TRANSVAGINAL SONOGRAPHY IN EARLY HUMAN PREGNANCY

(Transvaginale echoscopie in de vroege humane graviditeit)

PROEFSCHRIFT

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CHAPTER 1

GENERAL INTRODUCTION AND DEFINITION OF THE STUDY OBJECTIVES

1.1. INTRODUCTION

Efforts to employ transvaginal sonography as a method to visualise the internal genitalia and their contents already date from the late 1960's, when it was used to detect the embryonic heart beat and to study the female genital tract[1, 2]. It was reported that embryonic cardiac activity could be detected as early as 46 days menstrual age or 32 days after ovulation, which is much earlier than by then available conventional abdominal ultrasound techniques. However, the equipment was bulky and consisted of a large device producing A–mode images. Creation of two–dimensional images was extremely difficult and soon the method was forgotten[3].

It was only after the introduction of the grey scale technique and of real–time imaging in the mid–seventies that transvaginal sonography became feasible again. However, it took a considerable time before its value was rediscovered. This was probably due more to apprehension on behalf of the investigator than to lack of acceptance by the patient.

Although the significance of the transvaginal approach was recognised in the early eighties, notably in the German speaking countries and the United States, the major breakthrough came from IVF centres where it was first employed for the puncture of follicles and later for routine monitoring of induction of follicular growth[4-10]. It soon became clear that transvaginal sonography could give more detailed information in the field of early embryonic development and gynaecological disease. Recently, a host of data on the first topic has been reported[9-17]. Its role in late pregnancy is mainly determined by its accuracy in diagnosing placenta praevia[18, 19].

Transvaginal sonography, because of its superior resolution, has changed our concepts about normal and abnormal early pregnancy. More detailed evaluation of normal and abnormal early pregnancy first became possible

with the introduction of sensitive radio-immuno assays for human chorionic gonadotropin (hCG). Transvaginal sonography has opened the possibility of more accurate differentiation between early failure of intra-uterine gestation and ectopic pregnancy. More insight is needed into the synergistic role of transvaginal sonography and serial hCG determinations in the diagnosis and subsequent management of early pregnancy pathology.

The improved visualisation of the developing embryo permits imaging of organs shortly after their development. Its clinical application may, therefore, be determined by more accurate pregnancy dating and the detection of gross structural anomalies[20]. It is suggested that the neural tube can be reliably traced as early as eight weeks menstrual age[21]. Accurate information on renal size can now be obtained as early as 11 – 12 weeks menstrual age[22].

Transvaginal sonography has also been claimed to improve the procedure of chorionic villus sampling using an automatic puncturing device[23]. Its role as a routine application has not been determined yet.

Recently, the introduction of traditional pulsed Doppler and colour Doppler facilities in transvaginal sonographic equipment has provided the option of studying early fetal cardiovascular dynamics[24, 25].

1.2. DEFINITION OF THE STUDY OBJECTIVES

In this thesis attention will be focused on the role of transvaginal sonography in early pregnancy development. The objectives of the present study were:

a. to determine the safety of diagnostic ultrasound with particular reference to the transvaginal application,
b. to define the role of transvaginal sonography in:
   (i) the visualisation of normal early pregnancy development with emphasis on embryonic anatomy and biometrical aspects,
   (ii) the recognition, diagnosis and management of abnormal development, in particular missed abortion and ectopic pregnancy,
   (iii) the functional development of early pregnancy with special reference to the appearance and development of embryonic cardiac activity in single and multiple pregnancies under physiological and pathophysiological

circumstances.

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CHAPTER 2

TRANSVAGINAL SONOGRAPHY: Technical and Methodological aspects

2.1. INTRODUCTION

Ultrasonography is a technology which is gaining rapid acceptance by clinicians. The development of transducers for vaginal use was the result of the discontent with the information that could be obtained with the traditional abdominal scanning techniques leading to the manufacturing of transducers so small that they could be inserted into the vagina. This chapter deals with the technical and methodological aspects of transvaginal sonography and its patients' acceptability.

2.2. TECHNICAL ASPECTS

Optimal ultrasound imaging of the female pelvic organs is difficult to achieve. This is due to the pelvis being "crowded" with various structures of similar acoustic impedance. The distance from an abdominal probe to these organs is relatively large, precluding the use of frequencies higher than 5.0 MHz. This limits both axial and lateral resolution. Axial resolution is the minimum reflector separation required along the direction of sound travel so that separate reflections will be produced. The important parameter in determining axial resolution is the ultrasonic pulse length (spatial pulse length)[1]:

$$\text{axial resolution} = \text{spatial pulse length} / 2$$

Spatial pulse length can be decreased by increasing the frequency and/or reducing the number of cycles in each pulse. The latter is achieved by

increasing the transducer damping. When damping is reduced to a minimum (e.g. 2 – 3 cycles per pulse), the only way to improve axial resolution is to increase frequency. Lateral resolution is the minimum separation in the direction perpendicular to the course of the ultrasonic beam. This is the minimal distance between two reflectors that will produce two separate reflections when the beam is scanned across them. Lateral resolution is directly proportional to the beam diameter or width. Increasing the frequency reduces the beam diameter. A method of improving image quality is taking pelvic scans while the bladder is full. By introduction of a fluid–filled space, it is possible to observe more clearly some of the pelvic organs. However, it

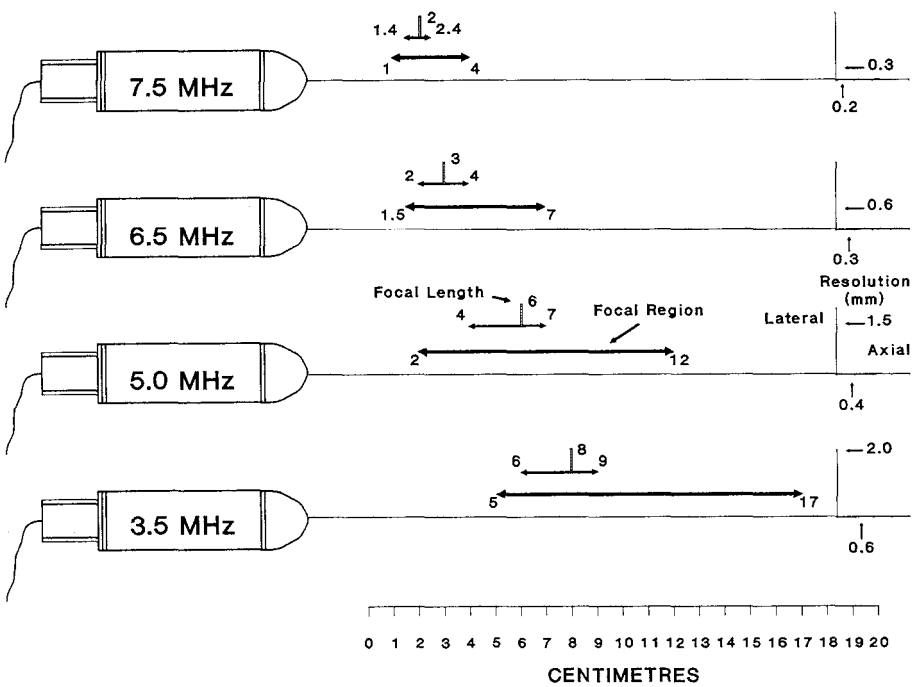


Figure 2.1. *A representation of the physical properties (axial and lateral resolution, focal length and focal region) of transducers with different emission frequencies.*

appears that a full bladder distorts the anatomy[2] and the problems discussed above are still present, so many fine details cannot be visualised. The concept of the vaginal transducer solved many of these problems and made it possible to obtain high-quality images of the pelvic anatomy. The main improvement is achieved by placing the ultrasonic probe closer to the pelvic structures. The relevant anatomical structures for transvaginal imaging are almost always within 9 cm of the vaginal fornices. The closer the object of interest, the higher the frequency that can be utilised and, therefore, the better the axial resolution. It is possible to increase the transducer frequency up to 7.5 MHz, while attenuation is still acceptable. Axial resolution is improved by 40 – 50% compared with the resolutions obtained with the conventional 2.5 to 5.0 MHz transducers used in abdominal scanning. Lateral resolution is also improved with the use of higher frequency, and stronger focusing is made possible by the proximity of the scanning head to the pelvic structures. A schematic representation of the physical properties of transducers with different emission frequencies is provided in Figure 2.1[3]. Transvaginal sonography is preferably performed with an empty bladder. A full bladder may displace most of the pelvic organs beyond the reach of the focal zone of the transducer. Moreover it will distort pelvic anatomy.

2.3. ACCEPTABILITY

The advantages mentioned above completely cover the objectives of the examiner in trying to arrive at a more accurate diagnosis. Before embarking on a new technique the question of patients' acceptability has to be answered. With the introduction of transvaginal sonography an inquiry was conducted into the acceptance of this new method. A total of 100 patients in which both transabdominal and transvaginal sonography was planned was fully informed about both sonographic techniques. It is noted that at that time most patients do not associate ultrasound scanning with a vaginal examination. The similarity between the vaginal transducer and a speculum was mentioned. Only three patients experienced slight discomfort while introducing the probe into the vagina. In general, transvaginal sonography was far preferred to

abdominal scanning. The answers revealed that this was mainly due to the fact that a full bladder was not necessary using the latter approach. Next to the discomfort experienced from pressure of the abdominal transducer on a tense bladder, this approach also means that patients have to drink about one litre of fluid one hour before the abdominal scan is performed. This may cause disruption of the daily routine, especially in patients who need serial sonographic evaluation such as monitoring of follicular growth. Particularly, these patients complained about this "necessary precaution" and they felt that it accentuated their (infertility) problem. Also the examiner may be faced with a problem. If a quick diagnosis is needed, there is no time to wait for a full bladder. Drinking can even be contraindicated when surgery is in question. In that case intravenous fluid application in combination with a diuretic drug or bladder filling through a catheter will provide a full bladder, but these are not the routine out-patient procedures.

2.4. THE EXAMINATION

For optimal imaging the following three main movements of the vaginal probe are available[4]:

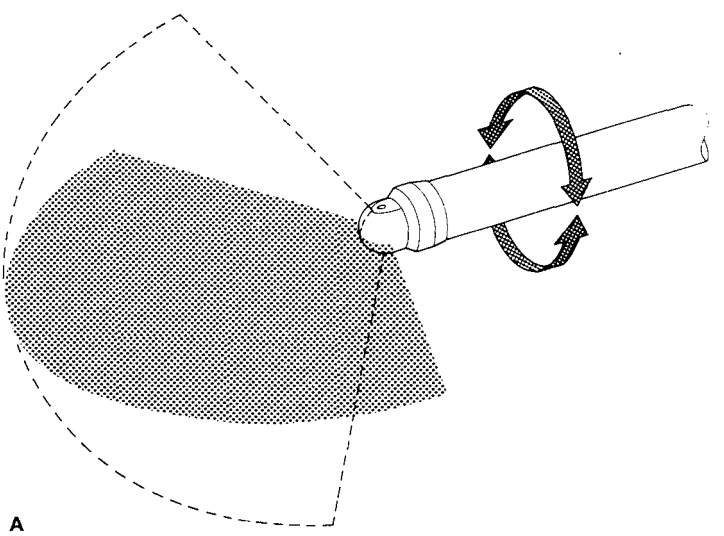


Figure 2.2a. *Rotating the probe along its longitudinal axis to get the right dimensions of a structure.*

1. rotating the handle slowly along the longitudinal axis of the probe to change the scanning plane along a 360 degrees range (Figure 2.2a),

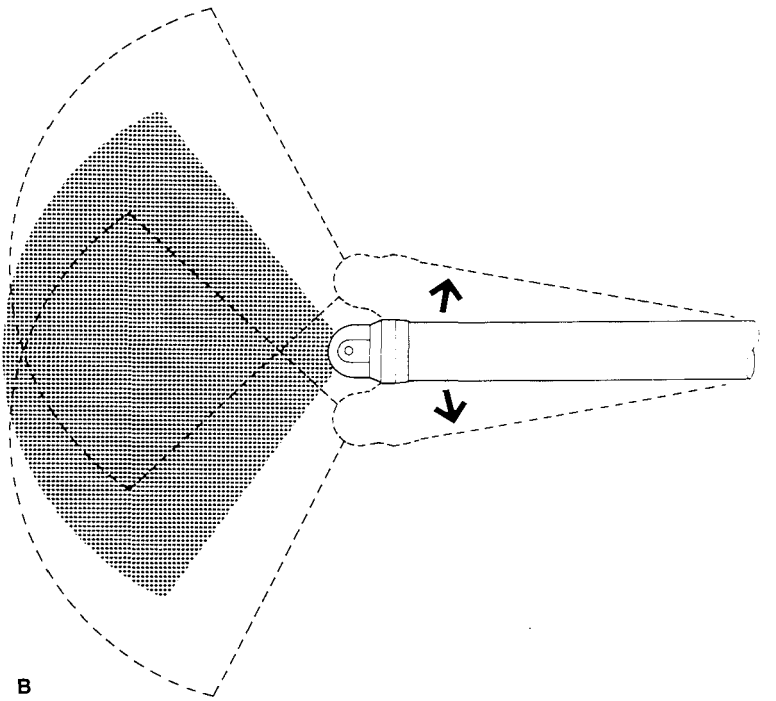


Figure 2.2b. *Angling or tilting of the probe to point at the structure of interest.*

2. tilting or angling the shaft by its handle so as to point the tip of the probe in any direction in the pelvis (Figure 2.2b),

3. pushing – pulling the entire probe to bring a deeper or a closer situated organ or structure into the focal region (Figure 2.2c).

Pelvic structures should always be studied in a horizontal and vertical plane (Figure 2.3a and 2.3b). The first structure on the path of the vaginal probe to its final position for scanning the pelvis is the cervix (Figure 2.4). If the cervix has to be scanned it may be done as the probe penetrates 3 cm into the

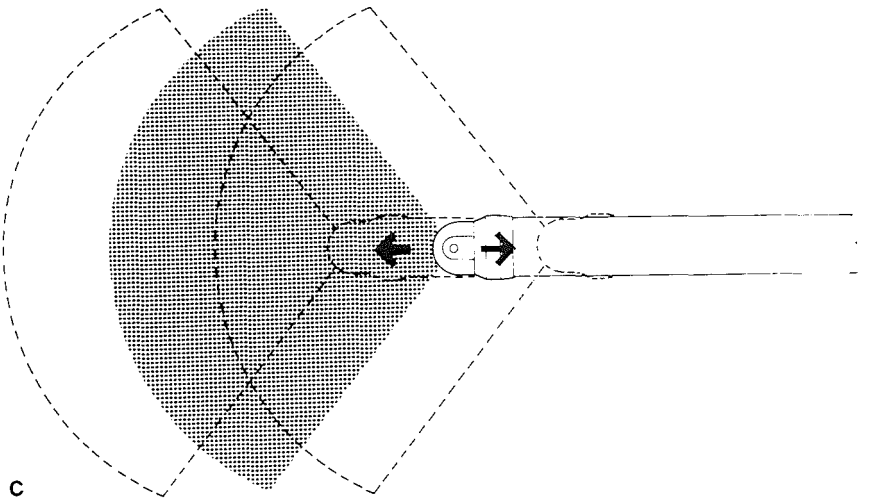
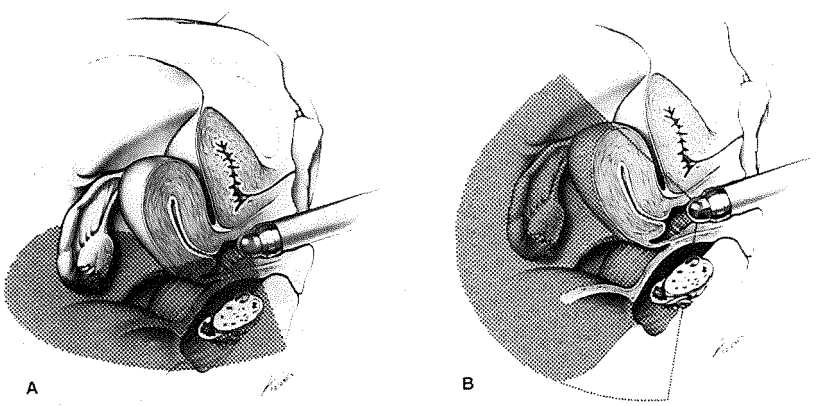


Figure 2.2c. *Pushing or pulling the probe to get deeper or closer structures in focus (2,a,b,c Reprinted with permission from Timor-Tritsch IE & Rottem (eds), Transvaginal sonography, 1988).*



Figures 2.3a & 2.3b. *Illustration of a horizontal (A) and a vertical plane (B) of scanning (Reprinted with permission from Timor-Tritsch IE & Rottem S (eds), Transvaginal sonography, 1988).*

vagina, so before the tip of the transducer reaches the cervix itself. The most

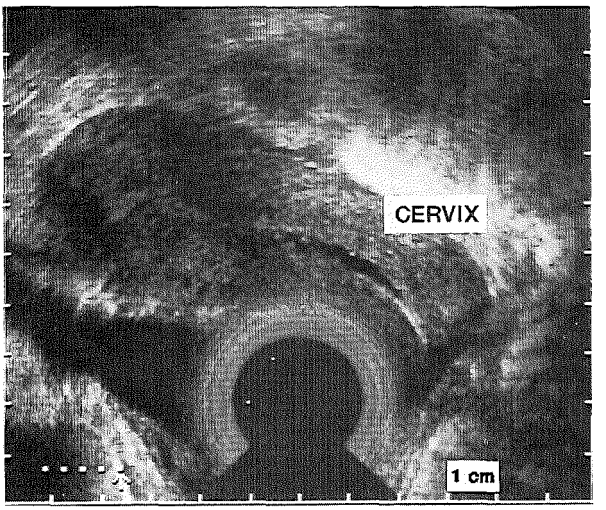


***Figure** 2.4. Sagittal plane of scanning of a normal cervix.*

prominent landmark in the pelvis is usually the uterus (Figure 2.5). If the

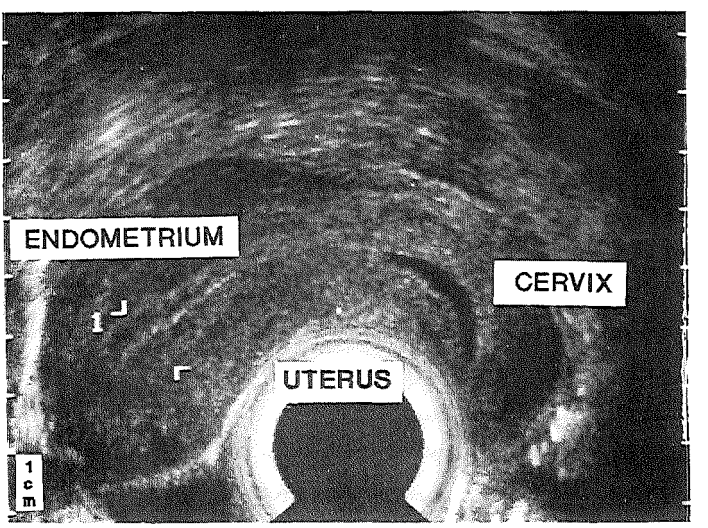


***Figure** 2.5. Sagittal plane of scanning of an uterus with thick pre-ovulatory endometrium.*

uterus is detected on the screen, systematic scanning should start. The direction of the probe and the plane of scanning (horizontal or vertical) gives

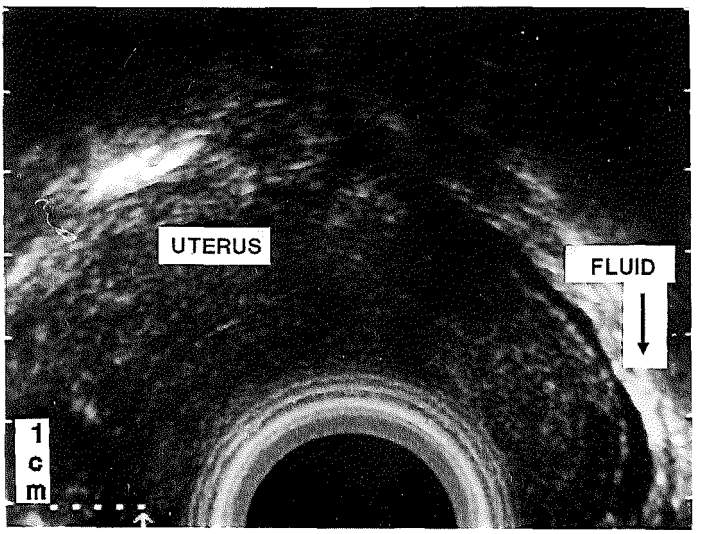


Figure 2.6. *Normal amount of fluid in the cul-de-sac just before or after ovulation.*

information about the position of the uterus (anteflexed or retroflexed, anteverted or retroverted). After the uterus and its contents are examined, the pouch of Douglas should be sought. In many cases there is some fluid present in the cul-de-sac (Figure 2.6). Free fluid outlines the posterior wall of the uterus and sometimes even the ovaries. The tip of the transducer should now be pointed toward the side of the uterus to visualise the adnexa. The ovaries are the most prominent structures (Figure 2.7). If the uterus and the ovaries are localised, the tubes may be scanned. They should be sought posterior to the imaginary horizontal plane drawn at the level of the endometrial line at the sides of the uterus. If they are normal and healthy or no fluid surrounds them, they cannot be visualised[5]. If pathological (e.g. dilated, thickened, fluid-filled or housing a gestational sac), they are usually recognised easily (Figure 2.8)[5]. The next step is to look for space-occupying structures in the

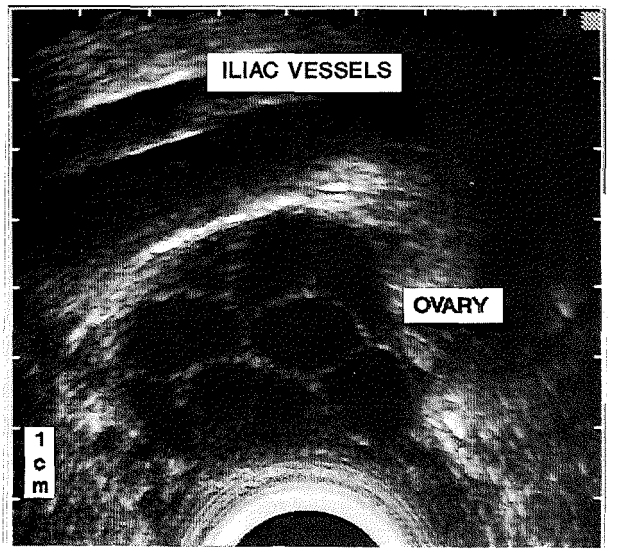


***Figure** 2.7. An ovary showing multifollicular development following stimulation with gonadotropins.*

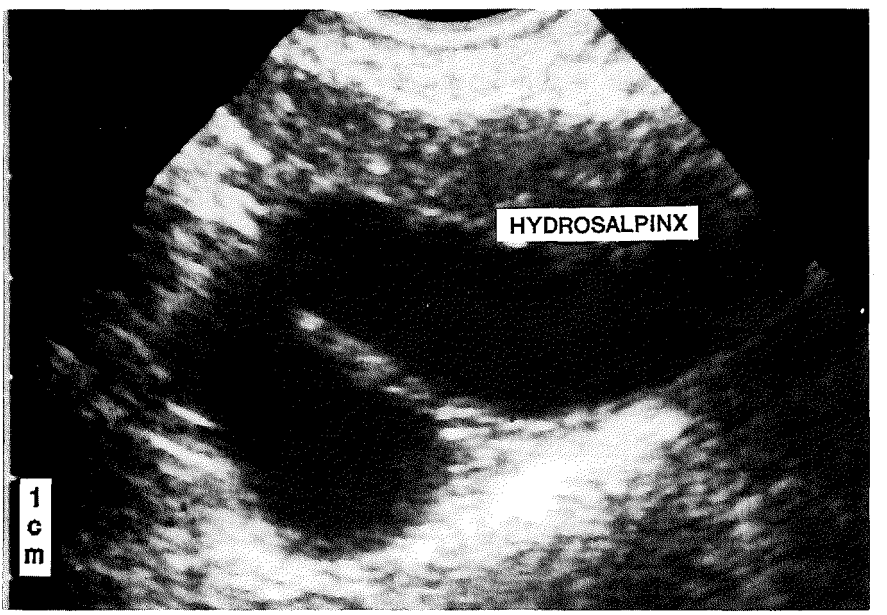


***Figure** 2.8. A fluid-filled, dilated Falloppian tube (hydrosalpinx).*

pelvis. The entire pelvis must be systematically "covered" by horizontal and vertical sonographic planes, and a thorough search should be made along them. The effective focal zone of high frequency transducers ends at 7 – 8 cm. One may see structures further away than 8 cm, but they appear "blurred" and are irregularly outlined. A fairly large mass may be missed by vaginal scanning if, because of its size, it does extend out of the reach of the vaginal transducer. If a pelvic mass has to be ruled out or is suspected, an additional transabdominal scan should be performed. Some special manoeuvres can improve the diagnostic potential of the technique[6]: The examiner may place his/her free hand on the lower abdomen to bring pelvic structures closer to the tip of the probe, as in a regular bimanual pelvic–abdominal examination. In case of pelvic pain, localisation of the point of maximal intensity may be attempted under direct vision and gentle pressure with the tip of the transducer. Diagnosis of pelvic adhesions becomes possible by the "sliding organs sign": the transducer tip is pointed at the uterus, ovaries, or any pelvic finding (e.g. ovarian mass, tubo–ovarian complex), and a gentle push–pull movement of several centimetres is started. If no adhesions are present, the organs will move freely in the pelvis. This displacement of organs is perceived on the screen as a sliding movement. One may, for instance, observe the free sliding of an ovarian mass over the lateral pelvic wall, which of course is static. In the case of a tubo–ovarian complex, the relative locations of the uterus, tube, and ovary will not change under the pushing motion of the probe, because of extensive adhesions preventing normal and physiological sliding of these organs.

2.5. TRANSVAGINAL ULTRASOUND EQUIPMENT USED IN THE PRESENT STUDY

1. Diasonics DRF 400 equipped with a 5.0 and a 7.5 MHz mechanical sector scan transducer. I_{spta} = 2.0 mW/cm^2 at a depth of 4.5 cm for both frequencies.

2. Toshiba SAL 77B equipped with a 5.0 MHz electronic convex sector scan transducer. $I_{spta} = 8.0$ mW/cm^2 at a depth of 4.5 cm.

3. Kretz Combison 310 equipped with a 5.0 and a 7.5 MHz mechanical sector scan transducer. $I_{spta} = 7.8$ mW/cm^2, depth not specified for both frequencies.

The ultrasound equipment mentioned under points 1 and 2 was used in the morphological, biometrical and dynamic studies, while the last apparatus was used in a few patients described in chapter 5.2. as well as for producing some photographic illustrations.

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CHAPTER 3

THE SAFETY OF DIAGNOSTIC ULTRASOUND WITH PARTICULAR REFERENCE TO THE TRANSVAGINAL APPLICATION

3.1. INTRODUCTION

Ultrasound is a mechanical wave phenomenon: by definition it is made up of sound waves with a frequency beyond the upper physiological auditory limit (20.000 Hz). Generally, medical ultrasound is performed with frequencies higher than 1.0 MHz. During propagation of the ultrasound pulse the structures of the tissues are made to vibrate on both the molecular and the cellular level. In addition to density (specific mass), the elastic properties of the tissues determine the propagation of ultrasound and its biological effects. These effects differ from those caused by ionising radiation (X-rays, gamma rays etc.). Ultrasound is neither ionising nor are its effects cumulative. The fact that it lacks mutagenic effects means that the cells suffer from reversible damage or are destroyed. Destruction is directly related to the radiation dose. Hence the biological effect of ultrasound may be viewed as an "all or none" phenomenon[1]. In general, the physical phenomena induced by ultrasound may be separated into thermal (heat generation) and non-thermal effects (radiation force, cavitation and microstreaming). A direct relationship between sound intensity and biological effects is not always present, moreover an increase in frequency does not always influence all effects uniformly. Consequently it is important to study the threshold values for the individual biological effects in order to draw up potential guidelines for safe use.

3.2. DIAGNOSTIC APPLICATIONS

The application of ultrasound in medical diagnosis is generally called echography. This comprises the imaging of anatomical structures and tissues

on a screen (echoscopy) as well as detecting the movement of structures and measuring flow in blood vessels (Doppler technique). A wide variety of equipment has been developed for both fields of application and various techniques are available for ultrasound imaging:

A–Mode: a one–dimensional image of the echo magnitude as a function of depth,

M–Mode: a one–dimensional image of the localisations of structures as a function of time,

B–Mode: a two–dimensional image of a section of the body through which the magnitude of the echo is transformed into luminosity on the screen.

There are two types of B mode equipment:

1. Static B Scanners: the transducer is manually moved over the patient's body. The image on the monitor lasts one or more seconds. These scanners have been used in the early years of ultrasound in medicine and they are completely replaced by real time scanners,

2. Real–time B scanners: the image is obtained in a short time, so that one to 50 images per second are produced and the echoscopist is continuously able to follow all changes caused by movements of the transducer or patient (fetus, embryo).

Real–time B scanners are also of two types based on the way in which the movement of the sound beam is obtained:

A. Mechanical real–time scanners:
the transducer makes a tilting movement or is rotated by means of an electric motor. These scanners provide a sector image,

B. Electronic real-time scanners:
they operate according to the "linear array" or "phased array" principle. The transducer consists of a series of small elements transmitting and receiving, either one after the other or in subgroups (linear array) or phased (phased array), resulting in a rectangular and a sector image respectively.

The Doppler apparatus for velocity measurements can be either of the "continuous wave" (CW) or the "pulsed Doppler" (PD) type of equipment. The latter can also be used for two-dimensional velocity imaging in the colour Doppler mode. We did not use Doppler equipment in our study. Therefore we will not discuss the specific fields of application.

3.3. THE BIOLOGICAL EFFECTS: physical aspects.

3.3.1. The generation of heat

The conversion of ultrasound energy into heat due to absorption may lead to a rise in temperature in the tissues. The absorption increases with a rise in frequency as well as a rise in ultrasound pressure (=intensity). This ratio is as follows[2]:

$$\delta W = 0.23 \, \alpha \, (f) \, I,$$

where δW = local generation of heat per second in a volume of 1 cm^3 ($J.cm^{-3}.s$),

α (f) = absorption coefficient in decibels per centimetre (dB/cm); in most tissues the coefficient is proportional to the frequency,

and

I = local average intensity per time unit in W/cm^2.

Biological effects may be expected when the temperature in the tissues exceeds 42 °C. Absorption in various tissues differs considerably, so that heat generation resulting from a specific intensity is also affected by the location and direction of the ultrasound beam. Since the intensity decreases

exponentially with penetration due to absorption for non-focusing transducers, the heat effect will be greatest in those tissues which lie closest to the transducer. Finally, the rise in temperature due to absorption also depends on the blood flow and on the property of the tissues to conduct heat (heat release). Experimental studies revealed that tissues in mammals exposed to diagnostic ultrasound intensity levels show no significant rise in temperature[3, 4].

3.3.2. Radiation force

Ultrasound generates a force on the surface between two tissues which may produce a biological effect. This radiation force may also cause effects in cell suspensions such as stasis and aggregation[5]. These phenomena usually occur when standing waves are generated, for example at the point of transition from soft tissue to bone.

3.3.3. Cavitation

Cavitation may occur during the use of a continuous wave (=CW) or pulsed wave (=PW). It can occur only if microscopically small gas bubbles are present in the tissue or blood. With CW ultrasound these bubbles may enlarge as a result of the radiation to a size that will cause resonance (stable cavitation). However, when pulsed ultrasound is used with a relatively high intensity the gas bubble may implode, resulting in locally very high temperatures (formation of radicals) and pressures (shock wave). This phenomenon is called transient cavitation. Some authors are of the opinion that, if the peak intensity of the ultrasound is less than 1500 W/cm^2 transient cavitation does not occur in-vivo[6, 7]. However, with a continuous wave intensity of 150 to 500 mW/cm^2 stable cavitation may occur in-vivo [5, 8].

3.3.4. Radiation torque

A mechanical effect that causes microstreaming is the so-called radiation torque, which produces a rotating movement of cells and even intracellular structures. Consequently microstreaming will be present around the cells and the cell wall may well be damaged by hydrodynamic shear stresses. There are indications that an oblique sound beam when directed at blood vessel walls

may produce microstreaming. A third cause of microstreaming is stable cavitation (also see 3.3.3.). High velocity gradients are established around the oscillating gas bubbles which may damage structures like blood cells. No direct evidence exists that microstreaming is of any importance in the range of intensities used in diagnostic ultrasound for in–vivo diagnosis[9].

3.4. COMBINED THERMAL AND NON-THERMAL EFFECTS ON TISSUES

Krizian has shown that the processes mentioned above when combined may influence each other[10]. When the temperature is raised, red blood cells are more prone to damage due to mechanical forces. In high intensity non–homogeneous sound fields the chance of red blood cells being damaged from the combined effects of elevated temperature due to absorption and mechanical forces provoked by the presence of pressure gradients, is much higher than when only one of these two processes influences the cells.

3.5. EXPERIMENTAL RESULTS AND DIAGNOSTIC CONDITIONS

The effects of ultrasound on biological systems have been described in approximately 800 publications. The American Institute of Ultrasound in Medicine (AIUM) Bioeffects Committee reviews periodically the literature concerning this topic and formulates guidelines for safe use[11]. The following facts should be taken into account:

A. The occurrence of a marked rise in temperature, stable cavitation, radiation force and (micro–) streaming has been observed only when continuous wave (Doppler) ultrasound of high intensities ($\geq$ 100 mW/cm^2)[11] or pulsed ultrasound of long pulse lengths ($\geq$ 1 microsecond) with high intensities ($\geq$ 100 mW/cm^2) was used[11]. Thus it is reassuring to realise that the pulsed ultrasound used in clinical situations is emitted in pulses of one microsecond followed by a pause of one millisecond. In an

examination lasting 15 minutes, the tissues are effectively exposed to ultrasound to a maximum of only one second. Therefore, medical diagnostic equipment should conform to the requirement that the intensity (I_{spta}) emitted does not exceed 100 mW/cm^2 as is recommended in a report of a committee of the Dutch Health Council[12].

B. The biological effects of ultrasound depend on local conditions during insonation. Certain cell types used in in–vitro experiments may be specifically sensitive to certain ultrasound effects.

C. Extrapolation of the results of in–vitro studies to cover clinical results possesses questionable validity. In–vitro studies may produce various artificial situations, such as very high intensities in the tissues closest to the transducer due to non–uniformity of the sound beam, and the development of static waves and focusing due to ultrasound reflection from curved structures.

D. A major problem arises if clinical studies are compared with animal studies. In the mouse an ultrasound intensity of 20 mW/cm^2 at the level of the maternal abdomen results in a virtually unchanged intensity at the level of the fetus. In humans, by contrast, a threefold reduction in intensity takes place between the maternal abdominal wall and the fetus[12]. In addition, the animal will lie absolutely still during the examination and the fetus will be fully insonated, whereas in humans the transducer is usually repositioned repeatedly.

3.6. THE PROBLEM STATED

The introduction of diagnostic ultrasound may be viewed as one of the most important developments in medical diagnosis during the past 25 years. Extensive studies have been carried out to evaluate the potential biological effects of ultrasound. Extrapolation of the results of animal studies or tissue culture experiments into the possible effects of echoscopy in pregnancy

presents a problem. In most publications no obvious evidence of any harmful effect of diagnostic imaging in pregnancy is provided, but it is still premature to state that it is harmless. There are no data available on possible deleterious effects of the transvaginal application of diagnostic ultrasound in early pregnancy. Before the start of our project we compared abdominal and vaginal real–time B mode transducers for the factors influencing the sound intensity (I) at the level of the embryo. The following factors have to be taken into account:

1. I_{sata}, I_{spta}, I_{sptp}, duration of the pulse and the pulse frequency.

2. Distance between transducer and embryo, frequency of the transducer (MHz) and the absorption of ultrasound in the different tissues.

Ad 1. There are, as already has been mentioned, different ultrasound systems with their own characteristics. The acoustic dosage is for example dependent on which area in a certain period of time is covered. Furthermore, intensity is determined by the pulse frequency and pulse duration. The intensity will have a peak value during the pulse, while the mean value in time is much lower. Three definitions are often used to indicate and define characteristics of transducers:

SATA Intensity: Temporal mean intensity averaged over the beam cross–sectional area in Watt/cm^2. This intensity is often reported by manufacturers (SATA: Spatial Average, Temporal Average).

SPTA Intensity: The local maximal and temporal average intensity in Watt/cm^2 (SPTA: Spatial Peak, Temporal Average).

SPTP Intensity: The local maximal and temporal peak intensity in Watt/cm^2. This intensity is of course higher than the SPTA or SATA value (SPTP: Spatial Peak, Temporal Peak).

Table 2.I presents a classification of the two most used definitions of intensity in relation to the three different diagnostic methods of ultrasound systems.

Table 2.I. Values for the intensities of the various diagnostic ultrasound systems (modified from Report No. 13 of the Dutch Health Council[12])

TECHNIQUE	I_{sata} (mW/cm^2)	I_{spta} (mW/cm^2)
Diagnostic * (pulse/echo imaging)	0.01–15	0.5–200
Doppler		
continuous wave	10–400	10–1000
pulsed Doppler	5–32	20–290

* The group diagnostic pulse/echo imaging includes both M-mode and real-time imaging systems.

The start intensities mentioned above can be the same for transducers for abdominal and vaginal application, but the intensity at the level of the object of study (embryo, fetus) may be different (see Ad 2.).

Ad 2. Figures 3.1 and 3.2 show the actual situation of transabdominal and transvaginal echography in early pregnancy. In order to calculate the sound pressure at the level of the embryo we used the graphics composed by Wells[13], collecting the data from other publications. The absorption of sound pressure in the different tissues involved is as follows:

Fat : 1 dB/cm/MHz,
Muscle : 2 dB/cm/MHz,
Water : 0.01 dB/cm/MHz.

In order to make the comparison not too complicated we assume that the abdominal and vaginal transducers have the same start intensity and a frequency of 3.0 and 7.0 MHz respectively.

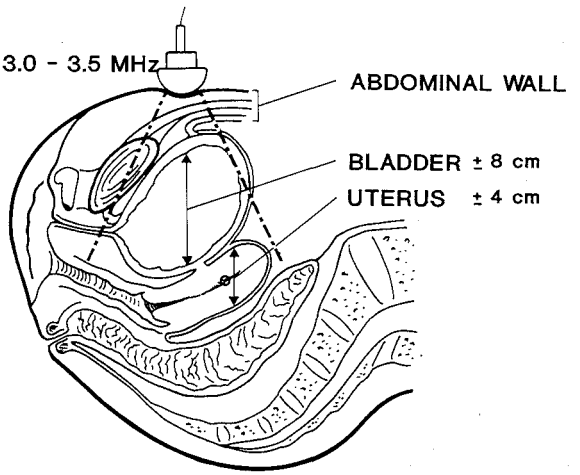

FIGURE 1

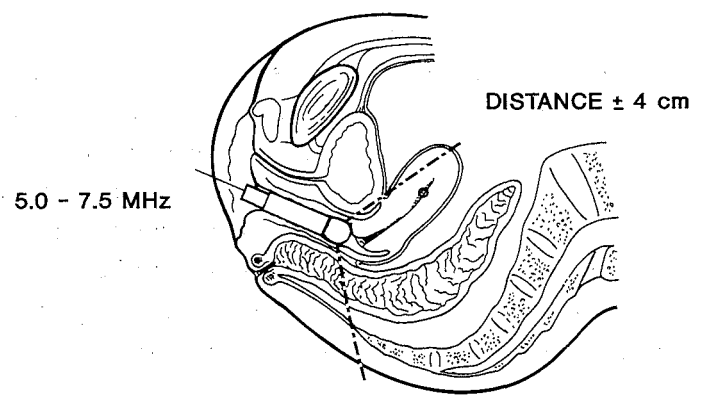

FIGURE 2

Figures 3.1 & 3.2. *Schematic representation of transabdominal (Figure 1) and transvaginal (Figure 2) sonography.*

Table 2.II. Absorption of sound intensity in dB in the various tissues employing transabdominal and transvaginal sonography in early pregnancy.

Transabdominal (3.0 MHz)			Transvaginal (7.0 MHz)		
Fat	3 cm	–9 dB	Uterus	4 cm	–56 dB
Muscle	2 cm	–12 dB			
Bladder	8 cm	–0.24 dB	TOTAL		–56 dB
Uterus	2 cm	–12 dB			
TOTAL		–30.24 dB			

From the data presented in Table 2.II one can conclude that compared with transabdominal sonography the application of transvaginal sonography results in higher absorption, mainly because of the use of a higher frequency which means a lower intensity at the level of the embryo. Recently introduced advanced techniques like beam narrowing (especially applicable to higher frequencies) and focusing can influence the result of the summation. However, these effects are relatively small and they will not affect the final conclusion. To our knowledge no studies are published in which the safety of mother and embryo following transvaginal sonography in early pregnancy is evaluated. Nevertheless, the literature on the effects of ultrasound on biological systems and the above presented calculation convinced us that our project would not have any deleterious effect on mother or embryo. The presented data indicate that early pregnancy scanning using high frequency transvaginal sonography is a safe procedure.

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CHAPTER 4

MORPHOLOGICAL AND BIOMETRICAL ASPECTS OF NORMAL EARLY PREGNANCY DEVELOPMENT

INTRODUCTORY REMARKS

Information on early morphological development of the embryo has been based on transabdominal ultrasound studies. Transvaginal sonography can be used earlier and a more detailed analysis of the embryo and its surroundings is possible. This is of clinical importance when a pathological development of pregnancy is suspected. This chapter consists of two parts. In the first part (4.1.) the role of transvaginal sonography in visualising embryonic structures as they develop during the first six weeks following conception (eight weeks menstrual age) will be determined. This will be preceded by an overview of what is known about early human embryonic morphology from animal and human post–mortem specimens. In the second part (4.2.), there will be a reappraisal of the embryonic crown–rump length measurement in early pregnancy using transvaginal sonography.

4.1. NORMAL MORPHOLOGICAL DEVELOPMENT IN EARLY PREGNANCY

4.1.1. INTRODUCTION

Transabdominal sonography has been used as an effective diagnostic and research tool in obstetrics. Its application is mainly in the second and third trimester of pregnancy. Its use in the first trimester is relatively limited and mainly diagnostic in nature. The introduction of the vaginal transducer which allows higher emission frequencies, leading to a better resolution has opened new possibilities in the study of early gestation. The present study describes in detail the information which can be gathered on embryonic morphology using transvaginal sonography in the first six weeks following conception.

4.1.2. SUBJECTS AND METHODS

Singleton and multiple pregnancies of 62 women (83 embryos) were studied. All patients became pregnant by means of IVF treatment; the duration of pregnancy was therefore exactly known. Twenty-three patients (28 gestational sacs, 28 embryos) were scanned from day 17 following follicle aspiration (FA) at two or three days intervals until day 23 after FA, then every day until the detection of embryonic cardiac activity. The other 39 patients (55 embryos) were examined daily from day 25 after FA until the appearance of embryonic cardiac activity, thereafter they were followed at intervals of two or three days until 42 days after FA. All examinations were performed using the Diasonics DRF 400 and the Toshiba SAL 77B, the former equipped with a 5.0 and a 7.5 MHz mechanical sector scan transducers and the latter with a 5.0 MHz electronic sector scan probe.

4.1.3. EMBRYONIC DEVELOPMENT

Before providing a detailed description of the morphological features of early pregnancy as visualised by transvaginal sonography an overview of the highlights of embryonic development during the first six weeks following conception will be presented[1].

WEEKS 1 and 2: fertilisation and implantation

Spermatozoa deposited in the vagina pass through the cervical canal, the uterine cavity and along the Fallopian tube to the ampulla, where fertilisation usually occurs. As the zygote passes down the Fallopian tube,

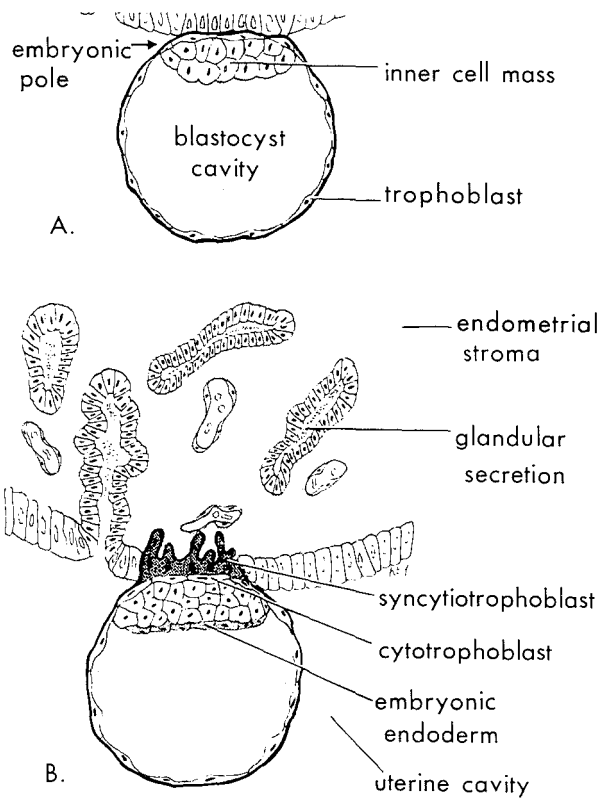

Figure 4.1.1. *Blastocysts containing the embryoblast, a blastocyst cavity and the trophoblast (Reprinted with permission from Moore KL (ed), The developing human, 1973).*

it undergoes cleavage in a number of small blastomeres. About 3 to 5 days after fertilisation, a ball of approximately 16 blastomeres, called the morula, enters the uterus. Soon a cavity is formed within the morula converting it into a blastocyst consisting of: 1) an inner cell mass (embryoblast), which gives rise to the embryo; 2) a blastocyst cavity and 3) an outer layer of cells (trophoblast) which encloses the inner cell mass and blastocyst (Figure 4.1.1). Implantation of the blastocyst begins at the end of the first week and ends during the second week. The entire process may be summarised as follows:

1. Zona pellucida disappears (days 4 – 5),
2. Blastocyst attaches to endometrial epithelium (day 6),
3. Trophoblast erodes epithelium and endometrial stroma (day 7),
4. Trophoblast differentiates into cytotrophoblastic and syncytiotrophoblastic layers (days 7 – 8),
5. Lacunae appear in syncytiotrophoblast (days 8 – 9),
6. Blastocyst sinks beneath surface of the endometrial epithelium (days 9 – 10),
7. Lacunar networks are formed by fusion of adjacent lacunae (days 10 – 11),
8. Trophoblast invades endometrial sinusoids, allowing maternal blood to seep into the lacunar networks and establishes the uteroplacental circulation (days 11 – 12),
9. Endometrial epithelium completely re–forms over the implanted blastocyst (days 12 – 13),
10. Marked decidual reaction occurs in the endometrium around the conceptus.

References to days are given only as guidelines and thus are approximations of the truth because early stages of implantation of the human blastocyst have not been observed. Most knowledge about early implantation is based on studies in the Rhesus monkey, but the process is thought to be essentially similar in man[2].

WEEK 3:

During this period rapid embryonic development coincides with the first

missed menstrual period. Major changes occur as the bilaminar embryonic disc is converted into a trilaminar embryo composed of three primary germ layers. The most important features of the third week are:

1. Formation of the primitive streak and the intra-embryonic mesoderm,
2. Notochord formation,
3. Neural tube formation,
4. Somite formation: division of the paraxial mesoderm into pairs of somites starts cranially by the end of the third week.
5. Intra-embryonic coelom formation: the coelomic spaces subsequently coalesce to form a single horseshoe-shaped cavity which eventually gives rise to body cavities.
6. Blood and blood vessel formation: blood vessels first appear on the yolk sac, on the allantois and in the chorion and develop in the embryo shortly

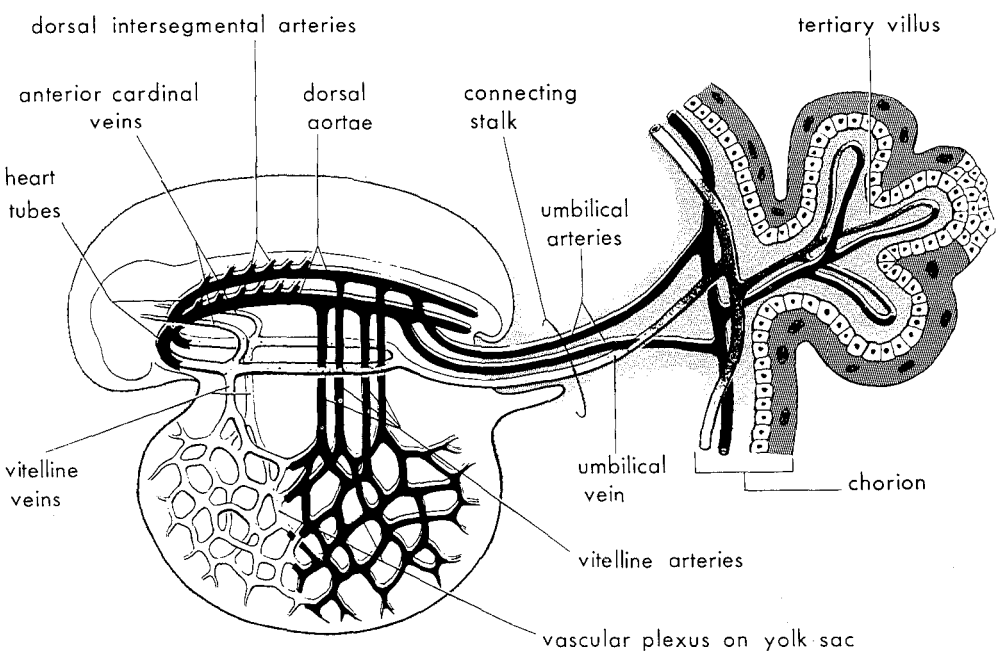

Figure 4.1.2. *Diagram of the primitive cardiovascular system in a 21-day embryo (Reprinted with permission from Moore KL (ed), The developing human, 1973)*

thereafter. At the end of the third week, the heart is represented by paired heart tubes which are joined to blood vessels in the embryo and in the extra-embryonic membranes (Figure 4.1.2).

7. Villi formation: primary villi become secondary villi as they acquire mesenchymal cores. Before the end of the third week, capillaries develop in the villi; this transforms them into tertiary villi.

WEEK 4:

Initially, the embryo is almost straight and the somites produce conspicuous surface elevations. The neural tube is closed opposite the somites, but is widely open at the rostral and caudal neuropores. By day 22 a single heart tube has formed. It elongates and develops alternate dilatations and constrictions. A slight curve is produced in the embryo by the head and tail folds and the heart produces a large ventral prominence (Figure 4.1.3). Three branchial arches are visible by 26 days, and the rostral neuropore is closed.

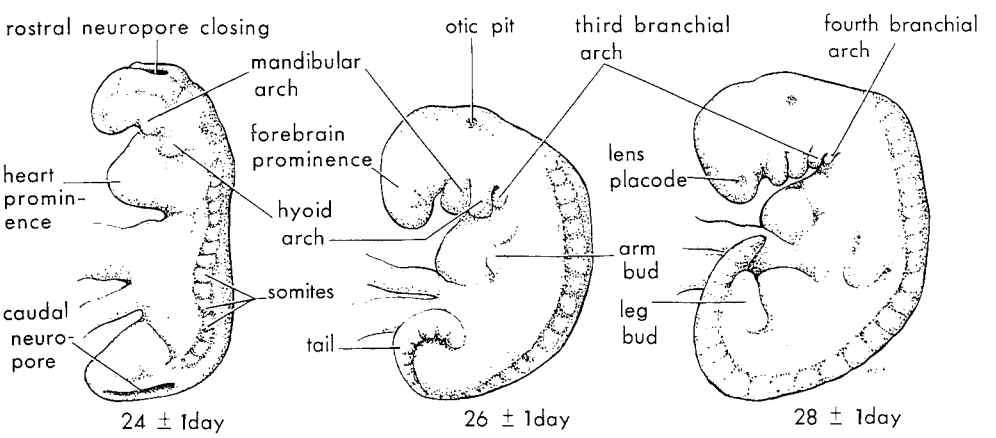

Figure 4.1.3. *A 24, 26 and 28-day embryo each showing a large ventral prominence caused by the heart (Reprinted with permission from Moore KL (ed), The developing human, 1973).*

WEEK 5:

Changes in shape of the body are minor compared with the fourth week, but growth of the head exceeds that of other regions. This extensive head growth is caused mainly by the rapid development of the brain. The face soon contacts the heart prominence (Figure 4.1.4). The limb buds show considerable regional differentiation, especially the fore limbs. The elbow and

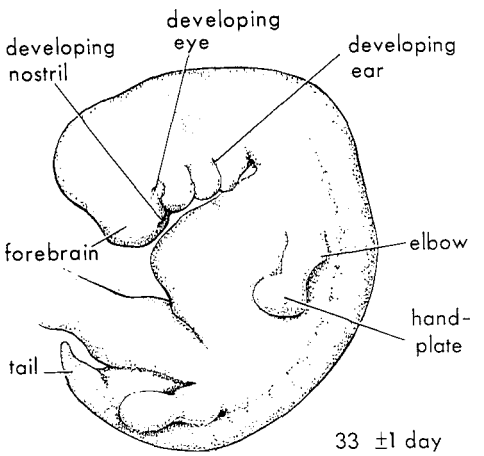

Figure 4.1.4. *A 33-day embryo: Note the contact of the face with the heart prominence and the limb buds (Reprinted with permission from Moore KL (ed), The developing human, 1973).*

wrist regions become identifiable and the paddle-shaped hand plates develop into digital ridges, called finger rays, indicating the future fingers. The development of the hindlimb occurs somewhat later than that of the forelimb in man. Several small swellings develop around the groove between the first two branchial arches; this groove becomes the external auditory meatus and the swellings eventually fuse to form the auricle of the ear. Largely because retinal pigments begin to appear the eye becomes more obvious.

WEEK 6:

The head is now much larger relative to the trunk and is more bent over the heart prominence. The head position results from bending (cervical flexure)

of the brain in the cervical region. By day 42 the trunk and neck begin to straighten. The somites are visible in the lumbosacral region until the middle of this week, but cannot be used as criteria for estimating age at that time. The communication between the gut and the yolk sac has been reduced to a relatively small duct, the yolk stalk or vitelline duct. The intestines enter the extra-embryonic coelom in the proximal region of the umbilical cord; this is called umbilical herniation. The limbs undergo considerable change during the sixth week. At day 37 the forelimbs project over the heart (Figure 4.1.5). By day 38 they have lengthened and flexed slightly so that the future fingers reach the nose. Notches appear between the rays in the hand plates, indicating the future fingers. By day 42 short, webbed fingers are present and notches appear in the foot plates between the toe rays.

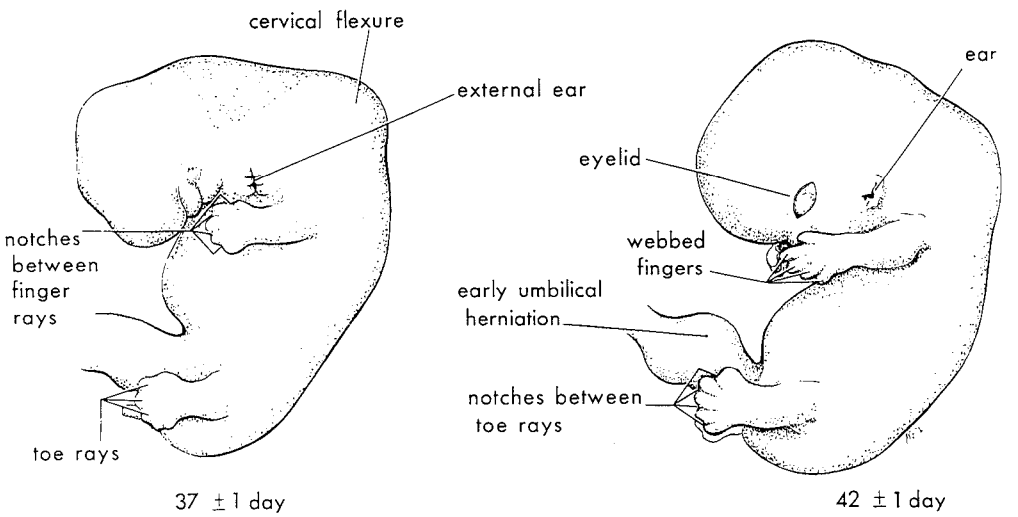

Figure 4.1.5. *A 37 and 42-day embryo: The forelimbs project over the heart (Reprinted with permission from Moore KL (ed), The developing human, 1973).*

4.1.4. SONOGRAPHIC FINDINGS

It will be obvious that no sonographic data of the first two weeks of embryonic development are available. The resolution properties of ultrasound

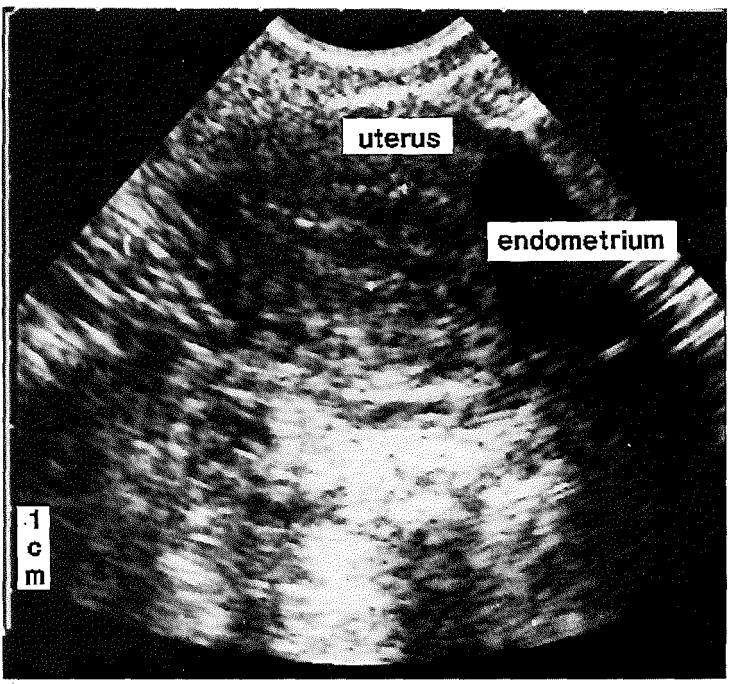

Figure 4.1.6. *Uterus with pre-ovulatory endometrium. The total thickness of the two layers of endometrium is 15 mm.*

waves, even of high frequency are far beyond the detection level of the size of the embryo and its surrounding structures at this stage of pregnancy. After ovulation the endometrium shows secretory changes. The pre-ovulatory appearance of the endometrium is illustrated in Figure 4.1.6. Close to ovulation the total sonographic thickness of the two layers of the endometrium is usually around 10 mm (range 7 – 15 mm). Its aspect at the end of the cycle, just around the expected menstrual period, is disorganised and inhomogeneous. However, the endometrium of a pregnant subject at that stage is thick (12 – 18 mm) and has a homogeneous and congested aspect

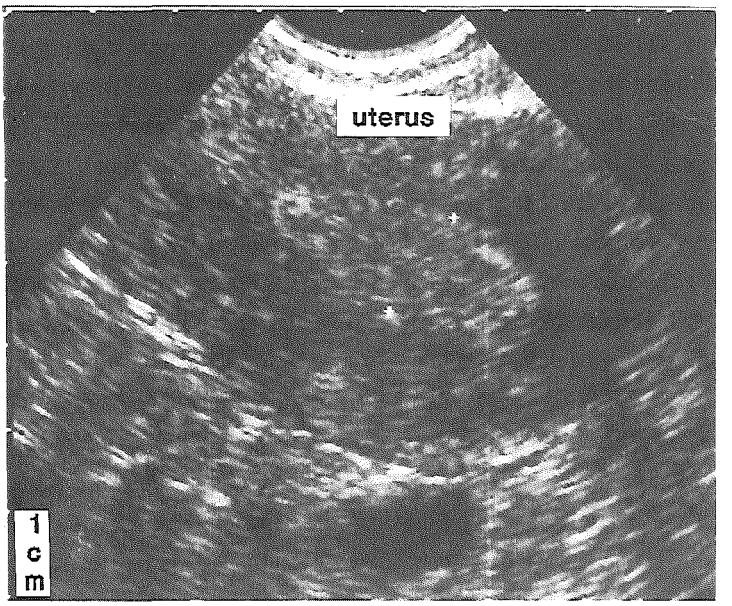

Figure 4.1.7. *Uterus with thick (15 mm) homogeneous, congested endometrium as can been seen in very early gestation.*

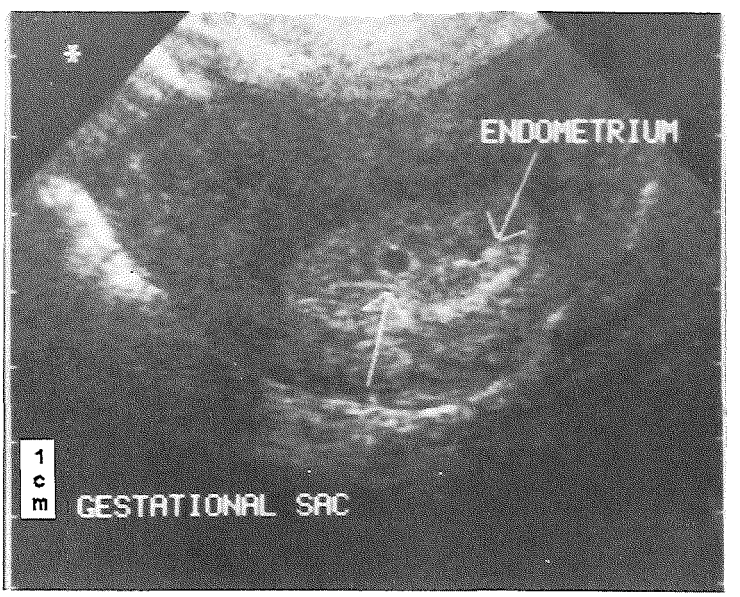

Figure 4.1.8. *Uterus containing a small gestational sac 19 days after follicle aspiration.*

(Figure 4.1.7). Soon after the menstrual period is missed i.e. between 16 and 19 days after follicle aspiration (median 17 days) a gestational sac can be observed (Figure 4.1.8). Between 20 and 23 days after follicle aspiration (median 21 days) the yolk sac can be identified, but the embryonic pole is not discernible yet. The size of the gestational sac varies between 7.5 and 9.2 mm (median 8.1 mm) at that stage. The embryonic pole becomes visible between 24 and 29 days (median 26 days) after follicle aspiration. The most striking evidence of the presence of a embryonic pole is the appearance of cardiac activity. The diameter of the gestational sac at that time varies between 9.0 and 11.4 mm (median 10 mm). The embryonic heart beat appears at a crown–rump length (CRL) between 1.2 and 3.0 mm (median 2.0 mm) and between 25 and 30 days following follicle aspiration (median 27 days). At this stage of pregnancy the embryo is closely attached to the yolk sac. This combined structure is always eccentrically positioned within the gestational sac (Figure 4.1.9). During the first few days when cardiac activity is present, the yolk sac

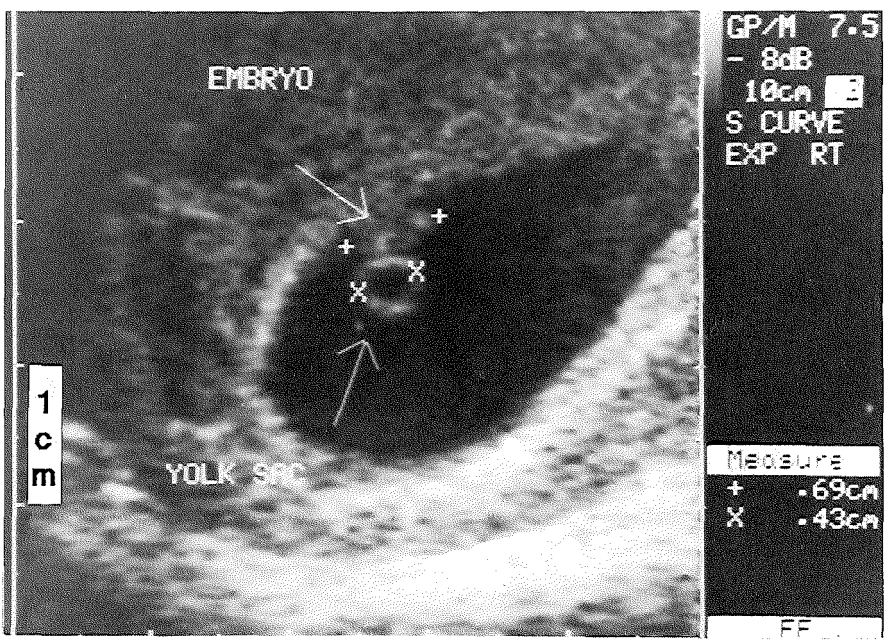

Figure 4.1.9. *Uterus with a gestational sac in which eccentrically a yolk sac and an embryo are visible (yolk sac diameter 4.3 mm and embryonic length 6.9 mm).*

is larger than the embryo (Figure 4.1.10) with a diameter of 3.2 to 5.2 mm (median 4 mm). Lacunar structures of 2 to 3 mm at one side of the gestational

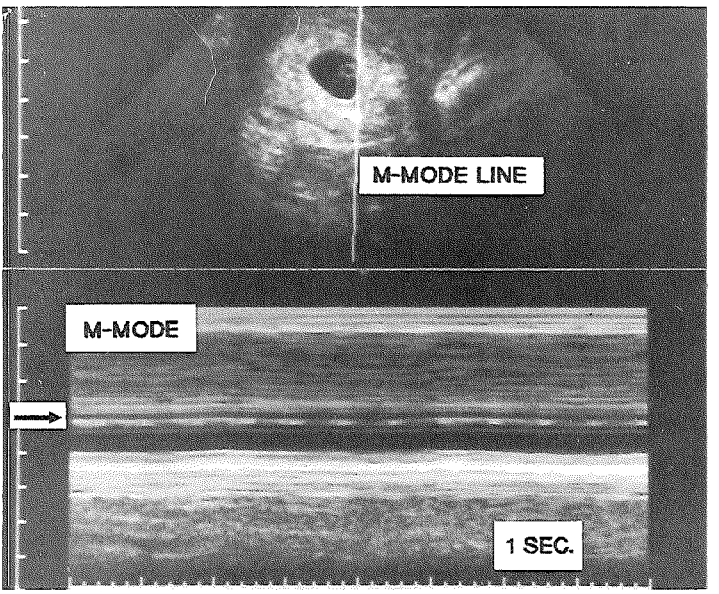

Figure 4.1.10. *M-mode registration of the embryonic heart rate (84 beats per minute) 27 days after follicle aspiration.*

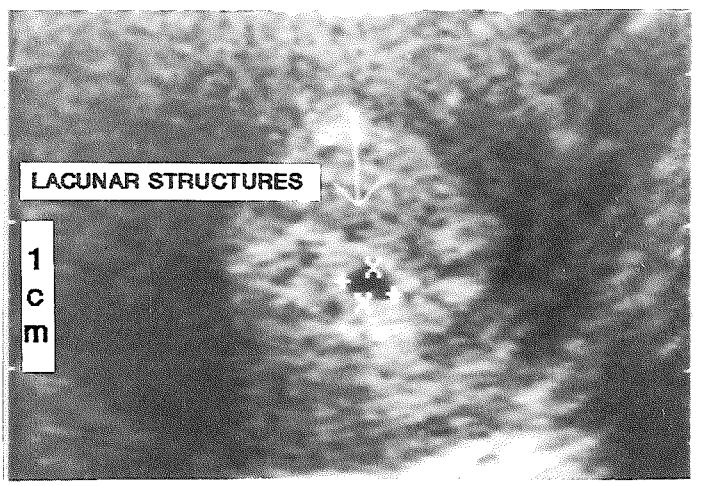

Figure 4.1.11. *Uterus containing a gestational sac. At one side (arrow) lacunar structures are evident.*

sac may be seen and the blood flow in these lacunar structures may be spotted suggesting syncytiotrophoblastic invasion of maternal vessels (Figure 4.1.11).

There is only a slight increase in the size of the yolk sac during the first six weeks following conception (0.5 to 1.5 mm; median 0.9 mm). The distance

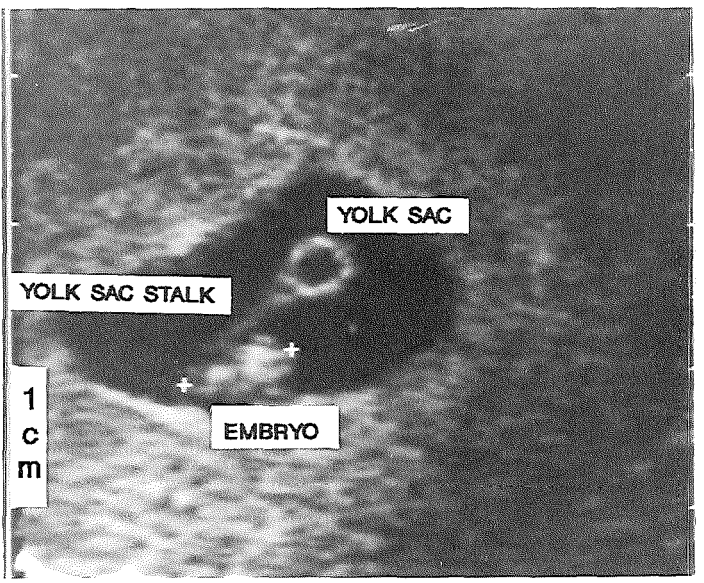

Figure 4.1.12. *A gestation, 33 days after follicle aspiration: the head of the embryo and the yolk sac stalk can be distinguished (embryonic length 8.5 mm).*

between the embryo and yolk sac increases. Sometimes the stalk of the yolk sac can be visualised (Figure 4.1.12). The head becomes distinguishable at the end of week 5 after follicle aspiration (Figure 4.1.12). In week 6 the relative echogenicity inside the developing head is a normal sonographic appearance (Figure 4.1.13). Close to the head, embryonic cardiac activity is present. This is caused by the heart prominence and the bending of the embryo. At more or less the same time the limb buds can be seen. In week 6 the amniotic cavity and the chorionic cavity can be identified. The yolk sac is situated in the chorionic cavity near the insertion of the umbilical cord. This structure

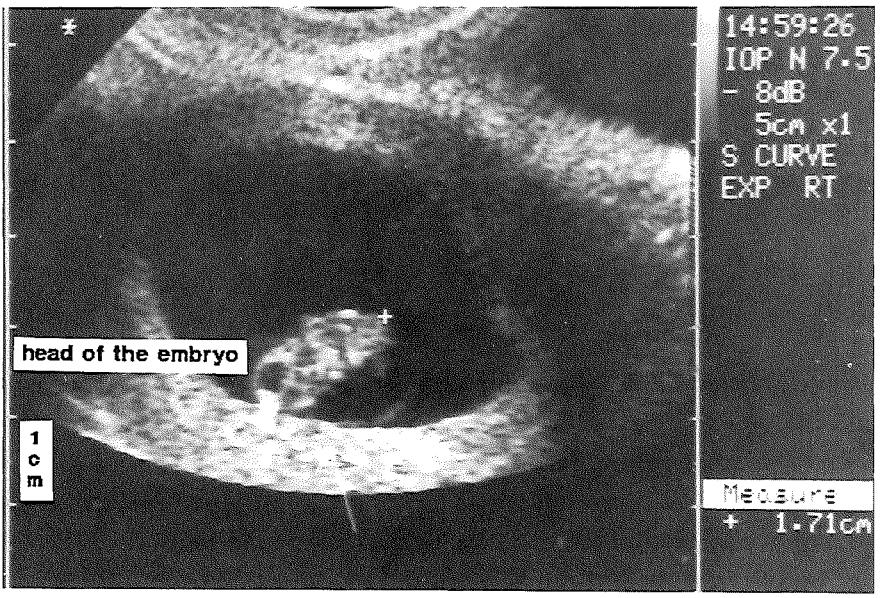

Figure 4.1.13. *A pregnancy 39 days after follicle aspiration: note the relative echogenicity of the developing embryonic head.*

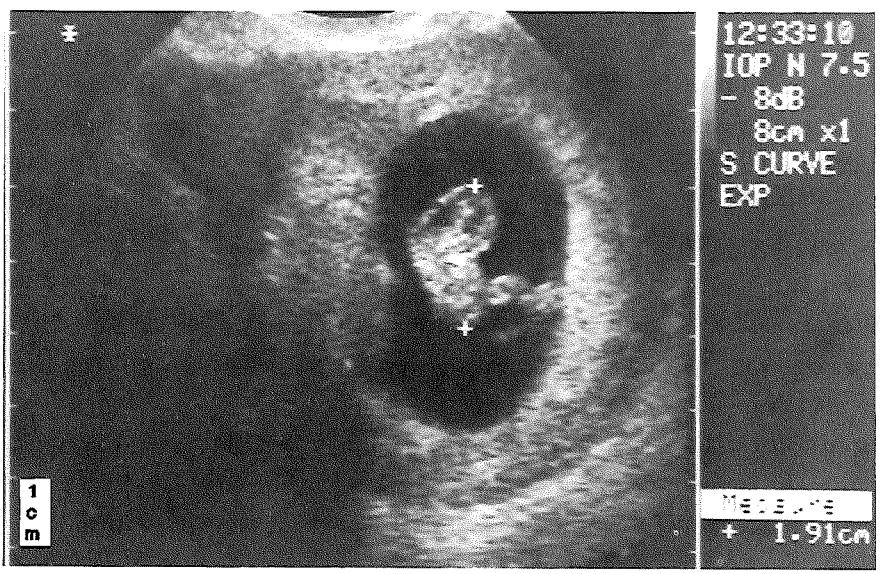

Figure 4.1.14. *The umbilical cord is visible in a gestation 41 days after follicle aspiration. This lateral view of the embryo shows a pronounced fourth ventricle in the rhombencephalon.*

becomes also visible at that time (Figure 4.1.14). A lateral view of the embryo sometimes clearly displays the rhombencephalon, with a relatively large cavity that later on becomes the fourth ventricle (Figure 4.1.14). In week 6 gross body movements can be identified for the first time. However, quantitative and qualitative assessment are not yet feasible.

4.1.5. DISCUSSION

Transvaginal sonography provides detailed information on early human pregnancy. We were able to examine a group of patients in which the duration of pregnancy was exactly known, since all gestations emerged from IVF treatment. The question as to whether these pregnancies can be considered as normal and the results can be extrapolated to in–vivo fertilised oocytes remains open for discussion. In this chapter the development of the embryo and its surrounding structures is described in a semi–quantitative way. We were able to visualise by means of transvaginal sonography relevant embryonic features like chorionic cavity, yolk sac, cardiac activity, the head and limbs about one week earlier than with the conventional transabdominal scanning technique. Due to the resolution properties if high frequency vaginal transducers, a gestational sac in–utero can be seen almost immediately after the menstrual period has been missed. This implicates that this method of scanning is almost as sensitive as the modern commercially available pregnancy tests. There is a similarity between embryonic and follicular development, in that the oocyte as well as the embryo develop in a fluid–filled compartment. The resulting echogenicity allows detection of these structures as soon as their size equals the resolution properties of the vaginal probe, which is between 1 and 2 mm[3-6].

Another cyst–like structure which becomes visible is the yolk sac. Although the human secondary yolk sac is non–functional as far as yolk storage is concerned (it would be better to speak of umbilical vesicle), its development is essential for several reasons. One assumes that it plays a role in the transport of nutrients during the second and third week while the utero–placental circulation is established[1]. Therefore, it acts as a primitive

placenta. From the third until the sixth week blood cells are formed in the yolk sac wall. Primitive germ cells appear in its wall early in the third week and subsequently migrate to the developing gonads, where they become spermatogonia or oogonia[1]. An embryo can not develop without a structure supplying the necessary nutrients in the early stages of development. Therefore, presence of the yolk sac is of crucial importance[6]. It grows slowly and the size of the yolk sac is not very useful in pregnancy dating[6-9]. Embryonic abnormalities may coincide with abnormal development or absence of this structure[9-11] (see chapter 5.1. & 6.3.).

One of the first signs of a viable pregnancy is the appearance of embryonic cardiac activity. Several investigators have shown the value of transvaginal sonography detecting the early heart beat[12-14]. Their data are in accordance with our findings (between 25 and 30 days after ovulation (median 27 days)). Only two authors reported embryonic cardiac activity as early as 21[15] and 22[16] days after conception. However, no mention was made as to how the date of conception was documented. The variability in the pre–ovulatory phase was not taken into account. As patients visited the clinic after the diagnosis of pregnancy was made, this early detection could well be due to early ovulation. Moreover, the size of the embryo is beyond the resolution properties of vaginal transducers and it can, therefore, be questioned if the heart tube already has started to contract at this stage of development[1]. DeCrespigny[12] examining 353 patients with a 5.0 MHz vaginal probe was always able to demonstrate cardiac activity if the mean diameter of the gestational sac exceeded 12 mm. Levi et al[13] always identified a yolk sac in gestational sacs measuring 8 mm or greater in diameter and cardiac activity in gestational sacs measuring over 16 mm in diameter. Bree et al[14] correlated size of the gestational sac with structures within that sac. A yolk sac was first seen in a gestational sac measuring between 6 and 9 mm and cardiac activity was seen in each patient with a gestational sac of $\geq$ 9 mm. The results of a study dealing with the dynamics of embryonic cardiac activity in relation to gestational age are presented in Chapter 6.

The results of our study indicate that transvaginal sonography is superior to transabdominal scanning techniques when the development of early pregnancy is studied. We were able to visualise embryonic structures in more

detail and about one week earlier in comparison with transabdominal sonography.

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4.2. THE CROWN-RUMP LENGTH IN EARLY HUMAN PREGNANCY: A REAPPRAISAL.

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4.2.1. SUMMARY

The crown-rump length (CRL) was measured by means of transvaginal sonography in 41 pregnancies resulting from in-vitro fertilisation (IVF). The embryonic crown-rump length could be determined as early as 25 days after follicle aspiration. A reference chart was constructed relating the CRL to the number of days following follicle aspiration. Comparison with the current CRL chart by Robinson and Fleming[1, 2] revealed that transvaginal sonography allows earlier and more accurate measurement of embryonic CRL.

4.2.2. INTRODUCTION

The reference curve currently in use for crown-rump length (CRL) measurements was derived from data collected with compound B-scan equipment[1,2]. Transvaginal sonography with its high resolution imaging might enable more accurate measurement of the CRL at an earlier stage of pregnancy.

The object of the present study was to establish whether transvaginal measurement of CRL could provide a more accurate reference chart for this dimension.

4.2.3. SUBJECTS and METHODS

Forty-one women who were pregnant following in-vitro fertilisation agreed to participate in the study. The IVF procedure provides exact information on the time of ovulation and embryo transfer and, therefore, the duration of pregnancy. The study was approved by the local ethics review committee. Mean maternal age was 34 years (range 24 - 40 years). A pilot-study in 23 women showed that the CRL could not be measured until day 25 after follicle aspiration. Therefore, CRL measurements commenced on day 25 in the present study. CRL was measured daily until embryonic cardiac activity appeared, then at 2 or 3 day intervals, depending on patients' availability and travelling distance, until day 42.

All patients were scanned between 10.00 and 12.00 a.m. using Diasonics DRF 400 and Toshiba SAL 77B transvaginal probes. The former was equipped with a 5.0 and a 7.5 MHz mechanical sector scan transducer and the latter with a 5.0 MHz electronic sector scan transducer. Scans were recorded by R.S. and C.A.M.J..

A reference chart (mean +/- 1 SD) was constructed of all the longitudinal CRL data. In 11 normal subjects with spontaneous pregnancies (15 embryos) four consecutive blind determinations of the CRL were obtained to establish the intra-observer variability of these measurements. Inter-observer variation was tested in 12 other normal subjects with single, spontaneous pregnancies.

Intra-observer variability was determined by the coefficient of variation; the inter-observer variation was established by the Wilcoxon two-tailed signed ranks test.

4.2.4. RESULTS

The intra-observer variability showed a coefficient of variation of 6.6 %. The

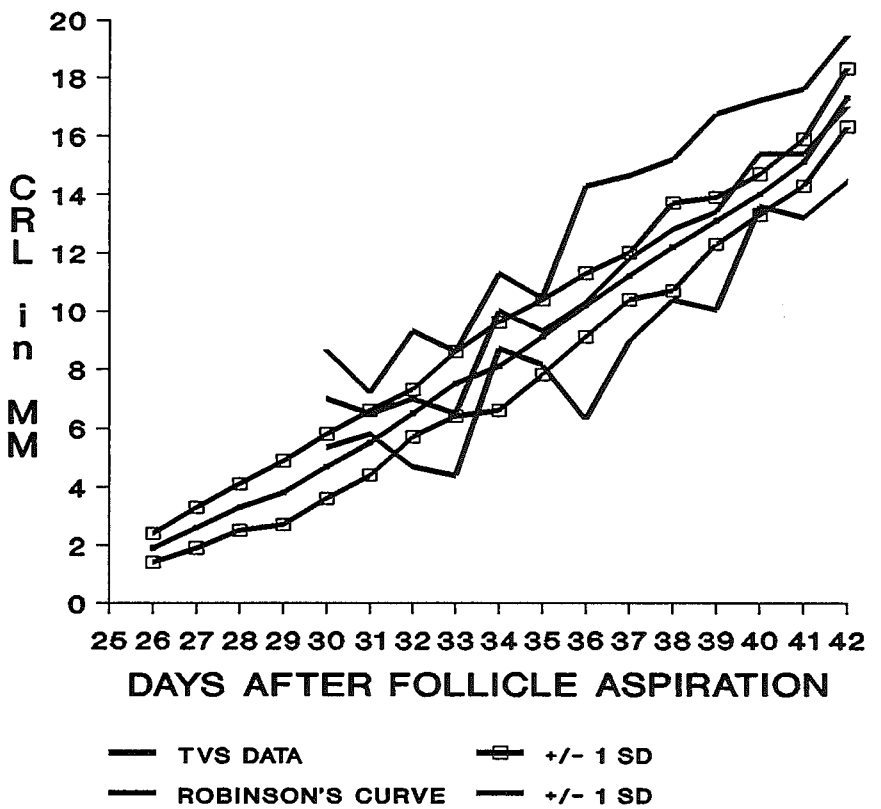

Figure 4.2.1. CRL data gathered with transvaginal sonography (TVS), superimposed with the data collected by Robinson & Fleming (1975).

inter-observer differences were not statistically significant ($p = 0.38$).

In eight out of 41 women we were unable to time the onset of embryonic cardiac activity and therefore we also missed the first CRL measurements, due to personal factors such as non-availability of the women when required. Therefore, incomplete data from eight women (five singletons and three twins) and the complete data from 33 women (21 singletons, nine twins, three triplets) were available for further analysis. In total 293 CRL measurements were obtained from 59 embryos. The number of CRL measurements varied between four and eight per embryo (median six) and between 10 and 30 per day of gestation (median 17). Figure 4.2.1 depicts the CRL reference chart derived from all the transvaginally recorded CRL measurements, compared with the reference chart by Robinson and Fleming[2].

4.2.5. DISCUSSION

Our transvaginal CRL measurements demonstrate an acceptable intra- and inter-observer variability. Particularly in the very early weeks of gestation there is a discrepancy between our data and those compiled by Robinson and Fleming[2]. We have referred, for comparison, to their original data instead of the chart constructed from linear regression analysis. Inclusion of part of the yolk sac may have been responsible for the larger CRL measurements in the beginning of the reference chart obtained by compound B-scan equipment. Degenhardt et al[3]. have also reported initially smaller CRL values using transvaginal sonography, though the number of CRL measurements per day of gestation was considerably smaller than that in our study: 225 measurements in 209 subjects between 6 and 13 weeks menstrual age. The smaller standard deviation displayed in our chart can be explained by (i) the high resolution capability of modern transvaginal transducers allowing more detailed identification of embryonic structures, (ii) the larger number of CRL measurements per pregnancy and (iii) knowledge of the exact duration of the pregnancy. The advantage of studying IVF pregnancies is that the precise time of ovulation (follicle aspiration) and embryo transfer are known. Whether the results can be extrapolated to spontaneous pregnancies is

uncertain. We included multiple pregnancies because the embryos behave like separate individuals with their own growth profile and developmental potential; in a number of cases we even observed a difference in the time of appearance of embryonic cardiac activity and in subsequent CRL values[4]. Of interest is that transvaginal CRL measurements were obtained approximately 4 days earlier than reported previously, which could allow earlier pregnancy dating in the event of an unreliable menstrual history[2]. We conclude from the present study that transvaginal sonography allows more detailed recognition of embryonic anatomy and, therefore, earlier and more accurate measurements of embryonic CRL. A larger transverse study, preferably in women with spontaneous pregnancies, should be initiated to construct a new reference curve for this scanning technique.

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CHAPTER 5

DIAGNOSTIC, MORPHOLOGICAL AND THERAPEUTIC ASPECTS OF ABNORMAL EARLY PREGNANCY DEVELOPMENT

INTRODUCTORY REMARKS

In this chapter the value of transvaginal sonography as a diagnostic tool in abnormal development of early pregnancy will be described. This chapter consists of two parts. In the first part (5.1.) clinical signs and symptoms of threatening abortion in relation to transvaginal sonographic findings in intra-uterine pregnancies are presented. Results will be compared with those reported in literature. In the second part of this chapter (5.2.) the role of transvaginal sonography in the diagnosis and management of ectopic pregnancies will be discussed. The combination of sensitive hCG determinations and transvaginal sonography allows to the identification of ectopic pregnancy at a stage when it still may be asymptomatic. This may lead to more conservative modes of management.

5.1. MORPHOLOGICAL ASPECTS OF ABNORMAL EMBRYONIC DEVELOPMENT

5.1.1. INTRODUCTION

Approximately 25% of women experience vaginal bleeding during early pregnancy. About half of these pregnancies will end in a spontaneous abortion[1]. Despite the fact that non-viable gestations ultimately abort, it is important to identify correctly these pregnancies before they lead to prolonged vaginal bleeding, infection and/or patient anxiety and to determine which patients merit dilatation and curettage[1]. Theoretically, the application of vaginal transducers with high emission frequencies and therefore high resolution imaging properties, should allow earlier and more detailed visualisation of normal and abnormal embryonic development than the standard transabdominal scanning techniques. Some authors compared the use of transabdominal and transvaginal sonography in normal and abnormal early gestation[2-4]. Others have published data concerning the transvaginal sonographic features in complicated first trimester pregnancies[5-9]. The results of these studies together with our own sonographic findings in 54 subjects who became pregnant following long-term infertility will be discussed.

5.1.2. SUBJECTS AND METHODS

Between April 1987 and February 1988 234 transvaginal sonographic examinations were performed in 54 pregnant women with vaginal bleeding and a closed cervical os indicating threatening abortion. Three groups could be recognised: (i) women who had become pregnant following IVF treatment (Group 1; N = 19); (ii) women with well-documented duration of pregnancy (Group 2; N = 21); and (iii) pregnant women with an unreliable menstrual history (Group 3; N = 14). In each woman four parameters were determined: a) the size of the gestational sac (mm) (= mean of longitudinal, transverse and

antero–posterior diameters) until the embryo became visible; b) the yolk sac diameter, also the average of three dimensions (mm); c) the crown–rump length (mm); and d) the embryonic heart rate (bpm).

All examinations were performed using the Diasonics DRF 400 and the Toshiba SAL 77B, the former equipped with a 5.0 and a 7.5 MHz mechanical sector scan transducer and the latter with a 5.0 MHz electronic sector scan probe. All examinations were recorded on video–tape.

5.1.3. RESULTS

Data on the numbers of the presence of the four above–mentioned parameters and the outcome of pregnancy are presented in Table 5.1.I.

Table 5.1.I. Pregnancy outcome of the study-group in relation to the presence of embryonic landmarks is noted.

	No. Pat.	Gest. Sac	Yolk Sac	Embryo	Card. Act.	Abortion	Cont. Preg.
Group 1	19	20	17	15	12	15	5
Group 2	21	23	20	18	16	12	11
Group 3	14	15	14	12	10	8	7
TOTAL	54	58	51	45	38	35	23

Group 1 = IVF patients, Group 2 = Patients with reliable menstrual history, Group 3 = Patients with unreliable menstrual history, No. Pat. = number of patients, Gest. = gestational, Card. Act. = cardiac activity present, Cont. Preg. = continuing pregnancy.

5.1.3.1. GESTATIONAL SAC:

Four twin pregnancies occurred in the group of 54 women and therefore, 58 gestational sacs were present for evaluation. A total of 163 mean gestational sac diameter measurements were performed. The number of examinations in one women varied between one and six (median 3). The mean gestational sac diameter ranged between 3 and 15 mm (median 11 mm). This parameter by itself was not a useful predictor of pregnancy outcome. However, the combination with other distinctive features of embryonic development (yolk sac and embryonic cardiac activity) enables one to distinguish normal from abnormal gestational sacs. Our data suggest that:

1. A yolk sac should be present if the mean gestational sac diameter exceeds 10 mm.
2. Embryonic cardiac activity should be present at a mean gestational sac diameter of 12 mm.

The appearance of a yolk sac and/or embryonic cardiac activity above these

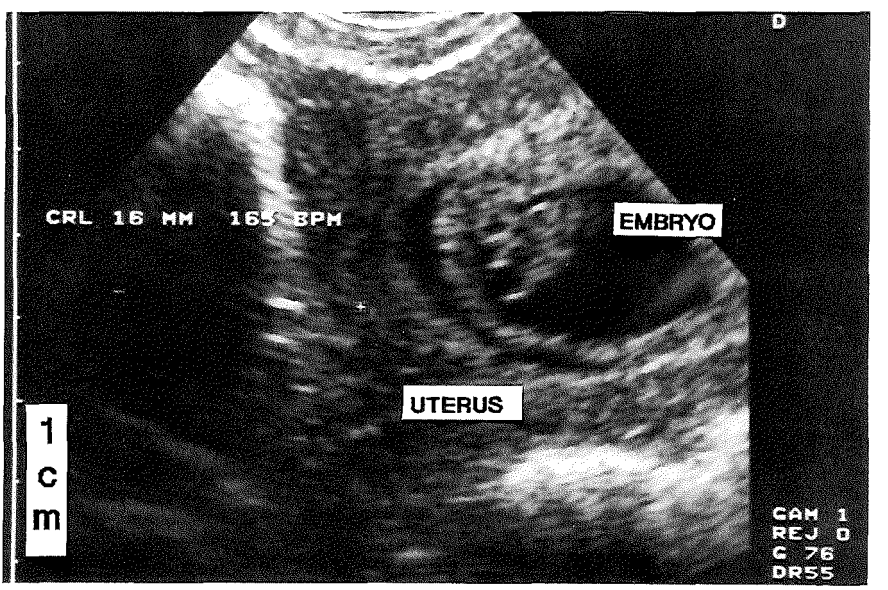

Figure 5.1.1. *Almost complete detachment of the gestational sac. The transonic space may represent blood.*

cut-off limits always resulted in first trimester abortion. A yolk sac and cardiac activity were documented in two gestational sacs measuring only 6 and 7 mm in diameter respectively. These pregnancies ended in first trimester abortion. A transonic area in the proximity of the gestational sac was observed in 37 out of 54 patients (68.5 %), giving the impression of at least partial trophoblast detachment (Figure 5.1.1). This feature alone was not of predictive value for pregnancy outcome, but if it was associated with a reduced heart rate and/or reduced CRL the pregnancy invariably ended in abortion.

5.1.3.2. YOLK SAC:

A total of 127 mean diameter measurements was performed in 51 yolk sacs (Table 5.1.I, Figure 5.1.2). The number of examinations ranged from one to

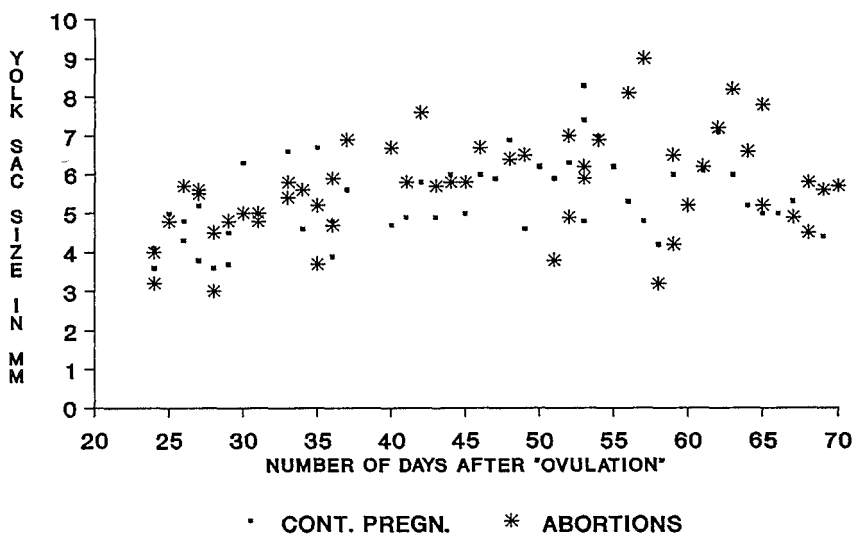

Figure 5.1.2. Serial mean yolk sac diameter measurements for continuing pregnancies and first trimester abortions with reliable menstrual histories.

five (median 3). The diameter ranged from 3 to 9 mm. Yolk sac diameter increased only slightly during the first trimester (range 0.5 – 3.2 mm, median 1.8 mm). Twenty–six yolk sacs consistently increased in size, 21 exhibited after an initial increase signs of involution and of four yolk sacs only one measurement was available. Last measurements of the mean yolk sac diameter were compared in continuing pregnancies and in pregnancies ending in abortion. There was no difference in mean value (5.3 versus 5.4 mm) or range (3.6 – 8.3 mm versus 3.0 – 9.0) (Figure 5.1.3). Yolk sac size was of no use in pregnancy dating (Figure 5.1.2). A collapse of the yolk sac was observed in one case. It coincided with development of an abnormal embryonic heart rate pattern and resulted in the birth of a male infant with Down's syndrome. This case is described in detail in chapter 6.3.

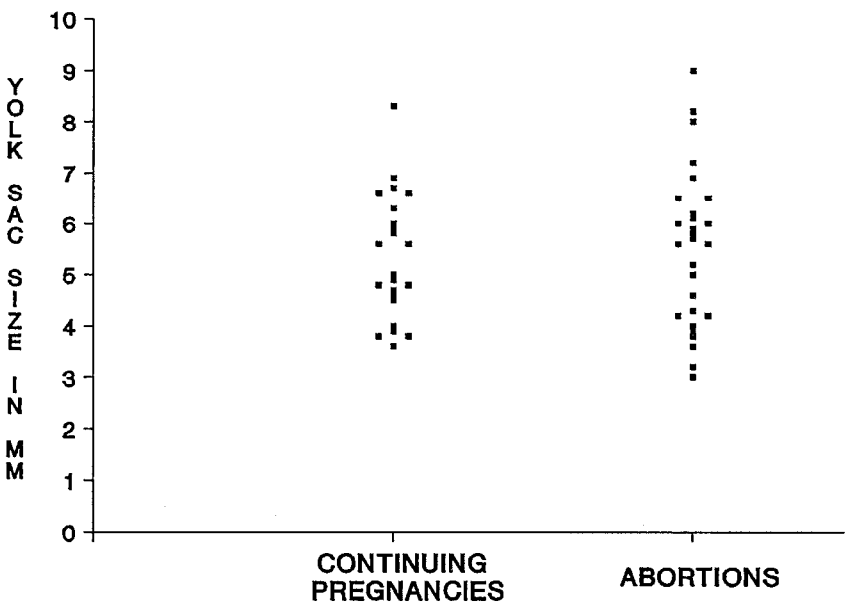

Figure 5.1.3. *Last measured mean yolk sac diameter of the continuing pregnancies and these which ended in first trimester abortions.*

5.1.3.3. CROWN-RUMP LENGTH:

The crown-rump length (CRL) was measured 143 times in a total of 45 embryos. The number of determinations per embryo varied between one and six (median 3). The CRL for the embryos of the continuing pregnancies all fell within the normal range of our reference chart (chapter 4.2.) below a menstrual age of eight weeks and of the curve of Robinson and Fleming[10, 11] between eight and 14 weeks. However, the picture was different for

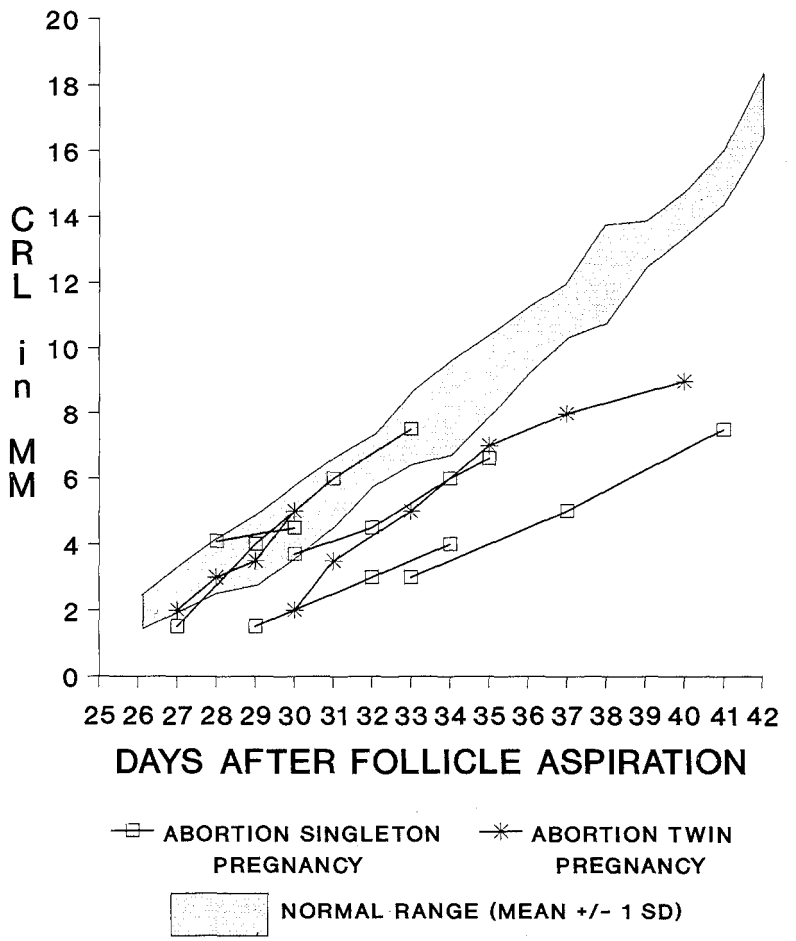

Figure 5.1.4. *CRL curves relative to the reference chart of Chapter 4.2 in pregnancies of six IVF patients (seven embryos) which miscarried in the first trimester.*

pregnancies which miscarried, especially when our own reference chart was employed. As an example may serve the CRL curves depicted in Figure 5.1.4 of the seven embryos with cardiac activity that arose from IVF treatment and miscarried. According to our reference chart two embryos exhibited normal growth, whereas the other five demonstrated a reduced growth rate.

5.1.3.4. CARDIAC ACTIVITY:

Normal and abnormal development of embryonic cardiac activity is discussed in chapter 6. Cardiac activity should be detected as soon as the embryonic pole has appeared. All continuing pregnancies were characterised by a very regular heart rate and absent beat to beat variation. In two pregnancies which miscarried an irregular heart rate was documented (Figure 5.1.5). The following day both embryos had died. At one occasion a regular heart rate pattern of 40 beats/min was observed. This embryo also died within 24 hours.

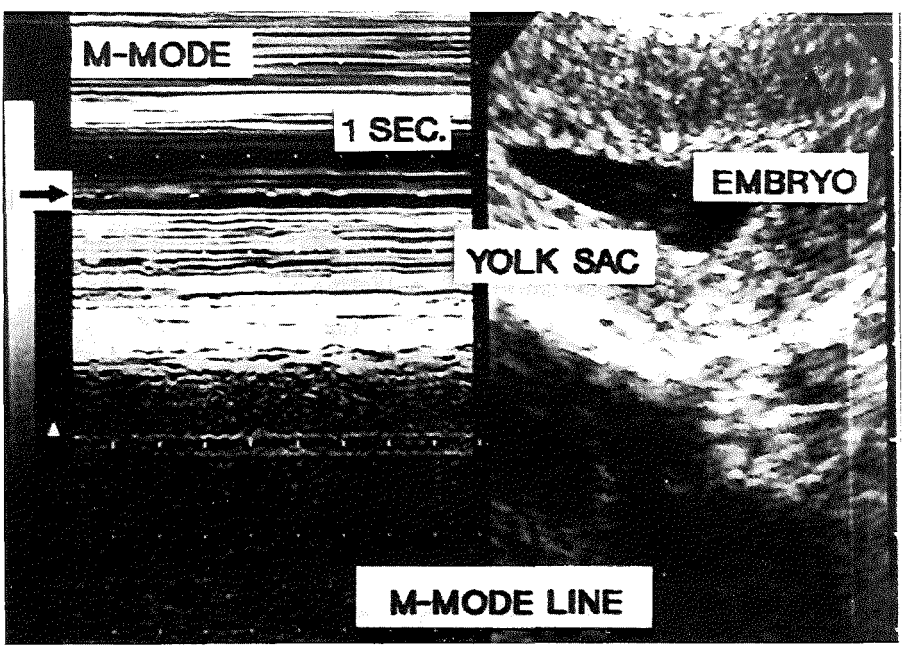

***Figure** 5.1.5. Combined real-time and M-mode registration of an embryonic irregular heart rate.*

5.1.3.5. PREGNANCY OUTCOME:

Six of the IVF patients (Group 1, seven embryos) aborted after embryonic cardiac activity was established and eight IVF patients aborted without demonstration of this viability sign. In three of those eight women only a gestational sac could be detected, in the other five women a gestational sac as well as a yolk sac could be visualised, but no embryonic pole. Five patients had vaginal bleeding but the pregnancy went on uneventfully. Seventeen of the 35 remaining patients (Group 2 & 3) eventually aborted (18 gestational sacs, 14 yolk sacs, 10 embryos of which in six cases at least once cardiac activity was established). In two women vaginal bleeding was explained by the so-called "vanishing twin". At the time of the sonographic examination cardiac activity was (already) absent in the second gestational sac in these cases. The growth profile of the remaining embryo was normal in both instances. In 16 women the pregnancy proceeded without any further complications.

5.1.4. DISCUSSION

Vaginal bleeding in the first trimester of pregnancy is a common clinical feature. Also in our highly selected group of patients it was associated with a high abortion rate (60%). It is logical to assume that transvaginal sonography is superior to transabdominal scanning in detecting abnormal early embryonic development on the basis of the proximity to the object of scanning and the application of high emission frequencies leading to a better image resolution. The superiority of transvaginal sonography in the early and detailed visualisation of embryonic structures and in biometry has been well documented (Table 5.1.II).

The normal sequence in appearance of the distinctive features of embryonic development (gestational sac, yolk sac, embryonic pole and cardiac activity) as established by transvaginal sonography is described in chapter 4.1. Deviations of this rather fixed developmental pattern may have considerable implications for fetal outcome. If at a particular gestational age a certain stage of development has not been reached, the pregnancy probably ends in an

Table 5.1.II. Summary of the studies comparing transabdominal versus transvaginal sonography.

Author	No. Pat.	Study Design	Object of Study	Parameters	Advantage	Disadvantage	Conclusion
Cullen et al[2]	120	Comparative study	Normal Pregnancies	Biometry, internal anatomy image clarity	Superior in obesity, < 10 weeks menstrual age, retroverted uterus	Limited maneuver-ability	Valuable tool in first trimester, complementing not replacing transabdominal sonography
Pennell et al[3]	38	TVS after inconclusive TAS	Normal, abnormal and ectopic pregnancies	Yolk sac, cardiac activity, absence or presence of intrauterine structures	Diagnostic in earlier stage pregnancy	Disadvantages not mentioned	Transvaginal sonography is more likely to be diagnostic in early pregnancy
Jain et al[4]	90	Comparative study	Positive pregnancy test, signs and symptoms of threatening abortion or ectopic pregnancy	Yolk sac, fetal pole, cardiac activity	More sensitive and earlier diagnosis	Disadvantages not mentioned	Transvaginal sonography is more sensitive in detection of early pregnancy and its complications

TVS = transvaginal sonography, TAS = transabdominal sonography.

abortion (Figure 5.1.6). A single sonographic examination may be conclusive about the outcome in pregnancies in which there is no doubt about pregnancy

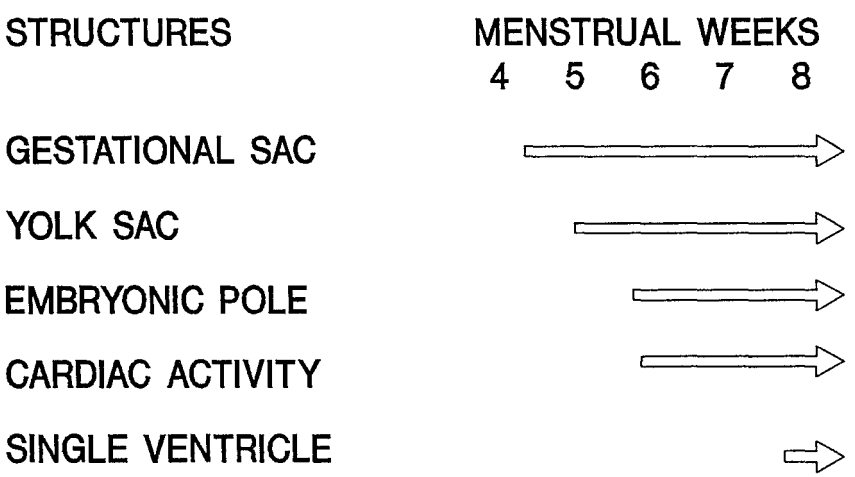

Figure 5.1.6. Appearance of embryonic landmarks relative to gestational age as visualised with transvaginal sonography.

duration, like those which emerged from IVF treatment or ovulation induction with gonadotropins. This is not so for women with an unreliable menstrual history. In these cases two scans with an interval of one week should reveal the correct diagnosis, since only then the growth pattern of the various components of embryonic development (gestational sac, yolk sac and crown–rump length) can be determined. Discussion on data in abnormal gestation from other authors relative to our own findings should focus on biometrical changes in each of the above–mentioned embryonic structures as well as the time dependent inter–relationship in the appearance of these structures including embryonic heart rate.

Mean gestational sac diameter alone was not helpful in the prediction of pregnancy outcome. Nyberg et al performing transabdominal sonography in 168 women with threatening abortion, defined some characteristics of abnormal gestational sac development[5]:

1. a gestational sac diameter ≥ 25 mm without an embryo;
2. absence of yolk sac at a gestational sac diameter ≥ 20 mm;
3. distorted shape of the sac;
4. thin (≤ 2 mm) and slightly echogenic or irregular choriodecidual reaction;
5. the typical "double sac" sign is lacking, at a sac diameter > 10 mm;
6. gestational sac positioned in lower uterine region.

Our own data demonstrate that some of the characteristics mentioned above should be redefined when transvaginal sonography is employed. Absence of the yolk sac at a mean gestational sac diameter of ≥ 10 mm and / or absence of embryonic cardiac activity at a gestational diameter of ≥ 12 mm indicates an abnormal development of pregnancy. Appearance of a yolk sac or cardiac activity above these limits is associated with a poor prognosis. The shape of the gestational sac and thickness of choriodecidual reaction are less important characteristics when transvaginal sonography is used, since they are regressive and consequently late signs of abnormal gestational development. A fluid-filled compartment with partial or complete detachment of the decidual ring may be seen and may actually reflect bleeding[9].

The present study shows that yolk sac size relative to gestational age is extremely variable. Increase in size was observed during the first trimester but a common pattern could not be detected. This is at variance with data reported by Reece et al[12] who assumed a curvilinear relationship with gestational age. They found a weak correlation ($r = 0.39$) between gestational age and size of the yolk sac. However, analysis of their data shows that the number of measurements are not equally divided over the entire period of study. Furthermore the study did not include observations before eight weeks of gestational age. The absence of a yolk sac has been observed in a pregnancy which resulted in embryonic death at 10 weeks menstrual age; the embryo exhibited spina bifida[13]. In our study, we did not observe any difference in yolk sac size between continuing and aborting pregnancies. In the light of these findings yolk sac size does not appear to be a sensitive predictor of embryonic integrity and pregnancy outcome. Too small as well as too large yolk sacs have been associated with impending spontaneous

abortion and karyotypic abnormalities[12, 14]. Rempen[15] did find yolk sacs with diameters exceeding 7 mm only in pregnancies with a pathological course.

A different picture emerged from embryonic CRL measurements. Here, reduced growth rate was highly associated with poor pregnancy outcome. Especially the combination of abnormal heart rate and reduced CRL relative to gestational age was a very strong predictor of abnormal pregnancy outcome.

Reduced heart rate may herald embryonic demise. The detection and development of normal and abnormal embryonic cardiac activity is discussed and described in detail in chapter 6.

Robinson[16] was the first who used transabdominal (compound B-scan) ultrasound in the diagnosis of early pregnancy failure. Our own data and reports of other investigators suggest that transvaginal real-time sonography allows one to recognise normal and abnormal morphological and functional development about one week earlier than previously has been described using the transabdominal approach[6-8, 17-20]. A single transvaginal sonographic examination can reveal a considerable amount of information on the prognosis and may be even conclusive when the exact gestational age is known and all available sonographic parameters are evaluated. The sequence in appearance of the relevant structures of early gestation should be followed. If, for example, the yolk sac is not present at day 25 following ovulation or not present in a gestational sac of which the mean diameter exceeds 10 mm, it will not appear any more and the pregnancy will end in abortion.

Correlation of sonographic findings with simultaneously determined maternal hCG levels has been found to be a useful method for evaluating early pregnancy complications. Nyberg et al[21] found that the serum hCG levels strongly correlated with gestational sac size up to a mean diameter of 25 mm. A disproportionately low serum hCG level relative to gestational sac size indicates abnormal development of pregnancy[21, 22]. Nyberg et al[23] performed transvaginal sonography in 84 women with suspected early pregnancy complications and determined hCG levels quantitatively. Their findings indicate that an intra-uterine gestational sac should normally be visualised when the hCG level (Second International Standard) exceeds 1000 IU/L. Bree et al[8] were able to detect a gestational sac by transvaginal

sonography in all patients with a hCG levels of 1000 IU/L (First International Reference Preparation). A level of 7200 IU/L was necessary before the yolk sac could be visualised and 10800 IU/L for the detection of an embryo exhibiting cardiac activity. This implicates that there should be concordance between the transvaginal sonographic findings and the hCG level.

It can be concluded from the results of the present study that transvaginal sonography allows the diagnosis of pregnancy failure by about one week earlier than with transabdominal sonography. Furthermore, disturbance of the sequence in appearance of the relevant structures of early gestation is indicative of poor pregnancy outcome.

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5.2. THE ROLE OF TRANSVAGINAL SONOGRAPHY IN DIAGNOSIS AND MANAGEMENT OF ECTOPIC PREGNANCY

5.2.1. INTRODUCTION

Ectopic pregnancy remains a diagnostic challenge to the gynaecologist. The clinal signs and symptoms are diverse. The fear of a potentially life threatening condition as well as the diagnostic problems have led to the fact that the diagnosis of ectopic pregnancy is made more frequently than it actually occurs[1]. Obviously there is an urge to diagnose an ectopic pregnancy as soon as possible to prevent calamities. Another advantage of early recognition is that if damage to the tubes and other structures can be minimised, the preservation of future fertility can be optimised and conservative modes of management can be pursued. In the past an ectopic pregnancy could only occasionally be diagnosed by transabdominal ultrasound. The introduction of transvaginal sonography has led to improved visualisation of ectopic pregnancy, even when it is asymptomatic[2-4]. Several comparative studies have been published showing the superiority of transvaginal to transabdominal scanning in diagnosing this entity[5-7]. In most cases ectopic pregnancy can be ruled out by demonstrating an intra-uterine pregnancy. An exception to the rule may be a pregnancy which emerged from IVF treatment. The combined presence of both intra-uterine and ectopic pregnancy may be seen following the transfer of more than one embryo[8,9].

The recent introduction of sensitive serum and urine hCG determinations with commercially available kits, makes it possible to diagnose the state of pregnant at a very early stage. Serum hCG levels have been found to correlate with transvaginal sonographic findings in normal pregnancy and as such these techniques can be used in evaluating early pregnancy complications. Several authors have tried to find discriminatory levels for the various embryonic landmarks[10-18]. A problem in evaluating these studies is the application of two different WHO standards for determining hCG levels: the first international reference preparation (IRP) and the second international

standard. Most investigators assume that the first IRP is equivalent to twice the value of the second international standard[19]. However, Fossum et al[17] found in their study only 34% higher values for the first IRP. An explanation for this finding has not been provided. Blumenfeld et al[18] report a lowest value of 420 IU/L at which a gestational sac could be visualised. Unfortunately they do not specify which standard preparation was used. If all the values for hCG are corrected to the first IRP the following discriminatory zones can be defined for transvaginal sonography[10-18]: a gestational sac should be identified when the serum hCG level exceeds 1000 IU/L, a yolk sac at a level that is equal or greater than 7000 IU/L, an embryonic pole above a level of 8500 IU/L and embryonic cardiac activity over 11000 IU/L. This implicates that there should be concordance between the transvaginal sonographic findings and the hCG level. If, for example a intra-uterine gestational sac can not be seen at a serum hCG level of 3000 IU/L an ectopic pregnancy is very likely to be present. This should not lead to immediate surgery, instead this early diagnosis should give one time to consider the best possible management. The combination of these sensitive diagnostic tools makes it possible to diagnose an ectopic pregnancy more often at a stage that it is still asymptomatic[2-4]. It is also possible that ectopic pregnancies are diagnosed that would have been missed altogether in the past, and would have resolved spontaneously. Many ectopic pregnancies turn out to be non-viable. Either this may be due to the quality of the trophoblast itself or to the inadequate site of nidation. However, viable ectopic pregnancies occur more often after IVF treatment, most likely because the mechanism of occurrence is different.

The scope of this chapter will be to present the results of a pilot study and to illustrate with case histories new management options in case of ectopic pregnancy with special reference to the advent of transvaginal sonography as a diagnostic tool and its use in the treatment of ectopic pregnancy.

5.2.2. SUBJECTS AND METHODS

Ectopic pregnancy was diagnosed by transvaginal sonography in 14 patients

in which clinical signs or symptoms were only mild (vaginal blood loss or abdominal pain without evidence of intra-abdominal bleeding) or even absent (Table 5.2.I). In nine of these patients the pregnancy was classified as vital

Table 5.2.I. Characteristics of the 14 patients with an ectopic pregnancy.

Vital Ectopic Pregnancies

Patient No	Age at Detection	Age at Puncture	Local MTX Dosage	hCG level at Puncture	Outcome
1	29 FA	29 FA	--	----	Resolution
2	26 FA	28 FA	--	9300 IU/L	Resolution
3	42 LMP	43 LMP	--	10200 IU/L	Salpingectomy
4	44 LMP	45 LMP	--	8300 IU/L	Salpingectomy
5	29 FA	30 FA	10 mg	8800 IU/L	Resolution
6	46 LMP	47 LMP	10 mg	12300 IU/L	Salpingectomy
7	40 LMP	42 LMP	10 mg	6800 IU/L	Resolution
8	39 LMP	42 LMP	10 mg	11400 IU/L	Salpingectomy
9	28 FA	28 FA	40 mg	15600 IU/L	Resolution

Non-Vital Ectopic Pregnancies

Patient No	Age at Detection	hCG level at Detection	Highest Level of hCG	Outcome
10	38 IUI	5000 IU/L	6500 IU/L	Resolution
11	50 LMP	3600 IU/L	3600 IU/L	Resolution
12	52 LMP	1400 IU/L	1600 IU/L	Tubal abortion
13	30 FA	3100 IU/L	3400 IU/L	Resolution
14	55 LMP	1700 IU/L	2900 IU/L	Resolution

FA = days following follicle aspiration, LMP = days from start of last menstrual period, IUI = days following intra-uterine insemination, MTX = methotrexate.

according to the following criteria: the presence of a gestational sac containing a yolk sac and embryo exhibiting cardiac activity outside the uterine cavity associated with rising hCG levels. Eight ectopic pregnancies were situated within the Falloppian tube and one in the intramural part of the right tube (No. 9, Table 5.2.I). In the remaining five patients the ectopic pregnancy was classified as non-vital because of the absence of embryonic cardiac activity and hCG levels below the lower limit for gestational age[20]. In one patient a vital intra-uterine pregnancy as well as a vital ectopic pregnancy was present (No. 1, Table 5.2.I). A total of five ectopic pregnancies occurred after IVF treatment, one following intra-uterine insemination of capacitated spermatozoa and eight spontaneously of which three during an infertility work-up.

According to the classification of ectopic pregnancies in vital and non-vital, transvaginal puncture was carried out or the natural course was monitored with transvaginal sonography and hCG determinations. In the vital ectopic pregnancies, except for patient No. 1, hCG determinations were carried out at least once before and at two or three day intervals in conjunction with transvaginal sonography after the transvaginal puncture. The hCG determination and scanning rate reduced as soon as the hCG level fell according to the half life time. In patients with a non-vital ectopic pregnancy the same follow-up scheme was employed. Three months after the puncture a hysterosalpingogram (HSG) was performed when suitable. In the hCG assays the first international reference preparation (IRP 75/537) was used as a standard.

All examinations were performed using the Diasonics DRF 400 the Toshiba SAL 77B and the Kretz Combison 310 equipped with a 5.0 and a 7.5 MHz mechanical sector scan transducer, a 5.0 MHz electronic sector scan probe and a 5.0 and a 7.5 MHz mechanical sector scan transducer respectively.

Puncture Technique: In all nine patients with a vital ectopic pregnancy transvaginal puncture was performed under sonographic guidance and under local analgesia (lidocain 1%, 10 ml). The procedure can be compared with a follicle aspiration which is common practice in oocyte retrieval in IVF programs. The embryo was aimed at in order to cause cessation of cardiac

activity, whereafter aspiration of the gestational sac and the yolk sac, if possible, followed. Transvaginal scanning was performed for at least another ten minutes to ensure that no cardiac activity reappeared. In the first four patients (No. 1 – 4, Table 5.2.I) puncture took place without instillation of the cytoreductive agent methotrexate (MTX) into the gestational sac. In the next four patients (No. 5 –8, Table 5.2.I) transvaginal puncture was followed by instillation of 10 mg of MTX into the aspirated gestational sac. In patient No. 9 40 mg of MTX was instilled.

5.2.3. RESULTS

The data of all 14 patients are summarised in Table 5.2.I. The crown–rump length of the embryos ranged between 3 and 6 mm. The first two patients (No. 1 & 2) displayed complete resolution of the ectopic pregnancy. The next two cases (No. 3 & 4) were less successful. Despite a correct puncture and cessation of cardiac activity, the hCG level started to rise again after an initial fall. Both patients had to undergo laparoscopy followed by laparotomy leading to salpingectomy of the affected side. In view of this and similar data reported by several other authors employing the aspiration technique alone[21, 22], as well as the suggestion made in by other reports that the instillation of substances such as, potassium chloride[23, 24], MTX[25-29], prostaglandin $F_{2\alpha}$[26] or E2[27] may improve the success rate, the next four patients (No. 5 – 8) were subjected to a different therapeutic approach. A dosage of 10 mg of MTX was instilled into the emptied gestational sac. However, in two of these patients (No. 6 & 8) an initial fall of the hCG level again was followed by a rise, and laparoscopy followed by laparotomy leading to salpingectomy of the affected side was required. In patient No. 9 with an intramural presentation of ectopic pregnancy a dosage of 40 mg of MTX was administered into the aspirated gestational sac. In addition, because of a plateauing of the hCG level one week after puncture and because of the intramural localisation of the ectopic pregnancy which is unattractive for surgical intervention, MTX was administered systemically.

In the five patients with a non–vital ectopic pregnancy no major problems

occurred. In one case (No. 12) symptomatic tubal abortion took place. Transvaginal sonography displayed the appearance of fluid in the pouch of Douglas indicating most likely the presence of blood which was confirmed by laparoscopy. Since the bleeding was self-limiting no further action was required. Tubal abortion occurred during the decline of the hCG level. In two patients (No. 5 & 7) without the suspicion of tubal pathology before the ectopic pregnancy occurred, a HSG was performed three months later. Both showed patent tubes. One of these patients became pregnant at a later stage, and transvaginal sonography revealed a vital intra-uterine gestation.

In order to illustrate the diversity in diagnosis and initially non-operative management of ectopic pregnancy five cases presented in Table 5.2.I will be described in detail:

Patient No. 1, aged 36 years was known with primary infertility due to inoperable tubal pathology. She had experienced an ectopic pregnancy in the first IVF treatment cycle, which was treated by conventional laparotomy and salpingectomy. In the subsequent cycle she became pregnant resulting in a vital intra-uterine pregnancy in conjunction with a vital ectopic pregnancy in the remaining tube. In an effort not to disrupt the intra-uterine pregnancy a puncture of the ectopic pregnancy as described above was performed. Following the puncture she was closely monitored by serial transvaginal ultrasound examinations; hCG determinations would not have been informative in this case. The ectopic pregnancy apparently resorbed completely and the intra-uterine pregnancy went on uneventfully and led to the birth of a healthy male infant at term by caesarean section due to cephalopelvic disproportion. At surgery no sequelae were found at the previous puncture site.

Patient No. 2, aged 31 years with primary infertility due to tubal pathology underwent IVF treatment. She became pregnant in the second cycle. Sixteen days after follicle aspiration (FA) a sensitive urine pregnancy test (ICON) was positive. Twenty-six days following FA, transvaginal sonography revealed a congested thick endometrium containing fluid (Figure 5.2.1); no intra-uterine gestational sac could be visualised. On the right side of the uterus a

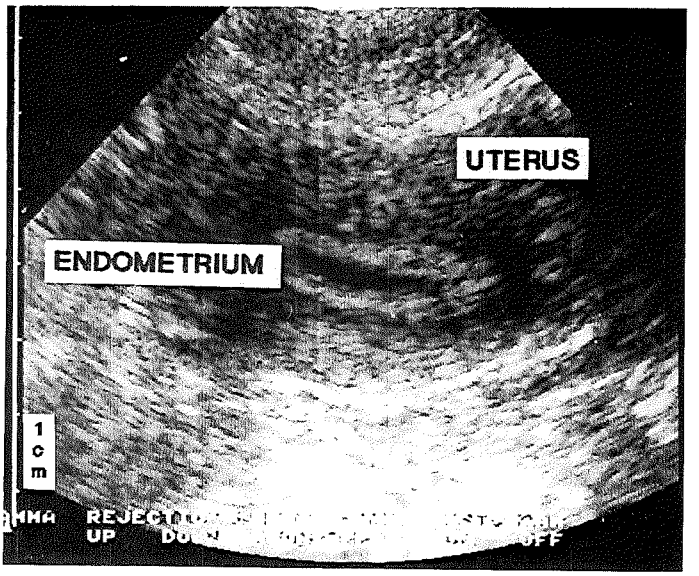

***Figure** 5.2.1. Uterus with thick endometrium containing fluid, leading to pseudo-ring formation.*

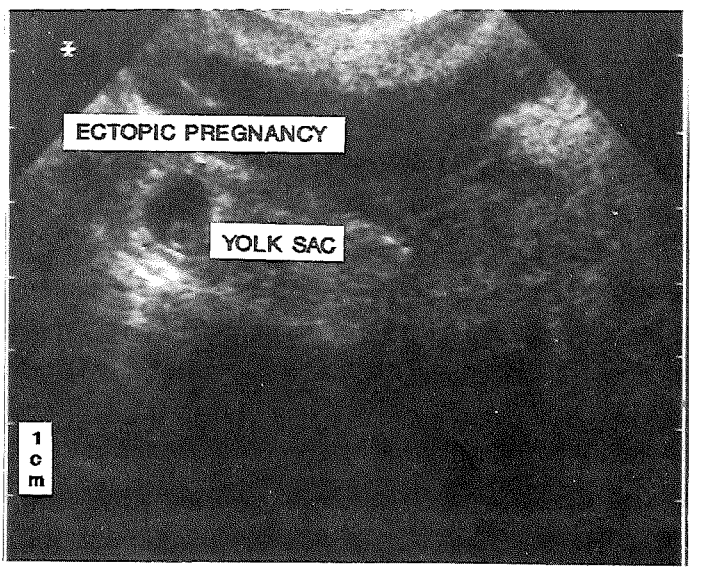

***Figure** 5.2.2. At the right side of the uterus an ectopic pregnancy (gestational sac containing a yolk sac) is present.*

gestational sac containing a yolk sac could be detected (Figure 5.2.2). The serum hCG level at that time was 6200 IU/L (first IRP). The ultrasound examination was repeated two days later and close to the yolk sac an embryo measuring 2 mm exhibiting cardiac activity at a rate of 96 beats per minute (BPM) was present. The hCG level had gone up to 9300 IU/L. A diagnosis of

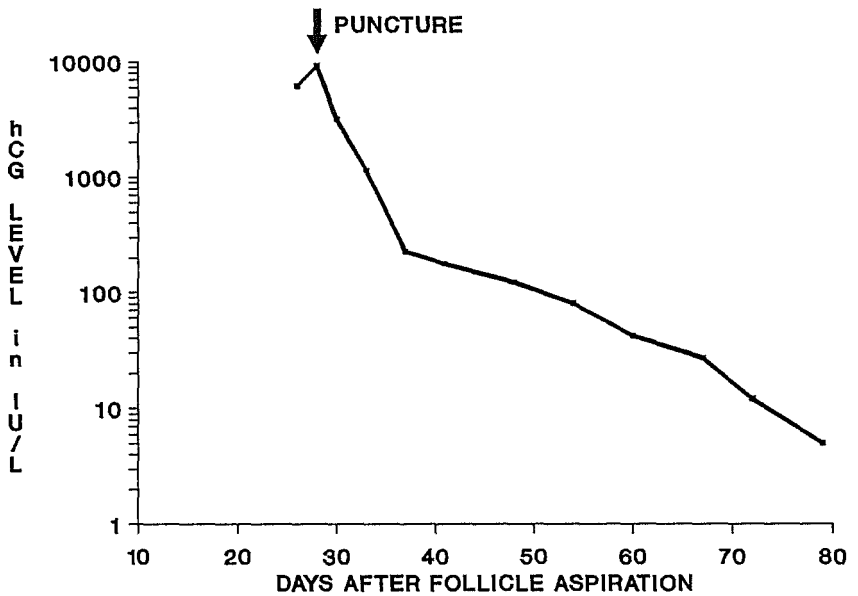

Figure 5.2.3. *Course of the hCG level of Patient No. 2.*

vital ectopic pregnancy located in the right tube was made. It was decided to aspirate the gestational sac by means of transvaginal puncture under sonographic control. There were no complications. The hCG level subsequently started to fall and was normalised six weeks after the puncture. The course of the hCG level is shown in Figure 5.2.3. Admission of the patient was only instituted for the first 24 hours following the transvaginal puncture. Further check-ups were carried out on an out-patient basis.

Patient No. 3, aged 27 years was referred by her general practitioner to the out-patient department because of vaginal bleeding and abdominal complaints

at a menstrual age of six weeks. The first pregnancy was uneventful and resulted in the birth of a healthy male infant two years before. A routine bimanual gynaecological examination showed no obvious abnormalities but

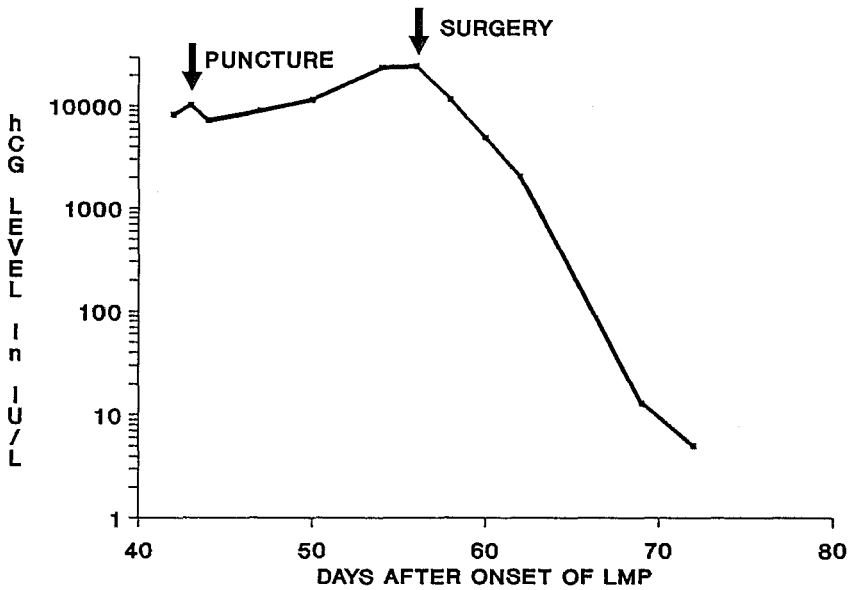

Figure 5.2.4. *Course of the hCG level of Patient No. 3. LMP = last menstrual period.*

was hindered by severe obesity. Transvaginal sonography revealed a vital ectopic pregnancy located on the left side of the uterus. Cardiac activity was present and the crown–rump length was 3 mm. Like in patient 1 the ectopic pregnancy was punctured under transvaginal sonographic guidance. After an initial fall of the serum hCG level one day after the puncture it started to rise again (Figure 5.2.4). The sonographic appearance of the punctured ectopic pregnancy showed no changes (Figure 5.2.5), although the hCG level was going up. The patient was admitted to the hospital, whereas clinical signs and symptoms were absent. The sonographic diagnosis was confirmed by laparoscopy. During manipulation, the left tube started to bleed and laparotomy was necessary whereby this tube had to be removed. During the

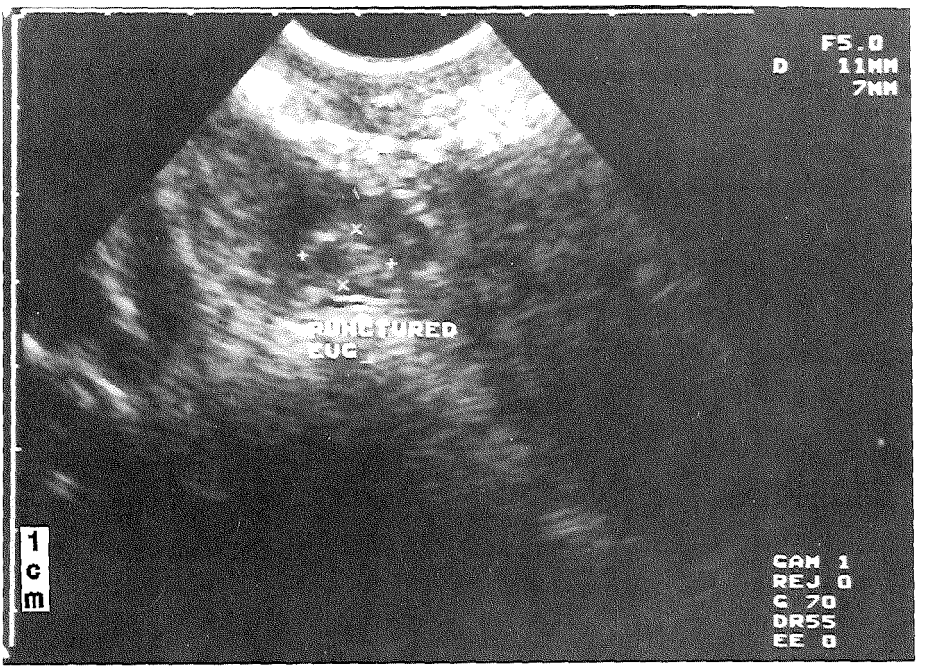

***Figure** 5.2.5. Transvaginal sonographic presentation of a punctured ectopic pregnancy (Patient No. 3). EUG = ectopic pregnancy.*

next four weeks hCG levels returned to normal Eight months later she became pregnant again. Transvaginal sonography performed at a menstrual age of five weeks demonstrated an intra-uterine pregnancy. An uneventful pregnancy resulted in the delivery of a healthy male infant.

Patient No. 9, aged 36 years with primary infertility caused by tubal pathology underwent IVF treatment. Three IVF treatment cycles in another hospital had not resulted in a pregnancy, although transfer of embryos of good quality took place in each cycle. In the fourth treatment cycle transfer of four embryos was carried out two days following FA. Fifteen days after FA a sensitive urine pregnancy test (ICON) was positive. The corresponding serum hCG level on that day was 235 IU/L. On day 19 following FA the level reached 1400 IU/L. Transvaginal sonography was performed 28 days after

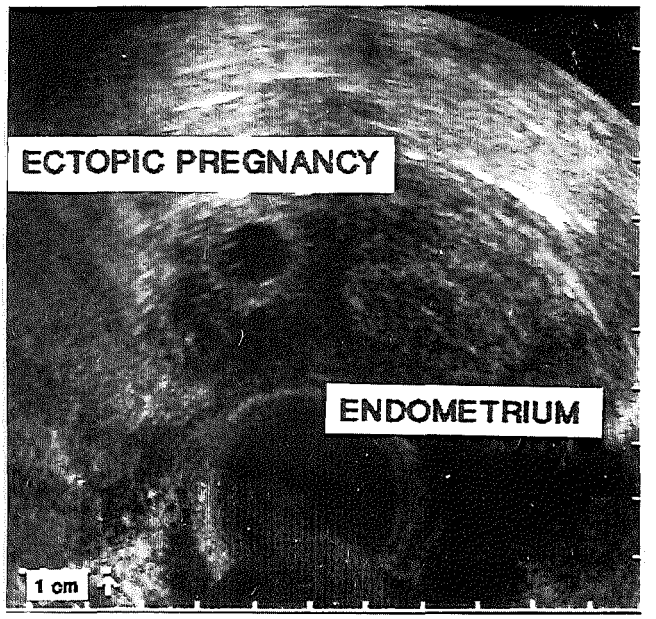

Figure 5.2.6. *Ectopic pregnancy localised in the intramural part of the right tube (Patient No. 9).*

FA. Inside the uterus a very thick homogeneous endometrium measuring 18 mm was present and outside the endometrium in the intramural part of the right tube a gestational sac with a yolk sac–embryo complex (Figure 5.2.6). The embryo measured 2 mm and displayed a heart rate of 112 BPM. A diagnosis of ectopic interstitial pregnancy was made. It was decided to puncture and to aspirate the gestational sac and to inject 40 mg of MTX locally. The intervention was without complications. The patient stayed in hospital for observation. Figure 5.2.7 depicts the course of the hCG level. In the first week there was a gradual rise in hCG level. It was decided to administer MTX systemically in three doses of 50 mg intramuscularly at three days intervals[28-35]. A leucovorin rescue scheme was included. The hCG level started to fall immediately and the patient was discharged from the hospital after the last MTX injection. Despite a further drop in hCG level, sonographically a cyst like structure developed at the site of the former ectopic pregnancy (Figure 5.2.8). Transvaginal puncture of this cyst was

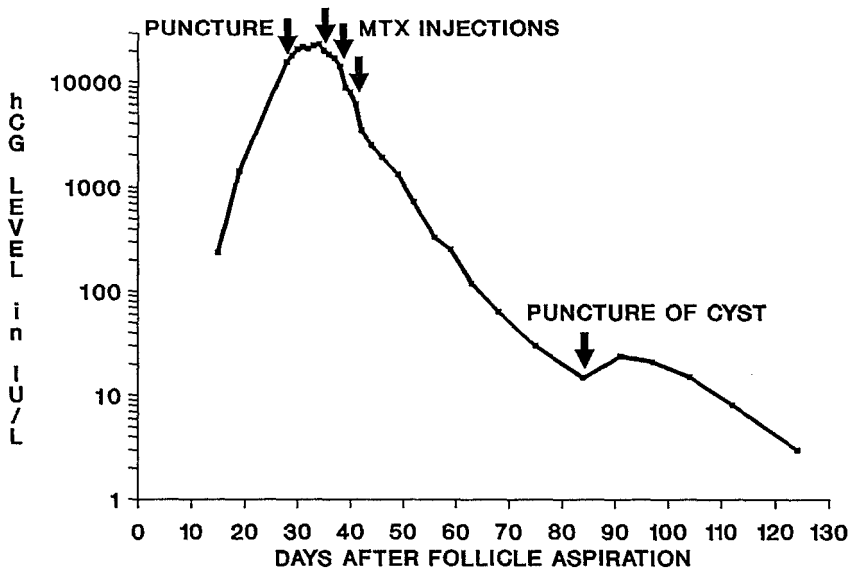

***Figure** 5.2.7. Course of the hCG level of Patient No. 9 (ectopic interstitial pregnancy).*

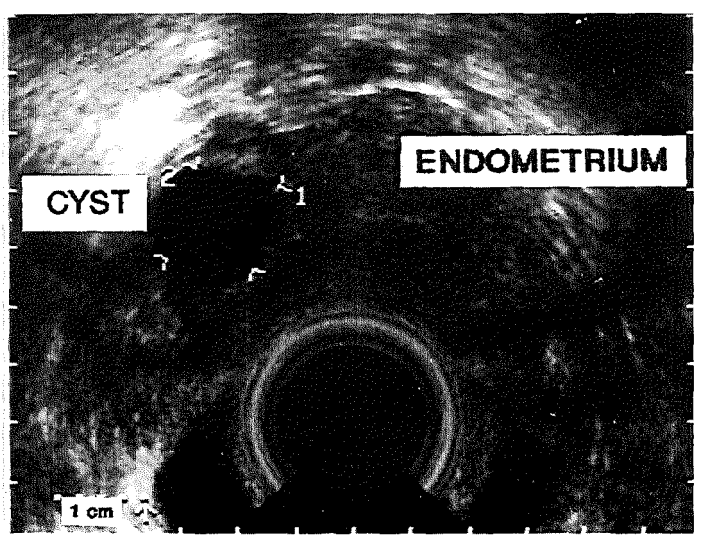

***Figure** 5.2.8. Cyst formation at the site of the previous ectopic interstitial pregnancy (Patient No. 9).*

followed by a transient slight rise in hCG level, but without any further treatment dropped to levels indicative of absent trophoblastic activity.

Patient No. 10, aged 37 years with primary infertility due to oligoasthenozoospermia was treated by means of intra-uterine insemination (IUI) of capacitated spermatozoa after ovulation induction with clomiphene citrate and hCG. IUI was carried out 40 hours after intramuscular injection of 10.000 IE of hCG. In the second treatment cycle a pregnancy occurred. On day 15 following IUI a urine pregnancy test (ICON) was negative. The amenorrhoea persisted and on day 24 the hCG level was 528 IU/L. On day 38 after IUI an ectopic pregnancy could be visualised with transvaginal sonography. The ectopic gestational sac contained no yolk sac or embryo (Figure 5.2.9). The hCG level on that day was 5000 IU/L. Because of these sonographic characteristics in combination with a hCG level of 5000 IU/L

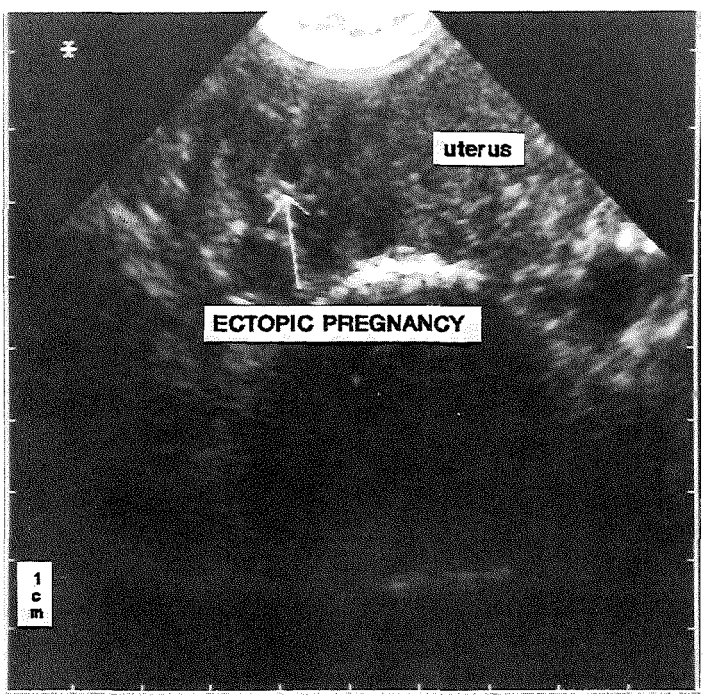

Figure 5.2.9. *Sonographic appearance of a non-vital ectopic pregnancy (Patient No. 10).*

indicating a non-vital ectopic pregnancy, it was decided to await spontaneous resorption under strict sonographic and biochemical monitoring. Figure 5.2.10

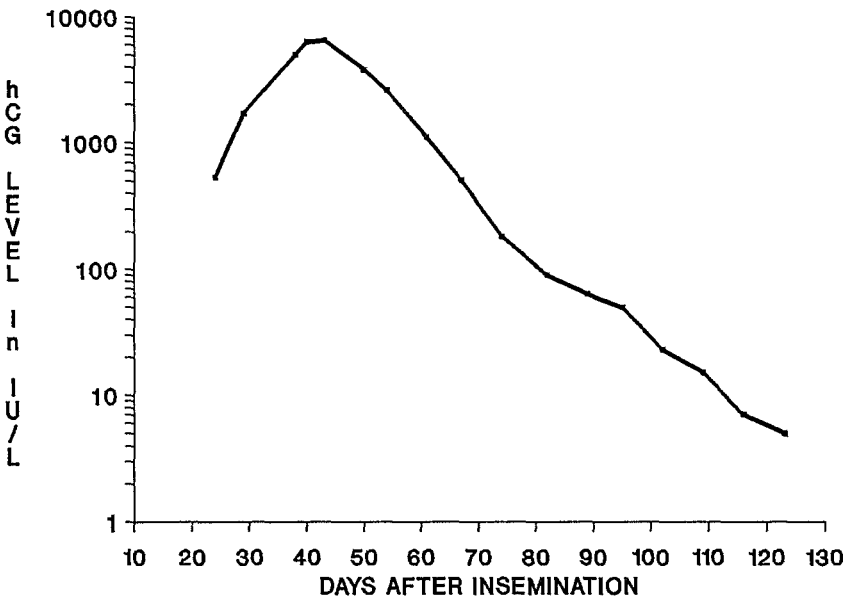

Figure 5.2.10. *Course of the hCG level of Patient No. 10.*

depicts the course of the hCG levels. Apart from mild irregular vaginal bleeding, the patient never revealed any sign or symptom of ectopic pregnancy. The cycle disturbance corrected itself after hCG levels had returned to normal.

5.2.4. DISCUSSION

Combined transvaginal sonography in combination with hCG determinations allow the diagnosis of ectopic pregnancy at a stage, at which the condition still may be asymptomatic[2-4]. This offers the opportunity to develop more conservative modes of management and should lead to a re-evaluation of the

surgical approach. On the other hand, there is the danger that early diagnosis may result in more surgical interventions. Such a development should be particularly undesirable in non–vital ectopic pregnancies, which otherwise would have been missed in the past and would have resolved spontaneously.

Transvaginal puncture was performed in nine patients in an effort to avitalise the ectopic pregnancy. However, in four out of nine patients no remission occurred and surgery was necessary at a later stage. Transvaginal sonography as a follow–up procedure after the puncture was not informative as illustrated by patient No. 3. It was still possible to visualise the punctured ectopic pregnancy but it did not provide enough information about the presence of residual trophoblastic activity. This appears to be better reflected by serial hCG level monitoring. Management of a vital ectopic pregnancy through transvaginal puncture is feasible, although management was unsuccessful in four out of nine cases with or without the instillation of MTX in the punctured gestational sac. It is apparently not possible to remove all vital trophoblastic tissue. The actual dosage of 10 mg of MTX may not be sufficient[27, 28]. However, Pansky et al[28] do not substantiate that the higher dosage of 25 mg of MTX yields better results than a dosage of 12.5 mg. In patient 9 the ectopic pregnancy was situated in the intramural part of the right tube and as such was not amenable to surgery due to increased risk of severe bleeding. The successful course in this case illustrates the possibility of combined local and systemic MTX therapy. This was also reported by Feichtinger & Kemeter[27].

We do not claim that transvaginal puncture of ectopic pregnancies is the therapy of choice in each patient, but since future fertility aspects are important it may be advocated in order to avoid surgery. Although four out of nine patients eventually underwent surgery, the other five did benefit from this conservative approach.

In the five non–vital ectopic pregnancies spontaneous resorption was awaited. However, one should be aware of the risks of tubal abortion, especially when hCG levels are falling. This emphasises the need for monitoring the hCG levels until they have returned to normal.

The place of systemic administration MTX in the management of ectopic pregnancy remains controversial. Failures and successes have been reported,

but the number of patients is too limited in our view to draw conclusions[30-37]. In a Dutch study of nine patients (10 ectopic pregnancies) the hCG level was below 500 IU/L in eight patients at a menstrual age of six to eight weeks, indicating the presence of non–vital or spontaneously regressing ectopic pregnancies[35]. It can be questioned, therefore, if it was justified to use a cytoreductive agent at these very low hCG levels. This also applies to studies reported by others in which systemic MTX treatment was implemented at hCG levels below 1000 IU/L at an amenorrhoea of six weeks or more. Moreover, at least two hCG determinations should be done in order to judge actual trophoblast activity before systemic MTX treatment is contemplated. Only Rodi et al[34] mention a rise or plateauing in hCG level as an inclusion criterium for systemic MTX treatment. The literature data concerning systemic MTX treatment in relation to ectopic pregnancy is summarised in Table 5.2.II.

Our own experience and review of the literature lead to the following suggestions:

1. In case of a non–vital ectopic pregnancy or uncertainty about the diagnosis, hCG assays and transvaginal sonography should be carried out every other day. In case of falling hCG levels await spontaneous resolution; in case of signs of tubal abortion laparoscopy may be necessary to exclude active intra–peritoneal bleeding.

2. In case of a vital ectopic pregnancy, transvaginal aspiration of gestational sac and contents should be performed under sonographic guidance and local application of a maximum of 50 mg of MTX. In case of subsequent rise or plateauing in hCG level, systemic MTX treatment should be considered.

3. At a CRL exceeding 10 mm the extensiveness of vital trophoblast may inhibit complete regression following gestational sac aspiration. Instead laparotomy is indicated.

In conclusion, transvaginal sonography is a valuable adjunct to hCG

Table 5.2.II. Literature review systemic MTX treatment.

Author	No. E.P.	hCG level Range (IU/L)	No. E.P. hCG < 1000 IU/L	MTX Dosage i.m.	C.A.	HSG + Result	Res.	Complications
Ory et al[33]	6	103-25410(1)	3	1 mg/kg/ 48hr/4x	un	np	5	1 Salpingectomy
Rodi et al[34]	7	127-15000(2)	5	1 mg/kg 48hr/4x	un	5, 4 open	7	2 "protracted courses"
Kooi et al[35]	10	20-17460(2)	9	50 mg/48hr 4-9x	un	1, open	9	1 laparotomy 2 months later
Stovall et al[36]	36	42-30966(2)	un	1 mg/kg/24hr 1-4x	1	np	34	2 laparotomy: hCG 15349 en 30996 IU/L (2)
Ichinoe et al[37]	23	80-16000 (urinary !)	9 ?	0.4 mg/kg/ 24hr/5x	un	19, 10 open	22	1 tubal rupture

E.P. = ectopic pregnancies, MTX = methotrexate, i.m. = intramuscularly, C.A. = presence of embryonic cardiac activity, HSG = hysterosalpingogram, Res. = resolution, (1) = 1st IRP, (2) = WHO standard not specified, un = unknown, np = not performed.

determinations and to laparoscopy in the diagnosis of ectopic pregnancy. Aspiration under sonographic control of an ectopic pregnancy with local instillation of MTX, followed by serial sonographic and biochemical (hCG) monitoring of trophoblast resorption seems a promising management procedure. However, its success rate in terms of complete resorption and tubal patency afterwards, as well as its cost–effectiveness relative to conventional surgical intervention needs to be evaluated.

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CHAPTER 6

FUNCTIONAL ASPECTS OF THE DEVELOPING EMBRYO

INTRODUCTORY REMARKS

Embryonic cardiac activity is the first sign of embryonic viability in early pregnancy. Knowledge about the presence of a viable embryo will reduce mental anxiety in those women who are at increased risk of pregnancy pathology. Serial documentation of embryonic heart rate in early pregnancy will provide more insight into the role of the sympathetic and parasympathetic system in determining cardiac rate and rhythm during embryonic development. Information is nowadays available on embryonic heart rate patterns prior to early abortion. Both aspects will be discussed in chapter 6.1. Observations done in IVF single pregnancies revealed differences in first appearance of embryonic cardiac activity. In chapter 6.2., IVF multiple pregnancies serve as a model for studying discrepancies in first appearance of cardiac activity under standardised maternal conditions. Finally, in chapter 6.3. a case report is presented in which an abnormal embryonic heart rate pattern recorded in early pregnancy was associated with Down's syndrome.

6.1. EMBRYONIC HEART ACTIVITY : APPEARANCE AND DEVELOPMENT IN EARLY HUMAN PREGNANCY

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6.1.1. SUMMARY

The appearance and development of embryonic cardiac activity was studied in early human pregnancies established by in-vitro fertilisation (IVF) using transvaginal sonography. Embryonic cardiac activity could be detected as early as 25 days after follicle aspiration. The range in appearance of embryonic cardiac activity in normal continuing pregnancies was 5 days. There was no correlation with maternal age, day of embryo transfer, or cell stage at embryo transfer. As the difference in appearance of embryonic cardiac activity was associated with a difference in crown-rump length whilst the subsequent growth curve was normal we ascribe these findings to different duration of the implantation stage. The later cardiac activity was detected the greater the risk of miscarriage. Reference curves were created relating embryonic heart rate to the number of days following follicle aspiration, the number of days that cardiac activity is present and the crown-rump length. In pregnancies ending in miscarriage heart rate patterns fell away from the reference curve. Embryonic factors seem to play an essential role in these observations.

6.1.2. INTRODUCTION

One of the first signs of a viable pregnancy is the appearance of embryonic heart activity. Using a standard abdominal ultrasound technique, Robinson[1] demonstrated this activity at 45 – 47 days of amenorrhoea. Ultrasound equipment has improved dramatically since then. The recent introduction of transvaginal sonography might enable detection of embryonic cardiac activity at a much earlier stage. The major advantage of this technique is that the distance between transducer and object of scanning, in this case the uterus and its contents is greatly reduced. This creates the opportunity to use high MHz frequencies with good resolving properties. Subjects' acceptance, despite previous reservation, has been remarkably good, especially as there is no need to endure a full bladder. The in–vitro fertilisation (IVF) procedure provides exact data on the time of ovulation (=follicle aspiration) and the subsequent embryo transfer, and therefore the duration of pregnancy. This allowed us to obtain more insight into the onset of embryonic cardiac activity and its development in early human pregnancy.

6.1.3. SUBJECTS and METHODS

A total of 47 women consented to participate in the study. All women became pregnant after IVF treatment in our clinic. The study was approved by the ethics committee of the institution. The procedure in our centre included the transport of oocytes to the laboratory of the Department of Physiology II at the Erasmus University of Rotterdam as has been described earlier[2]. The mean age of the women in the study group was 34 years (range 24 – 40 years). A pilot–study in 23 women showed that embryonic cardiac activity could not be detected before the 25th day after follicle aspiration. Based on this we designed the following protocol: transvaginal sonography was performed 25 days after follicle aspiration and repeated every day until embryonic heart activity appeared, then the women were scanned at intervals of 2 or 3 days until 42 days after follicle aspiration. The protocol implicates between five and eight recordings of cardiac activity for each embryo (median six). Two

days or 3 days were chosen at random depending on personal factors such as availability and travelling distance.

All the women were scanned between 10.00 and 12.00 a.m. using the Diasonics DRF 400 and the Toshiba SAL 77B vaginal probes. The former was equipped with a 5.0 and a 7.5 MHz mechanical sector scan transducer and the latter with a 5.0 MHz electronic sector scan probe. Scans were carried out by R.S. and C.A.M.J.. All observations were taped and when there was heart activity the frequency was nearly always documented by M–mode in conjunction with the real–time scan (Toshiba SAL 77B) (Figure 6.1.1). The embryonic heart rate was calculated from the M–mode or, if this was not possible, from the real–time image. Embryonic cardiac activity was defined as a distinct contractile pattern inside the embryo with a frequency different

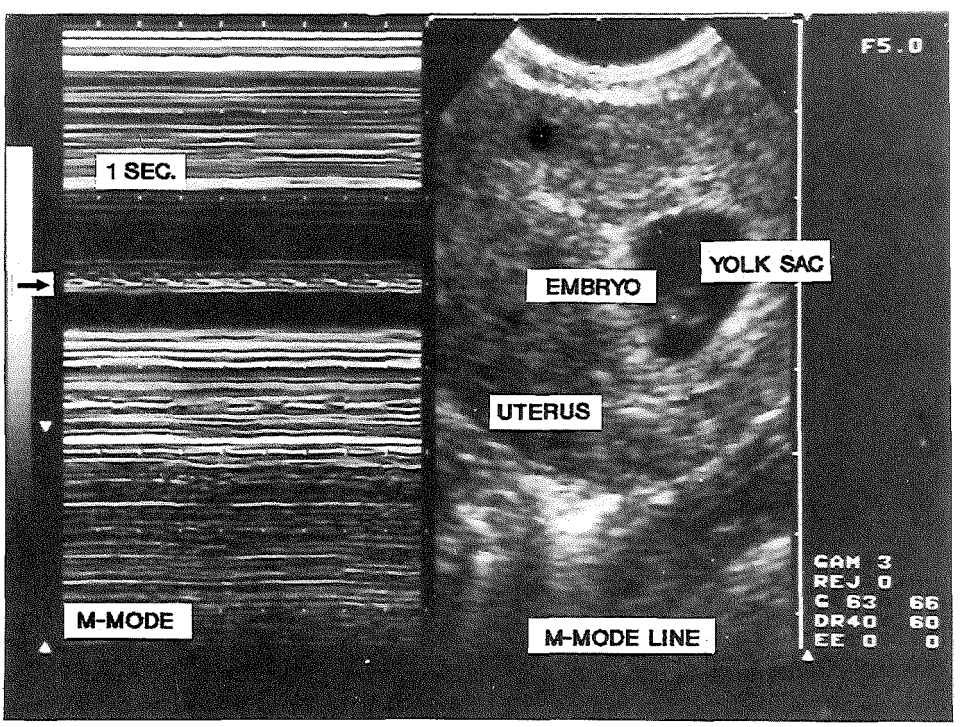

Figure 6.1.1. *Combined real–time and M–mode registration of an embryonic heart rate 29 days after follicle aspiration.*

from that of the maternal heart. In case of doubt both frequencies were counted simultaneously to be absolutely sure of registering the embryonic heart beat.

In 12 women the timing of onset of embryonic cardiac activity and the embryonic heart rate were determined by both R.S. and C.A.M.J. from the same M–mode recordings to exclude the possibility of inter–observer variability. Both observers also measured heart rate independently in 10 women at intervals of 15 – 60 min to rule out differences in heart rate over a short period of time.

The Kolmogorov–Smirnov two–sample test was used to test the distribution of embryonic heart rate and crown–rump length (CRL) of single and multiple pregnancies.

Embryonic heart rate was expressed as means +/– 1 SD. The Wilcoxon rank sum test was used to determine the difference in timing of onset of embryonic cardiac activity between continuing pregnancies and pregnancies which later miscarried in the first trimester.

6.1.4. RESULTS

There was complete agreement between the two observers for the timing of onset of embryonic cardiac activity. The inter–observer variation for embryonic heart rate determination from the same M–mode recordings was very low. The mean of the differences was 2.3 (SD 1.5, range 0 – 4, median 2). There was almost no variation in the embryonic heart rate over a short period of time (15 – 60 min). The mean of the differences was 2.9 (SD 1.7, range 0 – 4, median 2).

In eight out of 47 women we were not able to time the onset of embryonic cardiac activity due to personal factors such as non–availability on specific days and holidays of the women. This resulted in incomplete data from eight women (five singleton and three twin pregnancies) and complete data from 39 women (26 singletons, ten twins, three triplets) for further analysis. We included also multiple pregnancies, because so early in gestation development of embryonic cardiac activity and growth rate of CRL is similar in singleton

and multiple pregnancies. The cumulative frequency distribution for singleton and multiple pregnancies was virtually the same suggesting they can be regarded as one population (two-tailed P = 0.99). The mean of the absolute differences for embryonic heart rate (+/– SD) and mean CRL (+/– SD) were 2.42 (SD 1.5, range –4.4 to 5.1), 3.23 (SD 2.76, range –11.3 to 5.1), 0.37 (SD 0.27, range –1 to 0.6) and 0.29 (SD 0.28, range –0.7 to 0.6) respectively.

Figure 6.1.2 shows the distribution in the appearance of embryonic cardiac activity over the days after follicle aspiration. A difference of up to 5 days was seen in the continuing pregnancies. A continuing pregnancy was defined as a gestation with a well-developed fetus identified by ultrasound examination 11 weeks after follicle aspiration. The time difference in appearance of embryonic cardiac activity between the group of continuing pregnancies (33 women, 48 embryos) and pregnancies that miscarried in the first trimester (six women, seven embryos) was statistically significant (p = 0.0028). Figure 6.1.3 depicts embryonic heart rate relative to the number of

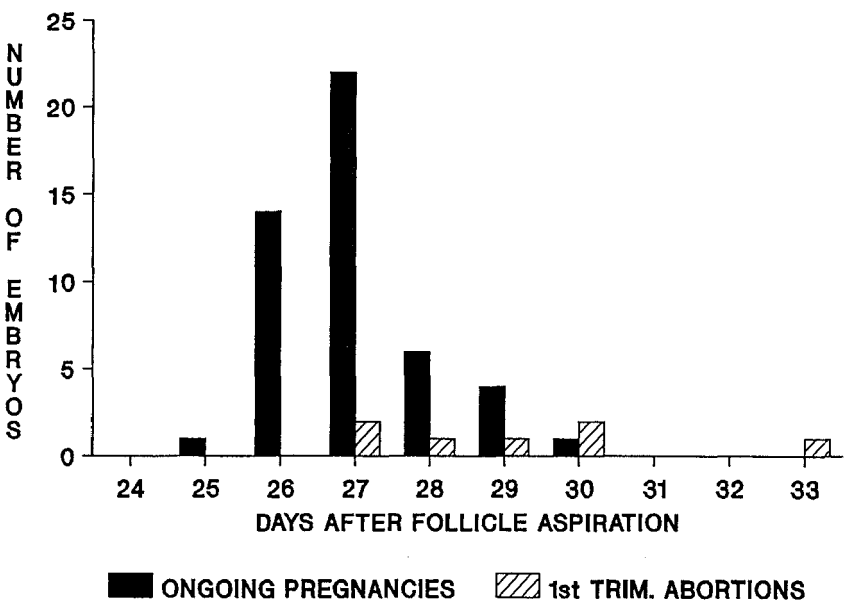

Figure 6.1.2. *The detection of embryonic cardiac activity in relation to the number of days after follicle aspiration.*

days after follicle aspiration. The number of observations per day ranged

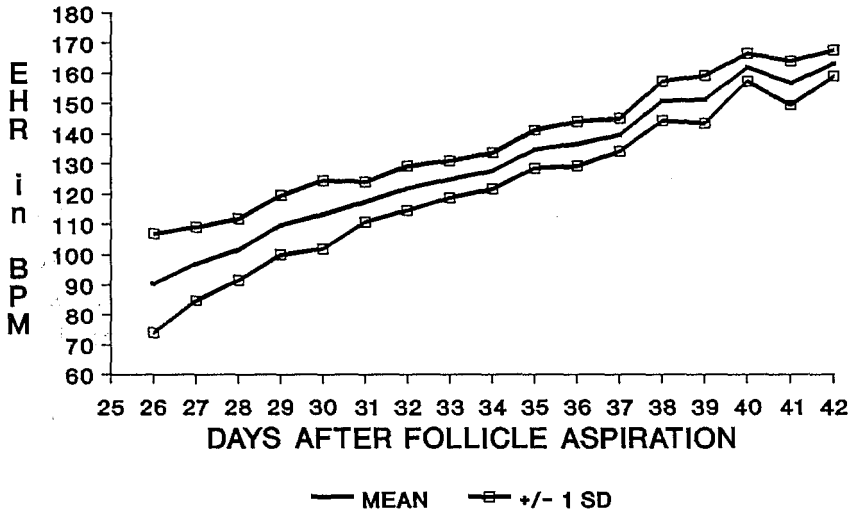

Figure 6.1.3. *Embryonic heart rate (EHR) (mean +/- 1 SD) in relation to the number of days after follicle aspiration. BPM = Beats per minute.*

between 10 and 31 (median 17). Mean embryonic heart rate rose from 90.5 (SD 16.5) beats/min on day 26 to 163.3 (SD 4.3) beats/min on day 42 after follicle aspiration. Figure 6.1.4 shows the embryonic heart rate relative to the number of days following the first detection of embryonic cardiac activity. The number of observations per day varied between 10 and 48 (median 24). Mean embryonic heart rate gradually increased from 92.5 (SD 12.9) beats/min on day zero to 161.0 (SD 5.3) beats/min on day 15. The developmental pattern of embryonic heart rate in the six pregnancies which miscarried (seven embryos) was always abnormal when compared with the continuing pregnancies (Figure 6.1.5). In three pregnancies abnormal heart rates preceded intrauterine death by a number of days or even weeks (range 1 – 17 in days). In Figure 6.1.6, embryonic heart rate is related to the ultrasonically

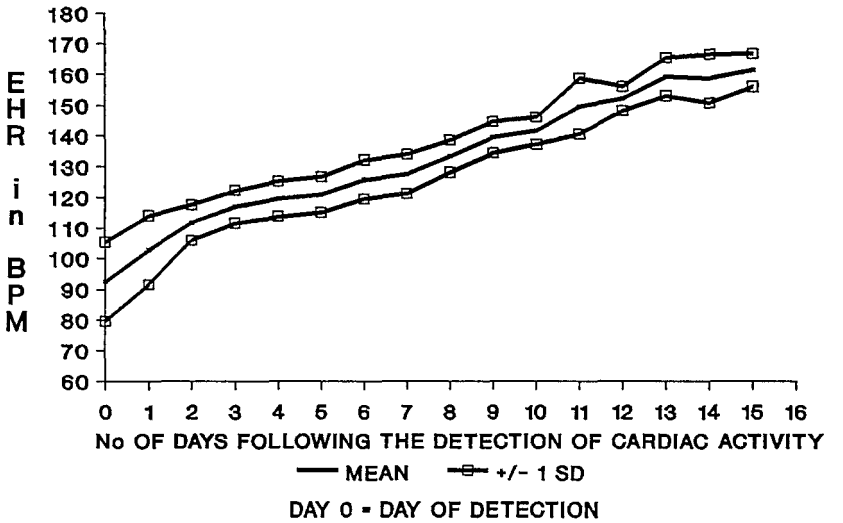

Figure 6.1.4. Embryonic heart rate (EHR) (mean +/- 1 SD) in relation to the number of days cardiac activity is present. BPM = Beats per minute.

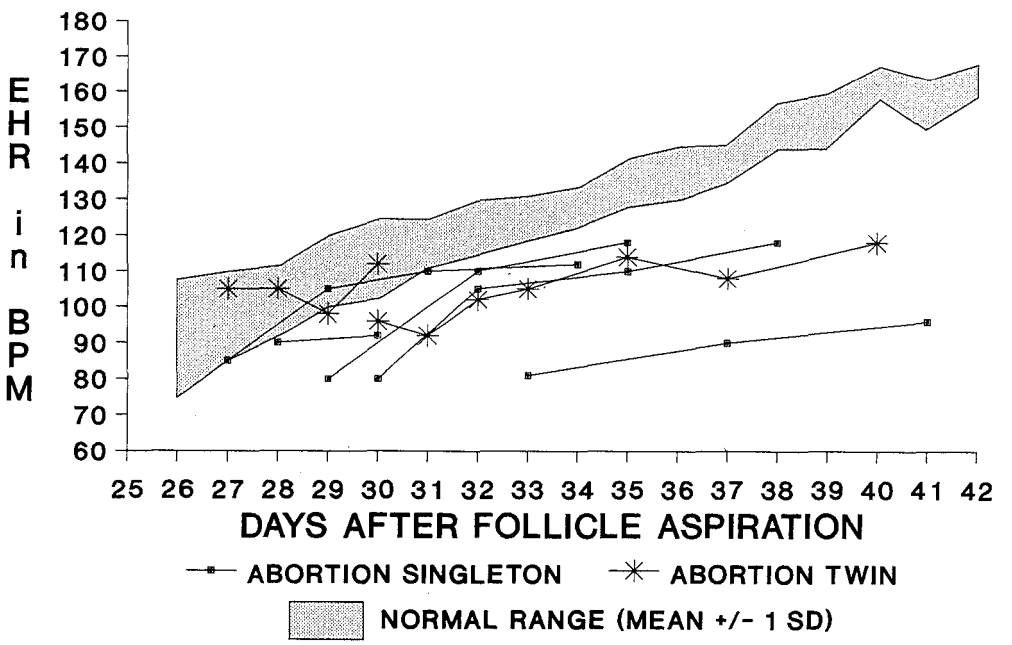

Figure 6.1.5. Embryonic heart rate patterns of all six first trimester abortions (seven embryos) relative to the normal range.

determined CRL. The number of observations per day ranged between 11 and

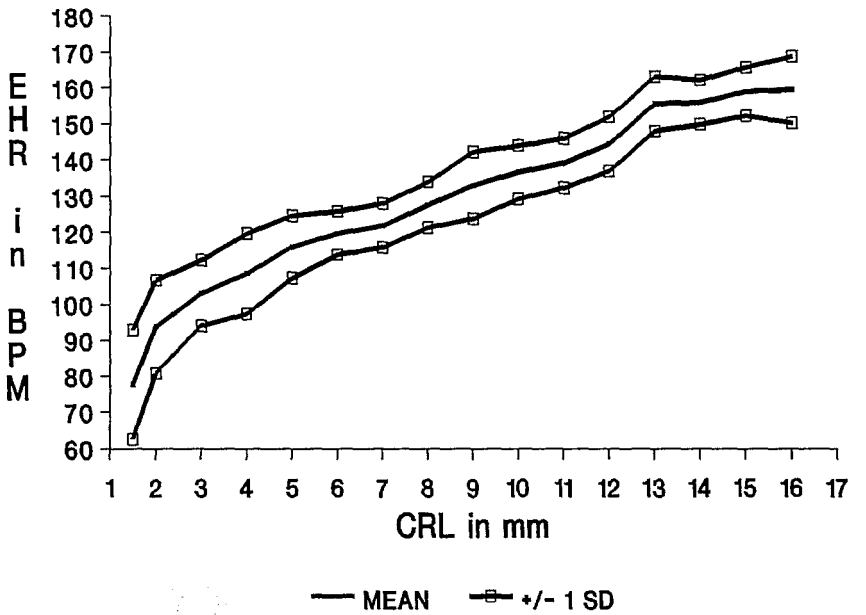

Figure 6.1.6. *Embryonic heart rate (EHR) (Mean +/- 1 SD) relative to the crown-rump length (CRL) in mm. BPM = Beats per minute.*

35 (median 26). Mean embryonic heart rate increased from 93.8 (SD 12.9) beats/min at a CRL of 2 mm to 159.0 (SD 6.7) beats/min at a CRL of 15 mm. The distribution of the heart rate determinations as well as the CRL measurements was normal.

In the present study embryo transfer was performed either 2 days (N=22) or three days (N=17) after follicle aspiration. There was no difference in timing of the first cardiac activity between these two groups (mean 27.3, SD 1.6 days compared with mean 27.0, SD 1.2 days after follicle aspiration). However, six of the seven embryos that were miscarried were transferred 2 days after follicle aspiration. Excluding the miscarriages, the corrected values are 26.7 (SD 0.7) and 27.0 (SD 1.3) days after follicle aspiration; the difference is not statistically significant.

No correlation was found between the cell stage at embryo transfer (two to eight cell stage) and appearance of first embryonic cardiac activity ($r = -0.16$).

Finally, there was a very weak correlation between the timing of first embryonic cardiac activity and maternal age ($r = 0.18$).

6.1.5. DISCUSSION

The more sensitive technique of transvaginal sonography has opened up a new field of research and provides the opportunity to study the very early stages in the development of human pregnancy.

The advantage of studying IVF pregnancies is that some of the variables such as time of ovulation (=follicle aspiration) and embryo transfer are exactly known. Whether the results can be extrapolated to spontaneous pregnancies is uncertain.

Most women in our study were older than average for pregnant women in the Netherlands, reflecting the population currently having IVF treatment.

Embryonic cardiac activity could be detected as early as 25 days after follicle aspiration. The spread of five days in the detection of heart activity in continuing pregnancies is considerable. Five days represents between 16.7% and 20% of the duration of pregnancy at that time. There is no clear explanation. Maternal age, the time between follicle aspiration and embryo transfer and the cell stage of the embryos at embryo transfer do not seem to play a significant role. Although the timing of embryo transfer after follicle aspiration does not influence the appearance of embryonic cardiac activity, in five of the six (one twin) pregnancies that miscarried the six embryos were transferred two days after follicle aspiration. This observation accords with the findings by Os van et al[3], who reported a statistically significant difference in the proportion of continuing pregnancies between embryos transferred two days and three days after follicle aspiration. Other factors, most likely embryonic, in conjunction with endometrial conditions at the sie? of implantation may play an important role in establishing a pregnancy. Moreover, in multiple pregnancies we often observed a variation in

appearance of cardiac activity between embryos, which emphasizes the importance of embryonic factors, rather than maternal variables which under these circumstances are constant[4]. The growth potential of the embryo itself seems to be very important in the struggle for implantation. It is possible that fast dividing embryos stand a better chance of implantation, and we examined whether this division potential was related to the day of first detection of cardiac activity[5], but we found no relation between cell stage at embryo transfer and the day at which cardiac activity was detected. Strong, normal embryos will implant fast, but it seems impossible to judge the quality of the embryos on morphological and cleavage characteristics alone[5-7]. If the asynchrony in appearance of cardiac activity in multiple pregnancies indeed reflects variability in embryonic rather than maternal factors, then it is easier to understand why a later appearance of embryonic cardiac activity is associated with a higher chance of miscarriage.

Transvaginal sonography did not only enable us to detect the embryonic heart beat one week earlier than reported previously, but we also found a certain pattern in the development of the embryonic heart rate. This was about 90 beats/min on day 26 and gradually increased to about 165 beats/min on day 42. Further improvement in ultrasound equipment may elucidate the rate at which the heart tube really starts functioning. Embryonic heart rhythm appeared to be very regular, however, quantification of the beat to beat variation from M-mode recordings was not feasible. The subsequent increase in rate may be an expression of the maturation of the nervous system or an adaptation to the size of the embryo or both. This may explain the observed relation between crown-rump length and heart rate at this stage of pregnancy. The pregnancies ending in miscarriage all showed an abnormal heart rate pattern in relation to our reference curve. This deviating pattern seems to be a reflection of the reduced growth capacity of the embryo. Heart rate may start off normal, but after a few days it falls away from the reference curve.

We conclude that in continuing IVF pregnancies the date of onset of embryonic cardiac activity may differ as much as five days. The later the onset of cardiac activity the greater the chance of miscarriage. The embryonic

heart rate preceding miscarriage may initially be normal or lower, but ultimately falls away from the reference curve. Environmental factors do not seem to play a significant role in these findings, so we believe the factors determining onset of cardiac activity and subsequent increase in embryonic heart rate are mainly embryonic in origin.

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6.2. ASYNCHRONOUS APPEARANCE OF EMBRYONIC CARDIAC ACTIVITY IN MULTIPLE PREGNANCIES FOLLOWING IN-VITRO FERTILISATION

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6.2.1. INTRODUCTION

Implantation is a continuing process that extends from day 5 or 6 up to about day 12 following ovulation. The embryo becomes a blastocyst on day 4 or 5 after fertilisation. At this stage it is capable, after hatching, of attachment to the endometrium and of implantation. In single pregnancies following in-vitro fertilisation (IVF) a difference up to 5 days in the appearance of embryonic heart activity has been demonstrated[1]. The factors determining rapid or slow implantation and subsequent development are disputed. Multiple pregnancies provide the opportunity to study different embryos in the same individual, thereby standardising for maternal influences. The objective of the present study was to establish a difference in the duration of the implantation stage in the human through documentation of the first appearance of embryonic cardiac activity in multiple pregnancies.

6.2.2. SUBJECTS and METHODS

Between September 1987 and April 1988 a total of twelve pregnant subjects consented to participate in the study. There were nine twin pregnancies and three triple pregnancies. All arose from IVF treatment, providing exact knowledge of the time of "ovulation" and transfer of the embryos and thus of embryonic age. Only pregnancies with normally developed fetuses detected by ultrasound examination 11 weeks after follicle aspiration (FA) were included.

All patients were scanned between 10.00 and 12.00 a.m. using the Diasonics DRF 400 and the Toshiba SAL 77B transvaginal probes. The former was equipped with a 5.0 and a 7.5 MHz mechanical sector scan transducer and the latter with a 5.0 MHz electronic sector scan probe. Following localisation of the embryos, a search for cardiac activity was made. If cardiac activity was

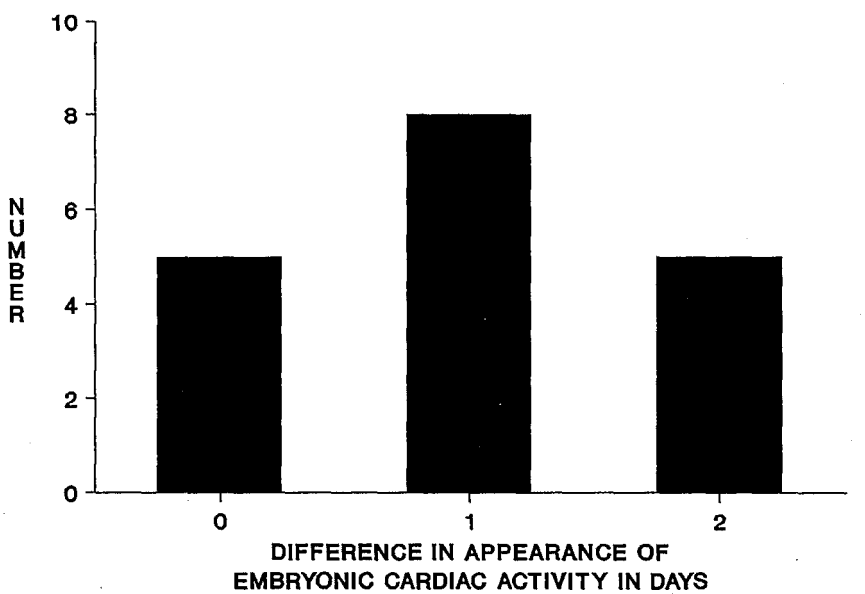

Figure 6.2.1. Difference in appearance of embryonic cardiac activity in days in IVF multiple pregnancies.

present the heart rate was documented by M–mode in conjunction with a real–time scan. The heart rate was calculated from the M–mode in beats per minute (BPM). Daily examinations for cardiac activity were started on day 25 after follicle aspiration. This was based on a pilot study in 23 women in which cardiac activity could not be detected before day 25 after follicle aspiration. Following documentation of first cardiac activity in all chorionic sacs within the same pregnancy, further examinations of the heart rate and crown–rump length (CRL) were carried out in 2 – 3 day intervals until 42 days after follicle aspiration.

6.2.3. RESULTS

Cardiac activity was first documented between day 26 and day 29 after FA (median day 27). The time difference in first appearance of cardiac activity

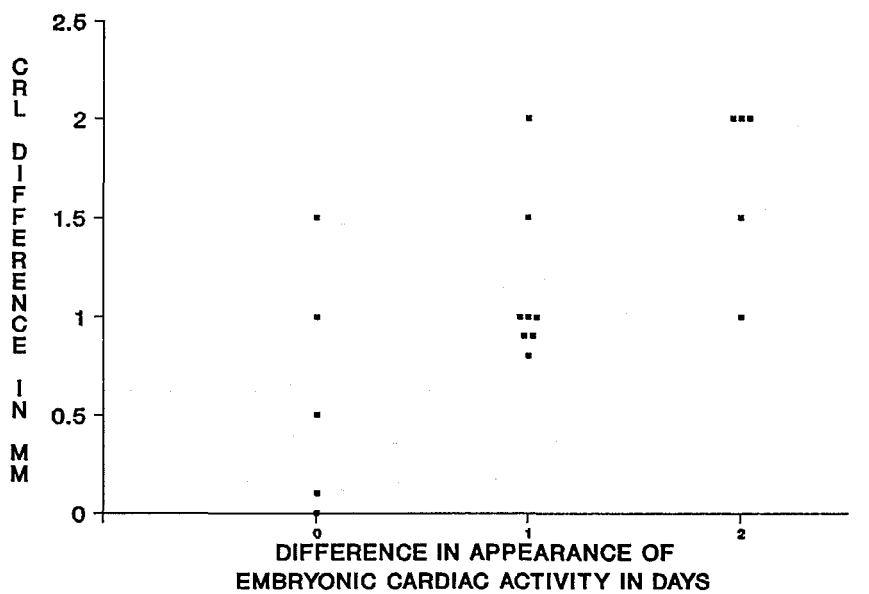

Figure 6.2.2. Difference in CRL relative to the difference in appearance of embryonic cardiac activity in multiple IVF pregnancies.

between different embryos in one and the same pregnancy varied between zero and 2 days (median 1 day) (Figure 6.2.1). A significant increase ($p < 0.05$

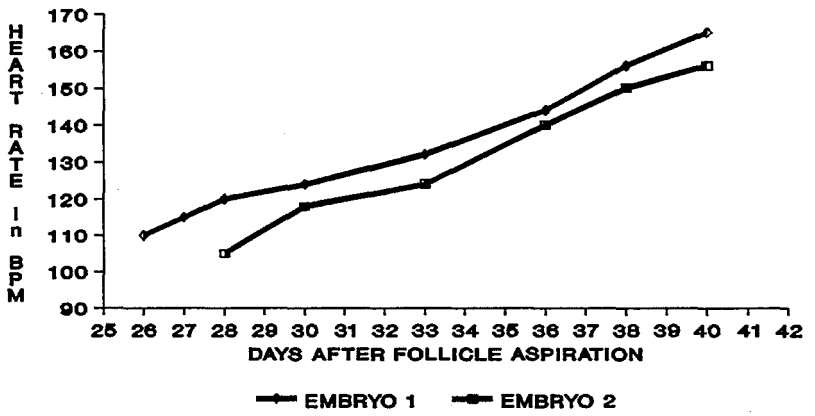

Fig. 3a

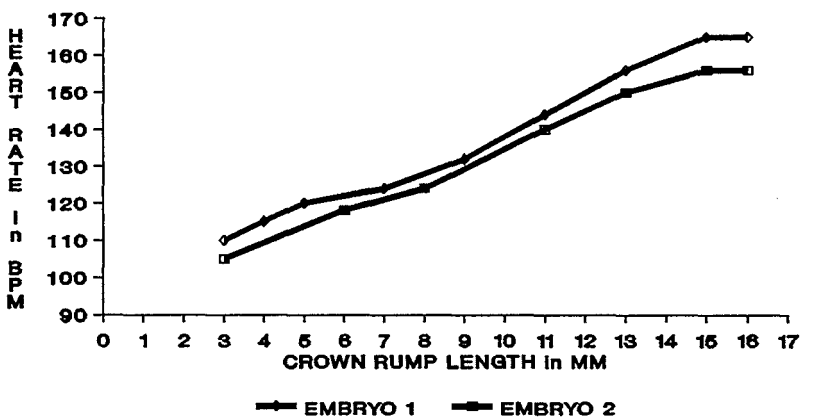

Fig. 3b

Figure 6.2.3. A twin IVF pregnancy: the difference in heart rate determined by the sequential onset of cardiac activity (figure 3a) is merely reduced after standardisation for CRL (figure 3b).

; Wilcoxon rank sum test) in CRL difference relative to the difference in appearance of embryonic cardiac activity was observed, when all multiple pregnancies were considered together (Figure 6.2.2). This is also demonstrated in Figure 6.2.3, in which for two embryos from the same pregnancy the difference in heart rate determined by the sequential onset of cardiac activity (Figure 6.2.3a) is merely reduced up to day 40 after follicle aspiration following standardisation for CRL (Figure 6.2.3b).

6.2.4. DISCUSSION

Until now it is impossible to judge, on morphological grounds alone the quality of oocytes after stimulation and harvesting, and the quality of the pre–embryos they produce after fertilisation[2]. Under in–vitro conditions the difference in developmental potential of pre–embryos has been documented from the cleavage rate[3, 4]. This finding cannot be extrapolated to the in–vivo situation because of the different environment in which the pre–embryos develop, in particular the quality of the endometrium. Implantation is the means by which the early embryo establishes a fixed relationship with respect to the uterus. The nature of the implantation process varies considerably between species and may include such events as the activation, spacing and orientation of the blastocyst, adhesion of the trophoblast to the luminal epithelium, invasion of the endometrial stroma and the development of a decidual response. The successful completion of these events involves a complex series of synchronised changes in the blastocyst and endometrium under the control of ovarian steroids. The very complexity of implantation makes this an extremely critical stage of early pregnancy.

A multiple IVF pregnancy offers the unique opportunity to study the development of two or even three embryos in the same subject, thus standardising for inter–individual differences such as maternal age, parity, and other environmental variables. The difference in appearance of cardiac activity between embryos from the same pregnancy may be determined by asynchrony in onset of maternal nutrient supply, although other factors such as micro–environmental differences cannot be ruled out. However, after

implantation the growth potential for each of those embryos was virtually the same, as reflected by the increase of CRL and the heart rate. We therefore suggest that the difference in first appearance of cardiac activity between embryos from the same pregnancy may be determined by the variable duration of the implantation stage.

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6.3. ABNORMAL EMBRYONIC HEART RATE PATTERN IN EARLY PREGNANCY ASSOCIATED WITH DOWN'S SYNDROME

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6.3.1. SUMMARY

The case history of a 35-year-old woman with a pregnancy following IVF treatment is presented. Transvaginal sonography revealed an abnormal embryonic heart rate pattern between 28 and 50 days following follicular aspiration. At term, a male infant with the characteristics of Down's syndrome was born. The diagnosis was confirmed by chromosome analysis. The implications of this observation are discussed.

6.3.2. INTRODUCTION

Several ultrasonographic signs have been described in second trimester fetuses at high risk for Down's syndrome[1-7]. Although parameters like a thickened nuchal fold, an increased biparietal diameter / femur length ratio and hypoplasia of the middle phalanx of the fifth digit are reported, there is no general agreement about the sensitivity and specificity of these signs[1-7]. A great disadvantage of these features is that they are recognised late in pregnancy. It would be of great value if a reliable parameter could be identified early in pregnancy which can indicate fetuses at risk for Down's syndrome or other chromosome abnormalities. Transvaginal sonography enables detailed information to be obtained about early morphological and physiological development of the embryo, because probes with high emission frequencies (5.0 – 7.5 MHz) and therefore high resolution can be employed. This case history illustrates that a deviating development of the embryonic heart rate was associated with a chromosome abnormality.

6.3.3. CASE HISTORY

A 35–year–old woman requested IVF treatment in our clinic. Infertility analysis in another hospital had revealed tubal pathology to such a degree that the chance of a spontaneous pregnancy could be regarded as nil. Following our routine controlled hyperstimulation scheme containing pure FSH, hMG, and hCG, oocyte recovery took place on day 12 of the cycle. Seven oocytes were obtained and three of them became fertilised. Transfer of the three embryos (8–,8– and 6–cell stage) was carried out three days after follicular aspiration. Fifteen days after oocyte collection, a pregnancy test (Predictor color) was positive. The patient consented to participate in a study on the appearance and development of embryonic cardiac activity. According to the study protocol daily transvaginal sonographic examinations were started on day 25 following oocyte recovery until the presence of embryonic cardiac activity was established, with documentation of the heart rate at two to three day intervals thereafter. The first embryonic heartbeats could be registered

on day 28 after oocyte recovery. The heart rate was calculated using combined real-time and M-mode registration of cardiac activity. The actual heart rate pattern was plotted in the reference curve for normal heart rate

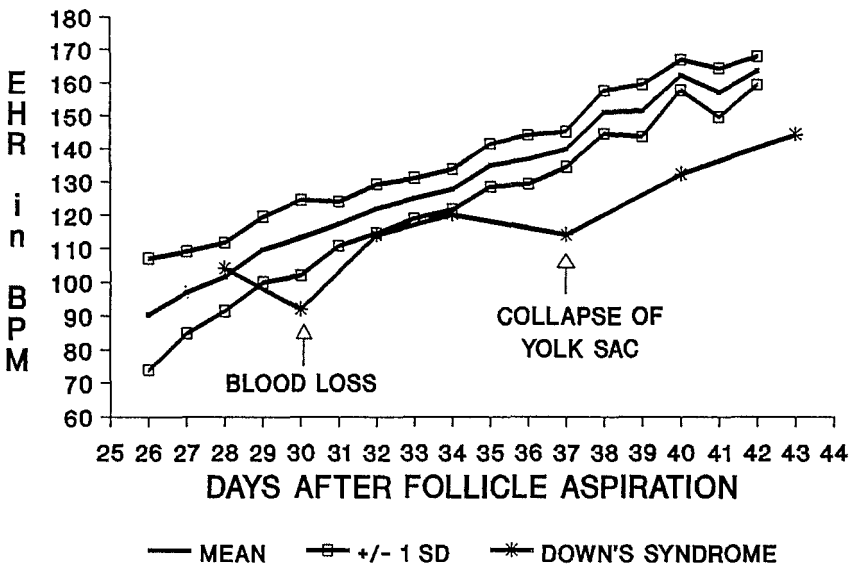

Figure 6.3.1. *Reference curve and serial plot of embryonic heart rate (EHR) in beats per minute (BPM) in a case of Down's syndrome.*

development[8] (Figure 6.3.1). On day 30, there was a slight vaginal blood loss, which was associated with a drop in heart rate below the lower limit of the reference curve. Following a slight recovery there was another marked drop in heart rate on day 37, which coincided with a sonographically visible collapse of the yolk sac (Figure 6.3.2). Whereas the yolk sac appeared to have regained its normal sonographic appearance three days later, the heart rate only returned to normal on day 50 according to the standard curve by Robinson and Shaw-Dunn[9]. From then, the pregnancy continued uneventfully. Transabdominal ultrasound examinations in the second and third trimester revealed no abnormalities and fetal growth was normal. There

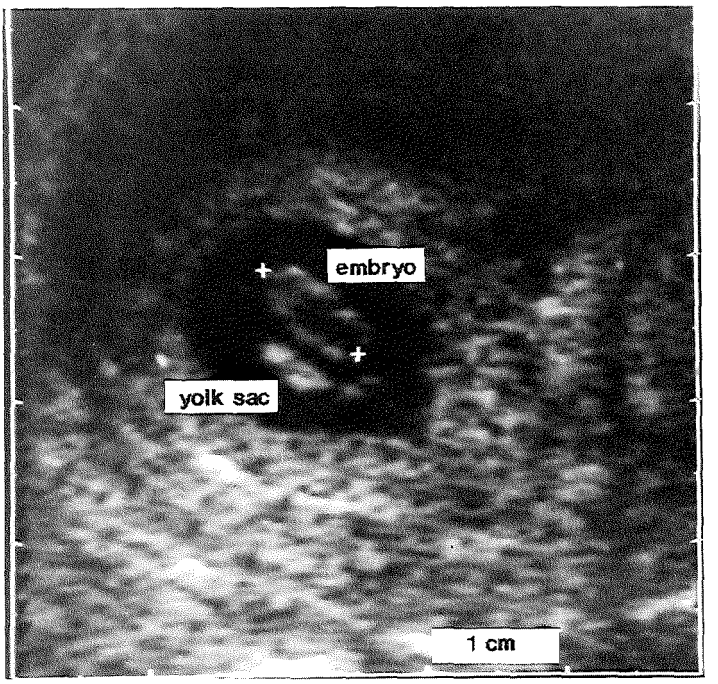

Figure 6.3.2. *Chorionic sac with embryo and collapsed yolk sac.*

was a normal biparietal diameter / femur ratio according to the reference curves by Campbell and O'Brien et al[10, 11]. At 39 weeks of gestation a male infant was born following forceps delivery because of persistent second stage fetal bradycardia. Apgar score at 5 min was 9, birth weight was 3060 g and the length 49 cm. The newborn exhibited characteristics of Down's syndrome. Chromosome analysis confirmed the presence of trisomy 21. There were no cardiac structural anomalies.

6.3.4. DISCUSSION

In this case history, abnormal development of the embryonic heart rate pattern was associated with Down's syndrome. To our knowledge this is the

first report of such an association. It would be interesting to know if this observation is just more than a coincidence. In a previous series of seven embryos with abnormal heart rate patterns between 25 and 42 days after oocyte recovery all embryos aborted in the first trimester[8]. Karyotyping in embryonic tissue was feasible in five cases, revealing an abnormal chromosomal pattern in two cases (45 XO and 46X–X + t(X;1)(q22;q23). Two cases showed a normal 46 XX chromosomal pattern and in one the tissue culture failed. In the case reported here vaginal blood loss and the temporary collapse of the yolk sac suggested threatening abortion. However, the pregnancy continued. It is common knowledge that spontaneous abortion is often associated with chromosome abnormalities. If there is indeed a close relation between early abnormal heart rate patterns and chromosome anomalies, chorionic villus sampling or amniocentesis should be offered in these cases. Further evidence is needed, until then the natural history of these abnormal early heart rate patterns should be studied.

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CHAPTER 7

CONCLUSIONS

Transvaginal sonography can be regarded as a breakthrough in the visualisation of early pregnancy development. It allows the use of transducers with higher emission frequencies, resulting in better image resolution.

The acceptance of this method by patients is excellent. Moreover, energy output at the level of the embryo is lower with transvaginal scanning than with transabdominal scanning.

Anatomical as well as functional aspects of gestational development can be evaluated earlier than previously described with transabdominal scanning. The diagnosis of vital and non-vital intra-uterine pregnancy can now be made as early as 6½ weeks menstrual age.

The introduction of transvaginal sonography allows more detailed visualisation of embryonic anatomy and, therefore, earlier and more accurate measurement of the crown-rump length (CRL). A reappraisal of the current reference chart for embryonic CRL demonstrates a smaller distribution of CRL measurements, allowing more accurate pregnancy dating. Moreover, transvaginal CRL measurements can be obtained approximately four days earlier than previously reported employing transabdominal scanning.

Transvaginal sonography is also helpful in determining the fate of those pregnancies associated with vaginal bleeding. A rather fixed sequence of distinctive features of embryonic development (visualisation of the gestational sac and yolk sac; appearance of cardiac activity; embryonic growth (CRL measurement)) has to be followed between definite time limits.

Transvaginal sonography in combination with sensitive hCG determinations permits the diagnosis of ectopic pregnancy before clinical signs or symptoms have appeared. This allows conservative management, which is of particular importance regarding future fertility. Transvaginal puncture under sonographic guidance with or without the instillation of cytoreductive agents may be successful. However, the failure rate of this approach is still too high to advocate general use. At present, the place of

transvaginal sonography in the various modes of conservative management of ectopic pregnancy has not been established clearly.

Embryonic cardiac activity is the first dynamic parameter that can be evaluated in early pregnancy development. The absence of cardiac activity after 30 days following ovulation or a heart rate falling away from the reference chart is associated with poor pregnancy outcome. A possible relationship between an abnormal heart rate pattern and chromosomal anomalies is suggested. Environmental factors do not seem to play an important role in the appearance and development of cardiac activity, as is illustrated by the variable onset of cardiac activity (difference up to two days) in multiple IVF pregnancies. Embryonic quality and subsequent growth potential seem to be the main determinant factors.

SUMMARY

Chapter 1

In this chapter the history of transvaginal sonography is summarised. Whereas the vaginal approach was already suggested in the 1960's recent electronic advances have made renewed introduction feasible. In the early eighties transvaginal sonography was mainly used as method for monitoring follicle growth and later also to recover oocytes in IVF centres. Since then transvaginal sonography is used in several diagnostic procedures in Obstetrics and Gynaecology, which are discussed shortly. The objectives of the studies presented in this thesis are defined.

Chapter 2

In this chapter the technical and methodological aspects of transvaginal sonography are described. The main characteristic of transvaginal sonography is the "different" approach, allowing to use transducers with high emission frequencies leading to high resolution images. An inquiry was conducted into the patient acceptability of this new technique. The results were favourable for transvaginal sonography in comparison with transabdominal scanning, particularly since no full bladder is necessary using the vaginal approach.

Chapter 3

In this chapter the safety aspects of ultrasound and transvaginal sonography in particular are described. Approximately 800 publications have been published until now about the biological effects of ultrasound. From this overwhelming amount of literature it can be concluded that sound intensities, which so far have been employed for diagnostic procedures, are harmless to the developing embryo, although unlimited use should be avoided. Transvaginal sonography is associated with lower energy levels reaching the

embryo than in the transabdominal approach under the assumption of the same start intensities.

Chapter 4

In the first part of this chapter (4.1) an overview is provided of embryonic development in the first six weeks following conception. This development is related to the transvaginal sonographic findings in normal developing gestations in this period. A number of embryonic landmarks (gestational sac, yolk sac and cardiac activity) can be visualised about one week earlier than previously reported. The second part (4.2.) shows that the reference chart currently in use for embryonic crown–rump length measurements derived from data obtained with transabdominal sonography (compound B–scan) needs to be adjusted, since it is possible to measure embryonic crown–rump length earlier and more accurate employing transvaginal sonography.

Chapter 5

In the first part of this chapter (5.1.) the transvaginal sonographic characteristics of abnormally developing intra–uterine gestations are described. The sonographic findings in a group of patients presenting with vaginal bleeding in the first trimester of pregnancy are described. Our own findings are compared with data reported by other authors. It is stated that the diagnosis of abnormal gestation can be made about one week earlier with transvaginal sonography. In the second part (5.2.) the role of transvaginal sonography in the diagnosis and management of ectopic pregnancy is discussed. The availability of sensitive hCG determinations in combination with transvaginal sonography permits the diagnosis of ectopic pregnancy before clinical signs and symptoms have appeared. This provides the opportunity to employ more conservative modes of treatment, which is especially important regarding aspects of future fertility. At present, the place of transvaginal sonography in the management of ectopic pregnancy

still has to be established. The significance of transvaginal puncture of ectopic pregnancies with or without systemic or local administration of methotrexate is discussed.

Chapter 6

This chapter consists of three parts. In the first part (6.1.) several aspects of appearance and subsequent development of embryonic cardiac activity in IVF pregnancies is described. A spread of five days in the appearance of cardiac activity was observed. The later cardiac activity can be visualised, the higher the chance of abortion. Also abnormal development of embryonic heart rate is associated with an increased risk of abortion. The factors determining the onset and subsequent development of the heart rate are most likely embryonic in origin. Environmental factors like maternal age, do not seem to play a significant role. Arguments for these assumptions are provided in the second part of this chapter (6.2.). In IVF multiple pregnancies a difference of up to two days in the appearance of cardiac activity between embryos was found in a number of cases. The determining factor in this observation seems to be a different duration of the implantation stage, since the growth of the embryos following the appearance of cardiac activity was virtually the same. In the third part (6.3.) a case history is presented in which abnormal development of the embryonic heart rate in early pregnancy coincided with the birth of an infant with Down's syndrome. The implications of this observation are discussed.

SAMENVATTING

Hoofdstuk 1

In dit hoofdstuk is de geschiedenis van transvaginale echoscopie beknopt weergegeven. De vaginale benadering werd reeds in de zestiger jaren nagestreefd, echter de recente elektronische ontwikkelingen maakten de feitelijke toepassing mogelijk en zinvol. In het begin van de jaren tachtig werd transvaginale echoscopie vooral gebruikt als methode voor monitoring van de follikelgroei en later ook om oöcyten te verkrijgen in IVF centra. Sindsdien heeft transvaginale echoscopie diverse toepassingen gevonden in de Obstetrie en Gynaecologie. Deze worden in het kort aangegeven. De doelstellingen van dit proefschrift worden gedefinieerd.

Hoofdstuk 2

In dit hoofdstuk worden de technische en methodologische aspecten van transvaginale echoscopie besproken. Kenmerkend voor transvaginale echoscopie is de "andere" benadering die het mogelijk maakt om transducers te gebruiken met hoge emissie frequenties waardoor een hoog oplossend vermogen wordt gecreëerd. Hiermee kan een beeld verkregen worden met veel details. Een enquête naar de acceptatie van deze nieuwe techniek door de patiënt viel zeer gunstig uit voor transvaginale echoscopie in vergelijking met transabdominale echoscopie.

Hoofdstuk 3

In dit hoofdstuk komen de veiligheidsaspecten van ultrageluid en transvaginale echoscopie in het bijzonder aan de orde. Er zijn tot op heden ongeveer 800 publikaties verschenen die betrekking hebben op de biologische effecten van ultrageluid. Uit deze overstelpende hoeveelheid literatuur kan worden geconcludeerd, dat ultrageluid met intensiteiten zoals die worden

toegepast voor diagnostische doeleinden in de zwangerschap niet schadelijk is, maar ongelimiteerd gebruik moet worden vermeden. Indien de geluids-intensiteiten die bereikt worden ter plaatse van het embryo voor transabdominale en transvaginale echoscopie vergeleken worden valt op, dat bij gelijke startintensiteit van de transducers deze intensiteit bij transvaginale echoscopie lager ligt dan bij transabdominale echoscopie.

Hoofdstuk 4

In dit hoofdstuk wordt in het eerste gedeelte (4.1.) een overzicht gegeven van de embryonale ontwikkeling in de eerste zes weken na conceptie. Deze ontwikkeling wordt gekoppeld aan de transvaginale echoscopische bevindingen in zich normaal ontwikkelende graviditeiten in deze periode. Het blijkt, dat een aantal embryonale structuren waaronder de vruchtzak en dooierzak, alsmede hartactiviteit ongeveer een week eerder kunnen worden gedetecteerd met transvaginale echoscopie. In het tweede gedeelte (4.2.) wordt aangetoond, dat de curve die voor de kruin–romp lengte tot op heden in gebruik is en werd samengesteld uit gegevens die werden verkregen met behulp van transabdominale echoscopie (compound B–scan) aanpassing behoeft, omdat het mogelijk is geworden om de kruin–romp lengte via transvaginale echoscopie eerder en nauwkeuriger te meten.

Hoofdstuk 5

In het eerste gedeelte van dit hoofdstuk (5.1.) wordt ingegaan op de transvaginale echoscopische kenmerken van zich abnormaal ontwikkelende intra–uteriene graviditeiten. De bevindingen bij een groep patiënten die zich met bloedverlies in het eerste trimester van de graviditeit presenteerden worden besproken. Onze onderzoeksresultaten worden vergeleken met de gegevens uit de literatuur. Ook de diagnose abnormale graviditeit kan met transvaginale echoscopie ongeveer een week eerder gesteld worden. In het tweede gedeelte (5.2.) wordt de rol van transvaginale echoscopie in de

diagnostiek en behandeling van ectopische graviditeiten beschreven. Door het beschikbaar komen van gevoelige hCG bepalingen in combinatie met transvaginale echoscopie is het mogelijk geworden om de diagnose ectopische graviditeit in een eerder, vaak nog asymptomatisch stadium te stellen. Hierdoor wint men tijd om meer conservatieve methoden van behandeling, waarbij gelet kan worden op aspecten van toekomstige fertiliteit, te overwegen. Het is nog niet geheel duidelijk welke plaats transvaginale echoscopie in deze zal gaan innemen. Transvaginale punctie van de ectopische graviditeit als therapeutische ingreep al dan niet in combinatie met lokale of systemische toediening van methotrexaat wordt besproken.

Hoofdstuk 6

Dit hoofdstuk bestaat uit drie gedeelten. In het eerste deel (6.1.) worden de verschillende aspecten van verschijnen en ontwikkeling van de hartactiviteit van het embryo in IVF graviditeiten beschreven. Er blijkt een spreiding te bestaan van vijf dagen in het optreden van hartactiviteit. Naarmate de hartactie later wordt vastgesteld des te groter de kans dat er een abortus optreedt. Ook een abnormale ontwikkeling van de hartfrequentie van het embryo lijkt samen te gaan met een verhoogde kans op abortus. De factoren die het begin en de daaropvolgende ontwikkeling van de hartactiviteit bepalen zijn waarschijnlijk in het embryo zelf gelegen. Omgevingsfactoren zoals bijvoorbeeld leeftijd van de moeder, lijken van ondergeschikt belang. Argumenten voor deze veronderstellingen worden aangedragen in het tweede gedeelte (6.2.) van dit hoofdstuk. In IVF meerling graviditeiten kon tussen de embryo's in een aantal gevallen een verschil tot 2 dagen worden aangetoond in het optreden van hartactiviteit. Dit lijkt veroorzaakt te worden door een verschillende duur van de implantatiefase, omdat de groei van de embryo's na het verschijnen van hartactiviteit praktisch hetzelfde was. In het derde gedeelte (6.3.) wordt een casus gepresenteerd waarbij een abnormale ontwikkeling van de hartfrequentie van het embryo vroeg in de graviditeit werd vastgesteld. Uiteindelijk werd er een kind geboren met het syndroom van Down. De implicaties van deze observatie worden besproken.

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