulatory follicle, but RI or PI remains high (RI > 0.55 and PI > 1.0). After ovulation PSV increases even more, and RI and PI decreases (RI ≤ 0.50 and PI ≤ 1.0) reflecting the angiogenic phenomena of the corpus luteum, achieving maximum expression around 7 days post-ovulation.

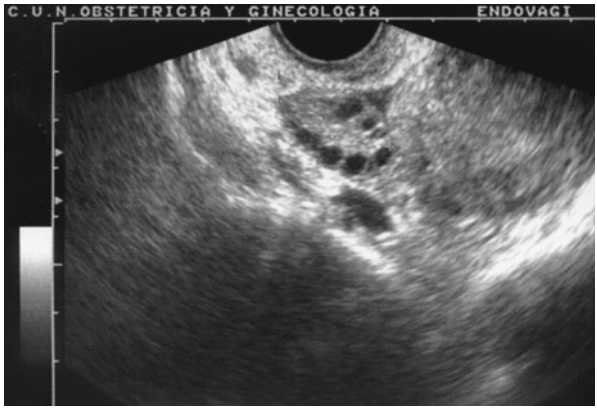

Fig. 2.8 Typical aspect of an ovary in early follicular phase as assessed by transvaginal ultrasound

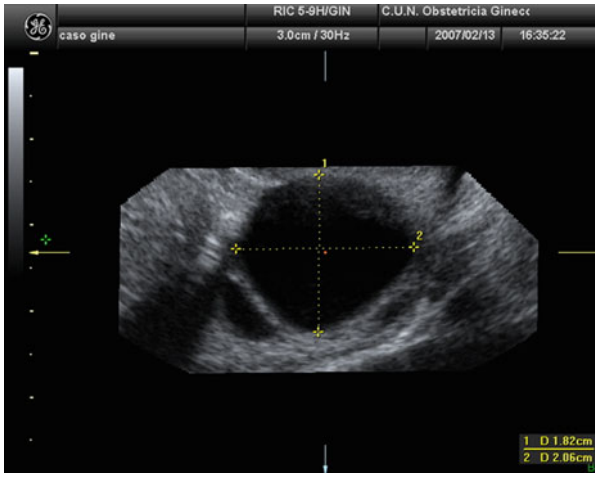

Fig. 2.9 Transvaginal ultrasound picture of a preovulatory follicle

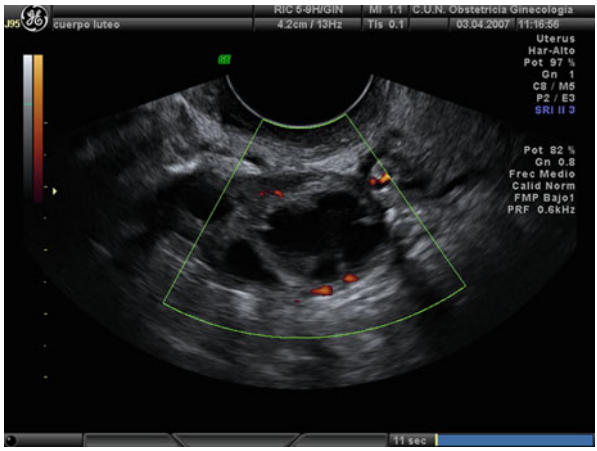

Fig. 2.10 Transvaginal ultrasound picture of a corpus luteum

Regarding the endometrium, this appears as a thin homogeneous stripe in early proliferative phase. Endometrial thickness increases through the proliferative phase and the endometrium becomes trilaminar (Fig. 2.11), because of the proliferative effect of the oestradiol produced by the dominant, preovulatory follicle. After ovulation, the endometrial thickness increases more being maximum (around 16–18 mm) 7 days post-ovulation. The endometrium becomes homogeneously echogenic (Fig. 2.12) because of the glandular secretory transformation produced by the progesterone produced by the corpus luteum.

However, in spite of superiority of ovarian dynamic assessment for evaluating ovarian function by ultrasound, the number of ultrasound scans needed makes it difficult to do so in daily practice. For this reason, at least two basic evaluations could be done for assessing ovarian function: first scan in early follicular phase (4th–7th day of the cycle) for assessing ovarian volumes and AFC and second scan

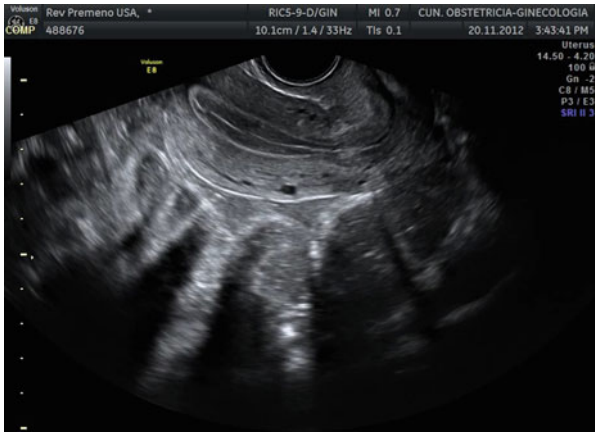

Fig. 2.11 Transvaginal ultrasound of a uterus showing a three layer pattern during proliferative phase

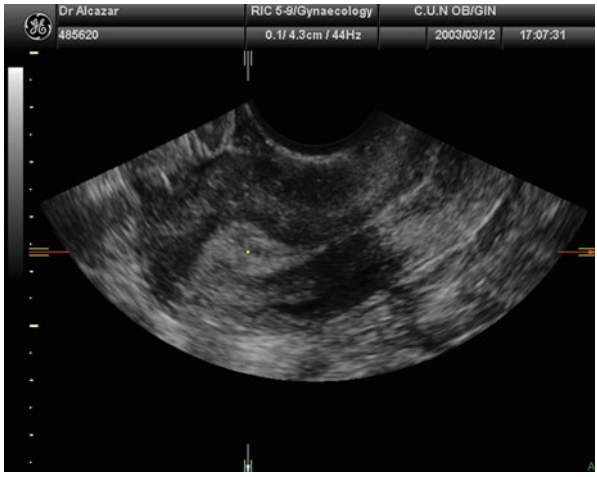

Fig. 2.12 Transvaginal ultrasound during secretory phase showing a homogeneous echogenic endometrium

in a theoretically 21st day of the cycle assuming an ovulation to occur by the 14th day, for assessing corpus luteum.

2.2.4.2 Anovulatory Disorders

Anovulatory Disorders, WHO Type I

As stated previously anovulatory disorders, WHO type I, are mainly due to hypothalamic/hypophysiary anomalies, either structural or functional.

In these women the ovaries commonly are small (ovarian volume <2–4 mL), and follicles can be seen scattered throughout ovarian stroma. AFC uses to be normal. The endometrium is thin (<4–5 mm) and homogeneous stromal ovarian flow is scanty [17, 18].

If ovarian and endometrial dynamics is assessed, no development of follicles is observed and endometrial stripe remains thin.

Anovulatory Disorders, WHO Type II

Polycystic Ovarian Syndrome (PCOS)

PCOS constitutes the most frequent cause of anovulation. Ultrasound criteria for diagnosing polycystic ovary (PCO) are based on ovarian volume stimulation and AFC.

Until recently, accepted criteria are based on the Rotterdam consensus [19]. According to this consensus, PCO should be diagnosed when ovarian volume is >10 ml and/or AFC is >12 follicle measuring 2–9 mm in at least one ovary.

However, recent studies have proposed a new AFC threshold for diagnosing PCO (>25 follicles per ovary) [20–23]. The androgen excess and Polycystic Ovary Syndrome Society have adopted this new threshold [24]. However, this criterion should be used when high-resolution transducers (>8 MHz) are used.

The assessment of ovarian stroma has been also proposed for diagnosing PCO [25]. However, the additional value of this parameter is very limited. Actually, a recent study has shown that AFC is much better than ovarian stroma assessment [26].

Ovarian stromal vascularisation (OSV) is another parameter proposed for diagnosing PCOS. Those studies analysing OSV have found that ovarian stromal vascularisation in PCOS women, using either color or pulsed Doppler techniques, is increased [27]. However, the lack of consistent and uniform data besides the absence of determined cut-off values for vascular indices make these parameters useless from the practical point of view for diagnosing PCOS.

Follicle development dynamic assessment shows that a dominant follicle does not appear throughout the cycle. The endometrial stripe may thicken up to 10–16 mm and usually with homogeneous appearance [28].

Luteinised Unruptured Follicle Syndrome

LUF has been associated with the ingestion of nonsteroidal anti-inflammatory drugs, endometriosis and pelvic adhesions. Its prevalence is not known. However,

the incidence in women with unexplained infertility undergoing intrauterine insemination has been reported as high as 25 % [29].

What happens in LUF syndrome is a failure in the ovulation due to a disruption of local paracrine mechanisms involved in the degrading of the follicle wall.

Therefore, ovarian volume and AFC are within normal range. Dominant and preovulatory follicle develops normally, but ovulation fails, even in the presence of ovulatory LH surge.

The follicle suffers luteinisation and progesterone is produced, albeit with levels of luteal insufficiency. However, angiogenic phenomena do not occur.

The diagnosis of LUF is done when ovulation is not confirmed during dynamic assessment of follicle development. The use of pulsed Doppler may be useful for confirming LUF since RI and PI remain high during "luteal" phase [6, 30] (Fig. 2.13), expressing the absence of angiogenic changes related to corpus luteum formation.

The endometrium usually thickens within normal range and becomes homogeneous in luteal phase.

2.2.4.3 Anovulatory Disorders, WHO Type III

This type of anovulation is due mainly to primary or secondary ovarian insufficiency, related to a depletion of primary follicles within the ovary.

Therefore, the main question to address in this type of anovulatory disorders is the so-called ovarian reserve. This point is addressed in Chap. 1 of this book.

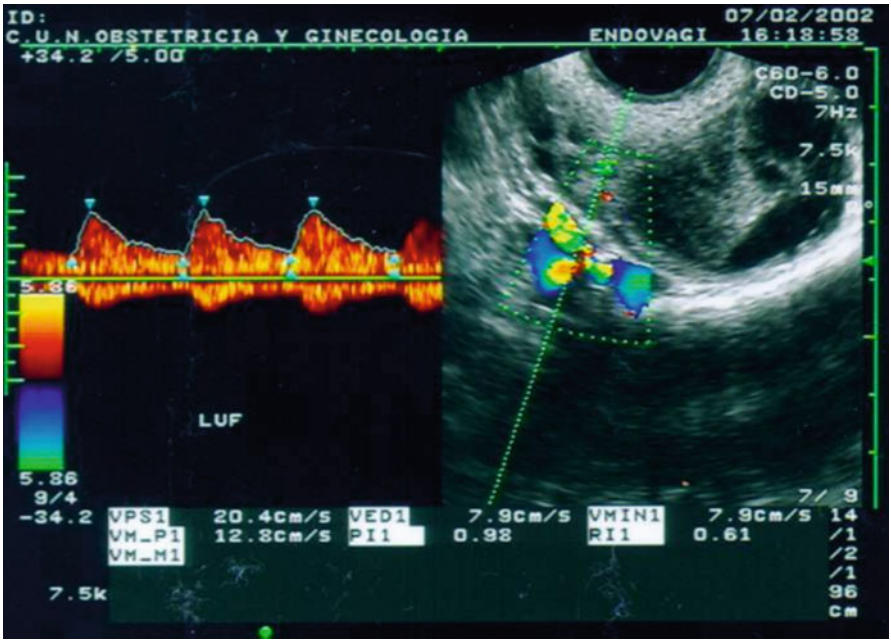

Fig. 2.13

2.3 Important Technical Tips

There are several important technical tips to be considered when evaluating the ovary and the endometrium in anovulatory disorders.

These technical tips are basically related to machine settings and depth.

The objective of the examination from the technical point of view is trying to get the maximum possible resolution. This is the main reason transvaginal ultrasound should be performed whenever possible, and transrectal ultrasound is the best alternative when TVS cannot be performed.

2.3.1 Depth

How far the structures under examination from the transducer is a very relevant issue, especially if Doppler is used, since Doppler signal is heavily affected by attenuation.

If the ovary and/or uterus are far from the transducer (>5 cm), a gentle pressure with the hand over the abdomen while performing ultrasound may get these structures closer to the transducer favouring image resolution.

2.3.2 Machine Settings

Machine settings are also important for achieving a good resolution image.

Transducer's frequency is the most important machine setting. It should not be less than 5 MHz and even higher frequency (8–9 MHz) is advisable. If Doppler is used, 5 MHz is an optimal frequency.

Gain is another important machine setting to be considered, especially if colour/power Doppler mapping is done. For greyscale evaluation low gain is initially advisable, increasing it in case of low-quality image. For colour/power Doppler assessment, it is recommended to increase gain until saturation and then reduce gain reaching sub-noise gain level.

Harmonics increases image resolution but penetration is lower. If the ovary and/or uterus are very close to the transducer, the use of harmonics is advised.

Other machine settings such as persistence, contrast and enhancement power do not usually need to be modified for improving image quality or resolution in most circumstance.

For Doppler assessment, other important parameters are:

A. Pulse repetition frequency (PRF). Since blood flow within the ovary and endometrium uses to be slow and the vessels are small, low PRF is advised (0.6–0.3 KHz).
B. Wall filter should be low (50 Hz).
C. Sample volume should cover the whole ovary.
D. Insonation angle is not relevant for assessing ovarian vascularisation since vessels are so small in that it is virtually impossible to ascertain vessel orientation.

E. Pulsed Doppler sample volume size should be adjusted to vessel calibre as better as possible. If not possible sample volume size of 0.7–1.0 mm is advisable.

2.4 Future Perspectives

It could be said that in current practice the use of ultrasound is essential for evaluating anovulatory disorders.

Future perspectives may be summarised in the following points:

(a) Confirmation that new ultrasound criteria for diagnosing PCOS are reproducible and better than those currently accepted
(b) Evaluation of reproducibility of new technical advances for AFC, such as sonoAVC™
(c) Evaluation of the impact into clinical practice of these new technical advances based on 3D ultrasound
(d) Development of logistic models based on clinical, ultrasound and laboratory parameters for improving our diagnostic ability of PCOS and even to distinguishing among different subtypes of this entity
(e) Development of logistic regression models based on clinical, ultrasound and laboratory parameters for improving our ability to assess ovarian reserve

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Tubal Diseases

3

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3.1 Background

3.1.1 Definition

Inflammatory process of the pelvis, or pelvic inflammatory disease (PID), is a frequent and serious yet treatable disease that can lead to abscess formation or pelvic fluid accumulation. When the abscess or the fluid remains into the tube, the pathology was called hydrosalpinx or pyosalpinx [1]. The condition can affect one or both fallopian tubes.

3.1.2 Epidemiology

It has been estimated that, in the USA, approximately 10–15 % of women in their reproductive years have had at least one episode of inflammatory disease of tubes and that at least 30 % of infertility problems and 50 % of ectopic pregnancies can be attributed to a previous episode of PID [2, 3]. The main agents responsible for the disease are *Neisseria gonorrhoeae* and *Chlamydia*. Hydrosalpinx is reported in the 2–3 % [4, 5] of cases of adnexal masses submitted to surgery.

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3.1.3 Symptomatology

Symptoms of acute tubal pathology are usually fever, purulent vaginal discharge, pelvic and abdominal tenderness, and laboratory findings of an inflammatory process. Clinical diagnosis of chronic hydrosalpinx, instead, is hampered by the lack of specificity of signs and symptoms. Usually, there are few symptoms noticed by patients although some women may suffer from abdominal or pelvic pain [1].

3.1.4 Hydrosalpinx and Infertility

Fallopian tube blockage is one of the factors of female infertility, and hydrosalpinx represents one of the causes of this pathology. In vitro fertilization (IVF) was originally developed to overcome the impediment created by tubal obstruction. However, the success of in vitro fertilization is significantly lower for women with hydrosalpinx compared to other causes of infertility, and bilateral hydrosalpinx yields about one-half of the rate of implantation of the unilateral form [6–9].

3.1.5 Therapy

Several surgical approaches have been discussed to improve the chances of a full-term pregnancy in patients with hydrosalpinges undergoing IVF. It was observed that implantation rates were improved after surgical treatment and had significantly better outcomes after salpingectomy [10]. Vaginal ultrasound-guided aspiration of hydrosalpinx is the simplest method of treating hydrosalpinges. Some studies show that aspiration of the hydrosalpinx fluid prior to an IVF procedure slightly increased implantation rate [11, 12]. Several studies reported that ultrasound-guided aspiration of hydrosalpinges at oocyte collection is an option for those who develop hydrosalpinges during controlled ovarian stimulation [13].

A new option for hydrosalpinx treatment is Essure. Several studies suggest the effectiveness of unilateral tubal occlusion caused by Essure microinsert in improving outcomes of in vitro fertilization treatment in cases of infertility associated with unilateral hydrosalpinx. Whenever laparoscopy is not recommended, hysteroscopic insertion of device seems the most effective option for management of hydrosalpinx before IVF [14, 15].

3.2 How We Do It

Laparoscopy is the gold standard in the diagnosis of this disease, but ultrasonography (US) should be proposed to allow appropriate patient counseling of further diagnostic or therapeutic interventions and to reduce the number of unnecessary laparoscopies [16].

In physiological conditions, the ultrasound study of the female reproductive organs does not allow direct visualization of the tube, but it is always possible to follow the usual anatomical course positioning the endocavitary transducer at the tubal corners and following the page of the broad ligament.

Over the years, based upon multiple publications in the literature, it has become clear that the transvaginal sonographic appearance of tubal inflammatory disease is typical and reproducible [17–27]. Pattern recognition may be used to make a specific diagnosis for hydrosalpinx that is defined by a tubular structure, ovoid or pear shaped, apparently separated from the ovary, but we have to distinguish between acute and chronic pathology.

At ultrasound in acute tubal inflammatory disease, the following findings can be present:

- Thick fallopian tube wall (more than 5 mm) (Figs. 3.1 and 3.2)
- "Cogwheel sign" (short-linear projections) (Fig. 3.3)
- Incomplete septa (septa that do not reach the opposite wall) with a tube filled by hypoechoic fluid (Fig. 3.4)
- Presence of tubo-ovarian complex (Fig. 3.5)
- Fluid in the cul-de-sac

At ultrasound in chronic tubal inflammatory disease, the following findings can be present:

- Thin fallopian tube wall (less than 5 mm) (Fig. 3.6).
- "Beads on a string" (small hyperechoic mural nodules) (Fig. 3.7).
- Incomplete septa (Figs. 3.8 and 3.9) with a tube filled by anechoic fluid.
- Tubo-ovarian complex and fluid in the cul-de-sac are less present [10].

Patel et al. [28] suggest that an additional finding as the "waist sign" (defined as diametrically opposed indentations along the wall of the cystic mass) (Fig. 3.10) is a useful additional finding associated with the presence of hydrosalpinx.

Using these findings, the transvaginal ultrasonography [4, 5, 16, 29] shows a very good accuracy in the evaluation of hydrosalpinx with very good values of positive and negative likelihood ratios as it is shown in Table 3.1.

Guerriero et al. [16] evaluate the diagnostic power of transvaginal sonography in the differential diagnosis of hydrosalpinx in 239 premenopausal patients with 256 adnexal masses, reporting a sensitivity of 93.3 % and specificity of 99.6 %.

The role of ultrasonography seems crucial also because only hydrosalpinges visible on ultrasound are associated with reduced implantation and pregnancy rates after in vitro fertilization as previously described [30, 31]. In addition in case of visualization at ultrasound of findings related to the presence of acute tubal inflammatory disease, also when symptoms are not present, we should suggest to avoid tubal patency evaluations and/or hysteroscopy.

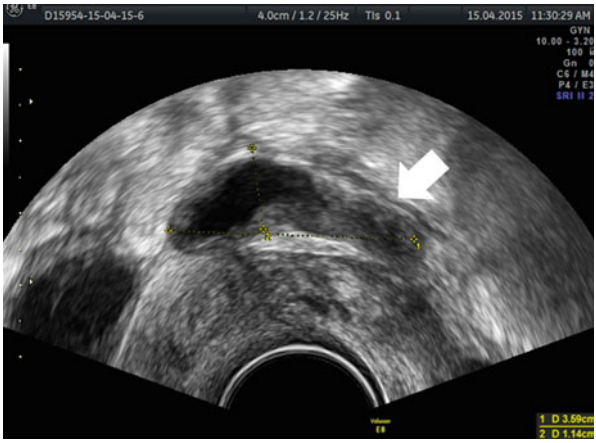

Fig. 3.1 A thick fallopian tube wall in case of acute tubal inflammatory disease

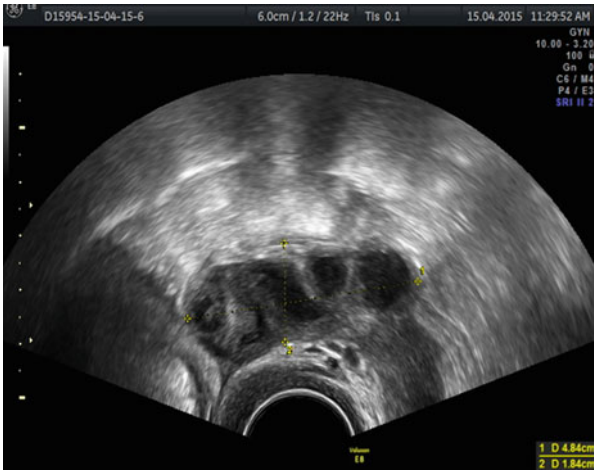

Fig. 3.2 Another thick fallopian tube wall in case of acute tubal inflammatory disease

3.2.1 Differential Diagnosis

In the case of an acute inflammatory process, to differentiate tubal inflammatory disease from an ovarian tumor is relatively easy and is determined by the acute inflammatory features of the pelvic disease. More complicated is the differential diagnosis of chronic hydrosalpinx from benign or malignant ovarian tumors including endometriomas, benign and malignant cystadenomas, cystadenofibromas, and paraovarian cysts. Dilated hydroureters have been misdiagnosed as fluid-filled fallopian tubes. The question arises when it is necessary to differentiate the "beads-on-a-string" sign of chronic tubal disease from small internal papillations and septa of an ovarian cystic. But in the case of a chronic hydrosalpinx, the mural lesions (beads on a string) are small, almost equal in size, and are distributed around the thin wall. On the contrary the papillary formations of an ovarian tumor are usually dissimilar

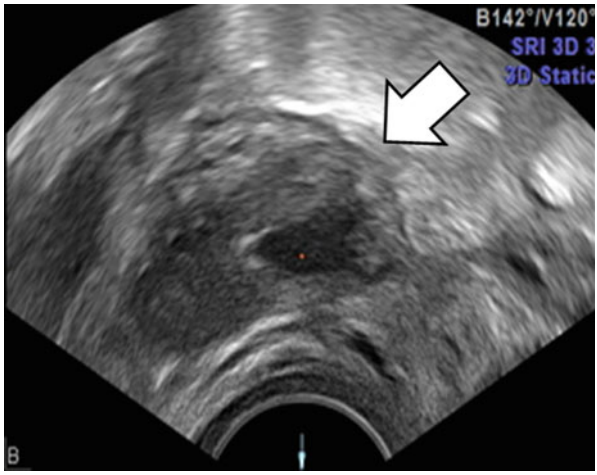

Fig. 3.3 A cogwheel sign (see *arrow*) in case of acute tubal inflammatory disease

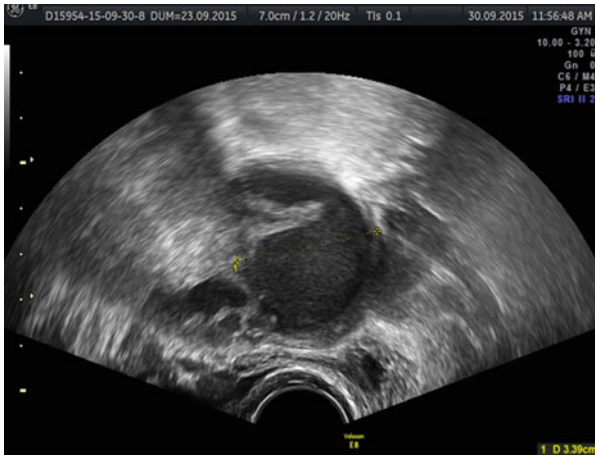

Fig. 3.4 An incomplete septum in case of acute tubal inflammatory disease with a tube filled by hypoechoic fluid

in size and located along the wall, which may show variable thickness with the presence of color Doppler signals inside. If the incomplete septa are present, these almost uniformly indicate the diagnosis of a fallopian tube since the true septa of ovarian tumors are very seldom, if ever, incomplete [1]. In some case the operator should also investigate the associated presence of deep endometriosis present in some cases.

3.3 Important Technical Tips

Tubal inflammatory disease can be identified by the following transvaginal sonographic technical aspects [1] (*see videos*):

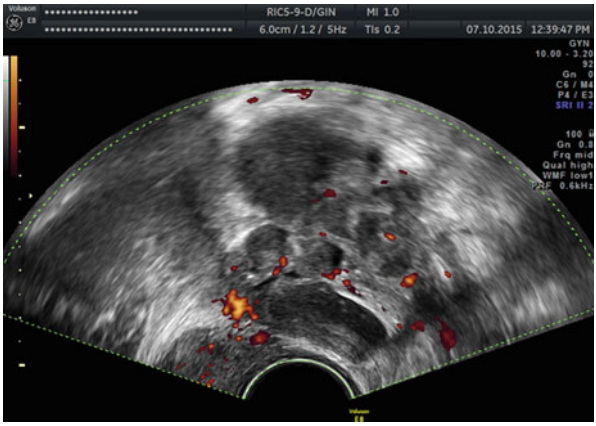

Fig. 3.5 A tubo-ovarian complex

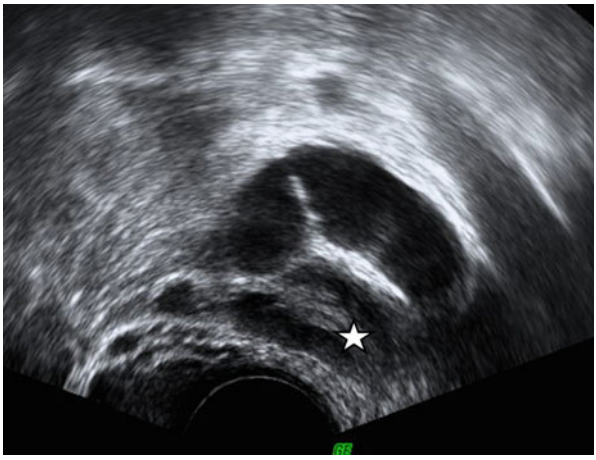

Fig. 3.6 A thin fallopian tube wall (less than 5 mm) in case of chronic tubal inflammatory disease surrounding the ovary (see *star*)

- The relationship with the ovary has to be evaluated pushing the tube with the vaginal probe and using the second hand that has to push on the pelvis (see videos 3.1, 3.2, 3.3, and 3.4).
- Shape: when the mass has been identified, the rotation in the longitudinal section has to show a pear-shaped, ovoid, or retort-shaped structure (see videos 3.1, 3.2, 3.3, and 3.4).
- Wall structure has to be evaluated by the rotation of the probe and following the enlarged tube for the presence of:
  Incomplete septa: that is hyperechoic septa that originate from one of the walls, without reaching the opposite wall (see videos 3.1 and 2); in some cases the mass can appear as multilocular, but rotating the probe the incomplete septum is visualized in the majority of cases (Fig. 3.11a, b).

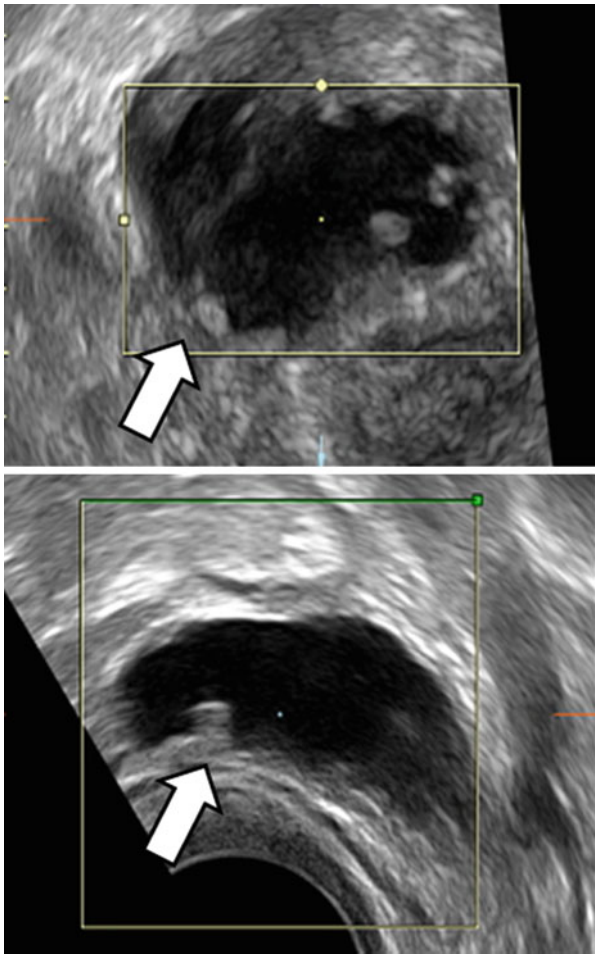

Fig. 3.7 The presence of beads on a string (small hyperechoic mural nodules) (see *arrows*)

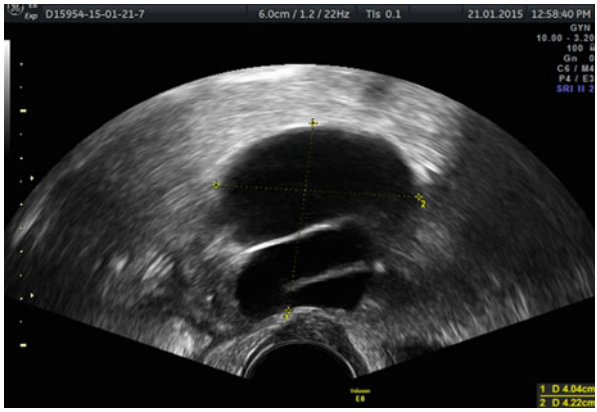

Fig. 3.8 An incomplete septum in case of hydrosalpinx

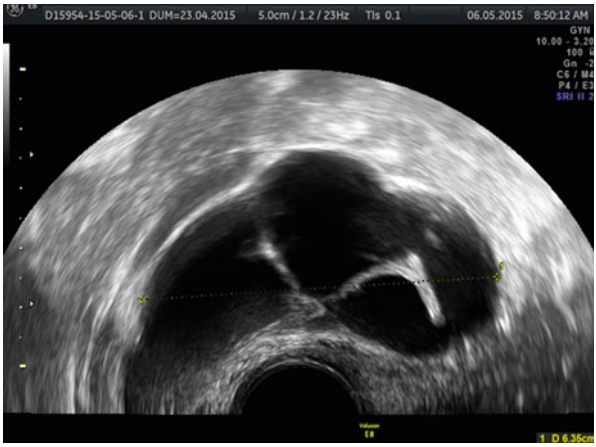

Fig. 3.9 Another incomplete septum in case of hydrosalpinx

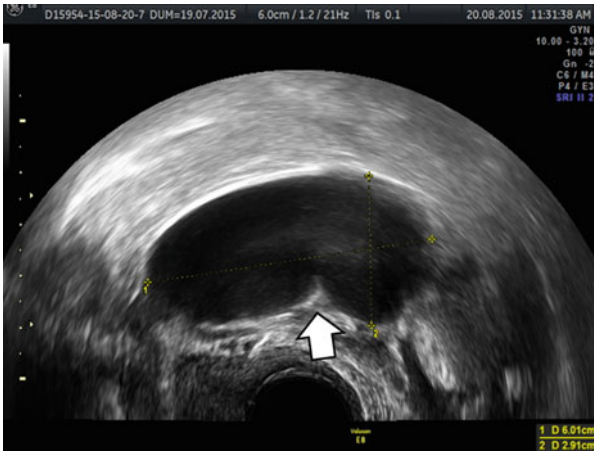

Fig. 3.10 The "waist sign" (defined as diametrically opposed indentations along the wall of the cystic mass) described by Patel et al. [28]

Table 3.1 The positive and negative likelihood ratio of transvaginal ultrasonography in the diagnosis of hydrosalpinx as reported in the literature

	LR+	LR−
Sokalska et al. (2011) [5]	38	0.15
Alcazar et al. (2011) [4]	58	0.21
Valentin (2006) [29]	99	0.01
Guerriero et al. (2000) [16]	93	0.07

"Beads-on-a-string" sign, small hyperechoic mural nodules seen on the cross section of the fluid-filled distended structure (see video 3.3); rotating the probe these structures are usually linear.

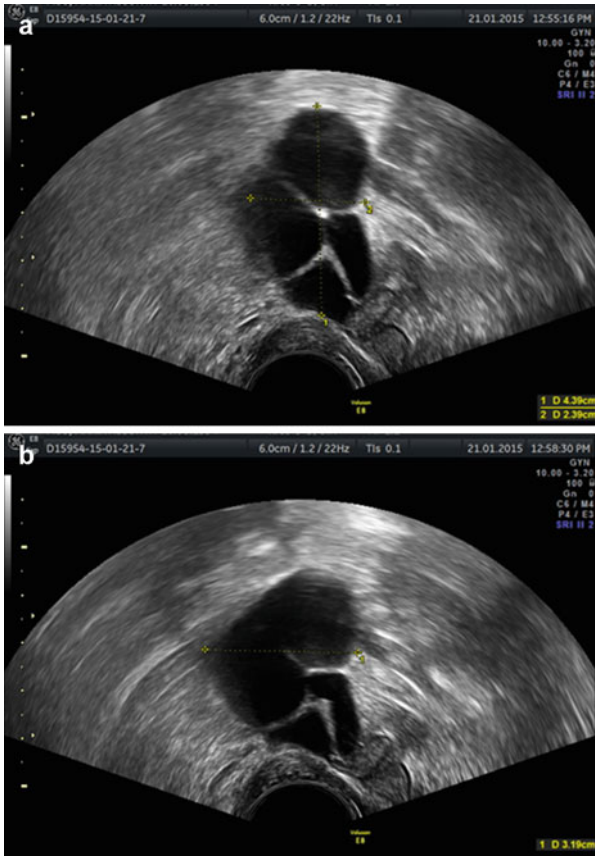

Fig. 3.11 This hydrosalpinx appears as multilocular (**a**), but rotating the probe in the incomplete septum is visualized in the majority of cases (**b**)

"Cogwheel" sign, internal profile coded for sonolucent cogwheel-shaped structure visible in the cross section of the tube with thick walls.

- Content: sonolucent or sometimes low-level echoes have to be searched.
- Push and pull with the vaginal probe to verify if it evokes pain.
- Push and pull with the vaginal probe to verify if there is mobility between pelvic structures.
- Extent of ovarian involvement defined as none, tubo-ovarian complex (see video 3.4) and tubo-ovarian abscess.
- Color Doppler: intense vascularization (color score 4) in case of acute tubal inflammatory disease; few spots in case of chronic tubal inflammatory disease [16] (Figs. 3.12 and 3.13).

Tubo-ovarian complex and tubo-ovarian abscess are clinically and sonographically distinct and deserving of different therapeutic approaches. The tubo-ovarian

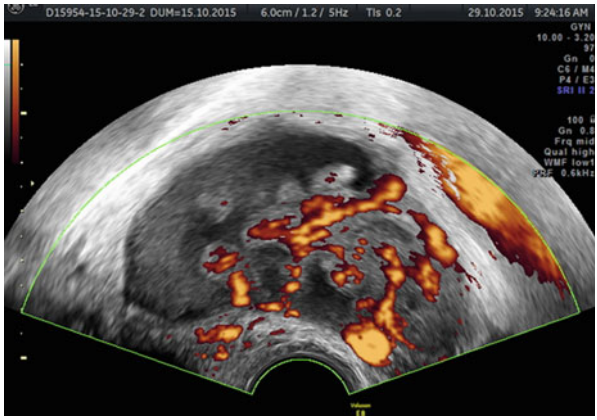

Fig. 3.12 Intense vascularization (color sore 4) at color Doppler in case of acute tubal inflammatory disease

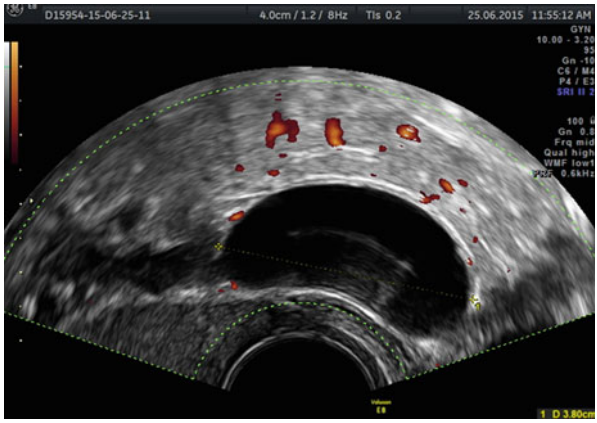

Fig. 3.13 Few vascular spots (color sore 2) at color Doppler in case of chronic tubal inflammatory disease

complex is a first step in the process which could lead to abscess formation. There are clear clinical and ultrasound features suggestive of acute PID. The ovaries and tubes are identified and recognized, but the ovaries cannot be separated by pushing the tube with the vaginal probe. The definition tubo-ovarian abscess should be reserved for a later phase in this acute pelvic process, when the total breakdown of the adnexal structures on one or both sides is seen.

A high level of confidence in the diagnosis of hydrosalpinx is reached when these combinations are identified in a cystic adnexal mass that does not have solid-appearing areas or features characteristic of hemorrhagic cyst, endometriomas, or dermoid cyst [28].

3.4 Future Perspectives

The diagnosis of hydrosalpinx, using 2D US, sometimes could be hard for the complicated anatomy of the tube reach of tortuous shapes which follow different directions.