The thickness of the uterine junctional zone

Comparison between fertile and infertile women

Leentje Dreesen

promotor :
Prof. dr. Lena DE RYCK
dr. L. MEYLAERTS

co-promotor :
dr. Ellen GIELEN

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Contents

Abbreviations

MRI:	Magnetic Resonance Imaging
US:	Ultrasonography
hCG:	human chorionic gonadotrophin
FSH:	Follicle-stimulating hormone
LH:	Luteinizing hormone
T:	Tesla
T_2 TSE:	T_2-weighted turbo spin echo
AP:	Antero-posterior
LL:	Laterolateral
SAS:	Statistical Analysis Software
SPSS:	Statistical Package for the Social Sciences

Preface

Deze thesis is het resultaat van 8 maanden stage op de dienst Radiologie van het Ziekenhuis Oost-Limburg te Genk. Een periode waarin ik onder meer nieuwe kennis heb opgedaan, in contact ben kunnen komen met mensen uit verschillende (medische) sectoren en nieuwe vrienden heb gemaakt. Werken aan mijn thesis was daardoor zeer plezant, interessant, maar laten we eerlijk zijn, soms ook een beetje frustrerend. Bij deze zou ik dus graag de mensen willen bedanken die mij tijdens deze periode geholpen en gesteund hebben.

Als eerste zou ik graag iedereen van de dienst Radiologie willen bedanken voor de aangename tijd. Dr. Liesbeth Meylaerts, mijn promoter, zou ik willen bedanken voor de kans die zij mij gegeven heeft om te werken aan dit interessant onderwerp, voor al de hulp en het advies. Uw kennis en beroepstoewijding hebben een enorme indruk op mij gemaakt. Een speciaal dankwoord gaat uit naar mijn co-promoter Ellen Gielen. Ellen, bedankt om er altijd voor mij te zijn, niet enkel met je kennis, maar ook als persoon. Je was er niet enkel op de momenten dat alles vlot verliep, maar ook wanneer het even tegen zat. Dat zal ik nooit vergeten. Jij verdient een standbeeld, een chocolade standbeeld☺ Ook de informatici, Jean-Marie, Joris en Pascal, bedankt om telkens weer mijn beelden op tijd klaar te zetten. Jullie zagen mij misschien niet graag met mijn papiertjes komen, maar toch heb ik daar nooit iets van gemerkt. Jullie stonden altijd klaar voor mij. Tot slot, Amber, Joris en het verplegend personeel die altijd voor een leuke afwisseling zorgden wanneer ik mijn baarmoeders even beu was. Amelie, jij bent een vriendin voor het leven!

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Abstract

De junctionele zone van de baarmoeder lijkt een rol te spelen in (in)fertiliteit. De eigenschappen van de bloedvaten en de dikte van de junctionele zone zouden de slaagkans van embryo-implantatie in de baarmoeder beïnvloeden. Ondanks verschillende verbanden van de junctionele zone met (in)fertiliteit, is het echter nog steeds niet duidelijk welke rol de zone precies speelt in (in)fertiliteit en tijdens welke menstruele fase. Er is, volgens onze kennis, zelfs nog nooit een vergelijkende studie, door middel van magnetisch resonantie beeldvorming (*imaging*, MRI), uitgevoerd tussen fertiele en infertiele vrouwen wat betreft de junctionele zone. Het belangrijkste doel van deze studie was om na te gaan of er significante verschillen bestaan tussen de junctionele zone dikte van fertiele en infertiele vrouwen.

Om de hypothese te testen dat infertiliteit in verband kan worden gebracht met abnormaliteiten in de junctionele zone dikte, ondergingen 30 nullipara, 17 primipara en 22 infertiele vrouwen tussen 19-33 jaar een MRI onderzoek. De dikte van junctionele zone werd gemeten op sagitale T_2-gewogen turbo spin echo beelden, na injectie van buscopan en zonder vetonderdrukking. Metingen werden uitgevoerd tijdens de drie menstruele fases en op acht locaties in de baarmoeder. Elke studiegroep werd bovendien onderverdeeld in vrouwen die wel en geen contraceptie namen. Indien nodig werden de data gecorrigeerd voor leeftijd en baarmoedervolume.

De resultaten tonen aan dat een normale junctionele zone gelijk of kleiner is dan 5 mm in dikte. Over de invloed van de menstruele cyclus, en dus van oestrogenen, op de junctionele zone dikte kon er echter geen consensus worden bekomen. Tijdens de luteale fase was de zone significant dunner in nullipara vrouwen die contraceptie gebruikten dan in de niet gebruikers. Dit kan wijzen op een belangrijke rol van de luteale fase, de fase waarin de voorbereidingen van de baarmoeder op implantatie beginnen, in fertiliteit en op het feit dat de junctionele zone niet te dun mag zijn tijdens deze fase. Deze veronderstelling werd verder ondersteund door de bevindingen in de infertiele en primipara vrouwen, waar respectievelijk een dunner junctionele zone en een significant dikkere zone werd gemeten.

Hoewel het onmogelijk was om standaard junctionele zone diktes te definiëren voor de diagnose van infertiliteit, suggereert deze studie dat de junctionele zone dikte een rol speelt in (in)fertiliteit, voornamelijk tijdens de luteale fase. Tevens werd aangetoond dat conclusies over de junctionele zone dikte enkel gemaakt mogen worden op basis van junctionele zone metingen.

Abstract

The uterine junctional zone may play a role in (in)fertility. It is, for example, assumed that the characteristics of the blood vessels and the thickness of the junctional zone affect the success rate of embryo implantation in the uterus. However, despite various links of the junctional zone with (in)fertility, it has still not been precisely described which role exactly the zone plays in (in)fertility and at what stage of the menstrual cycle. Moreover, to our knowledge, a comparative study between fertile and infertile women concerning the junctional zone has never been performed so far by magnetic resonance imaging (MRI) examination. The main goal of this study was to find out whether substantial differences exist between the junctional zone thickness of fertile and infertile women.

To test the hypothesis that infertility might be linked to abnormalities in the thickness of the junctional zone, MRI measurements were performed in 30 nullipara, 17 primipara and 22 infertile women between 19-33 years of age. The junctional zone thickness was measured on sagittal T_2-weighted turbo spin echo images after buscopan injection and without fat suppression. Measurements were performed during the three different phases of the menstrual cycle and at eight different uterine locations. In each study group, a subdivision was made between contraceptive and non contraceptive users. When necessary, data were corrected for age and uterus volume.

The results show that a normal junctional zone is equal or less than 5 mm in thickness during the menstrual phase. There was, however, no consensus about the effect of the menstrual cycle, and thus oestrogens, on the junctional zone thickness. During the luteal phase, the junctional zone was significantly thinner in contraceptive using nullipara women than in the non contraceptive users. This might suggest that the luteal phase, i.e. the phase at which the uterine adaptations in preparation to implantation start, plays an important role in fertility and that the junctional zone should not be too thin during this phase. This assumption was further supported by the findings in the primipara women, i.e. a significantly thicker junctional zone, and the results obtained in the infertile women, i.e. a thinner junctional zone.

Although it was not possible to define standard junctional zone thickness values for the diagnose of infertility, this study suggests that the junctional zone thickness may play a role in (in)fertility, mainly during the luteal phase. It was also indicated that conclusions about the junctional zone thickness should only be made on junctional zone measurements.

1. Introduction

Infertility has become a growing problem during the last decade, with an estimated prevalence of about 14% in Europe. Infertility is defined as the inability to conceive after one year of unprotected and frequent intercourse and can be attributed to any abnormality in the female or male reproductive system. Female infertility can have different causes, among others ovulation disorders, tubal factors and uterine problems [1, 2]. Two commonly encountered uterine abnormalities associated with infertility are congenital uterine abnormalities and acquired uterine abnormalities. The congenital abnormalities consist of six classes of mullerian duct anomalies, which are all caused by interruption during the development or the fusion of the mullerian ducts. These paired structures are designed to form the uterus, cervix and upper two-thirds of the vagina during development. Acquired uterine abnormalities refer to two uterine disorders, leiomyomas and adenomyosis, which involve the uterine layers [3].

The uterus, located in the lower pelvis behind the urinary bladder and in front of the rectum, is a pear-shaped muscular organ, whose principal functions are to receive and house the embryo during pregnancy and to expel the foetus at the termination of pregnancy. The uterus consists of two main parts: the cervix and the corpus, with a ratio of 1:2 contributing to the total uterine length in women of reproductive age (Figure 1). The corpus can be subdivided in the middle body and the upper fundus. The region between the body and the cervix is called the isthmus. The uterus also consists of three main zones: the endometrium, the myometrium and the perimetrium (Figure 1). The endometrium is a mucosal layer, which changes daily throughout the menstrual cycle under the influence of oestrogen and progesterone. It participates in the implantation and the nourishment of the embryo. The perimetrium is a serous membrane and the myometrium, a thick smooth muscle layer, can be subdivided into two parts: the circular arranged inner one-third of the muscle layer, i.e. the junctional zone, and the longitudinal arranged outer two-third of the muscle layer [4-6].

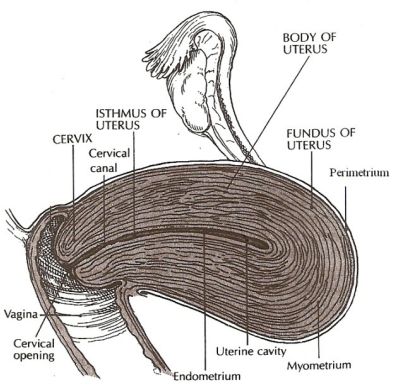

Figure 1: The different parts and zones of the uterus

The uterus consists of two main parts: the cervix and the corpus. The corpus can be subdivided into the upper fundus and the middle body. The isthmus is the part between the body and the cervix. The uterus consists of three zones: the inner endometrium, the middle myometrium and the outer perimetrium [4].

The uterine junctional zone may play a role in (in)fertility. First, the zone is a hormone-dependent structure that governs uterine contractions, i.e. uterine peristalsis, which are primarily regulated by oestradiol and progesterone [7]. Kunz et al. (1996) proved that the peristalsis is involved in sperm transport [8]. By means of hysterosalpingoscintigraphy it was shown that during the periovulatory phase of the menstrual cycle, the contractions have a cervico-fundal pattern by which the spermatozoa are rapidly directed to the fallopian tubes. Ijland et al. (1997) showed a link between junctional zone contractility and embryo implantation [9]. Conception cycles were found to be associated with a reduction in overall contractility when compared with non-conception cycles. Second, studies have shown that disorders of the junctional zone, such as uterine leiomyomas and adenomyosis, are linked with infertility [3, 7, 10]. Leiomyomas, i.e. benign smooth muscle neoplasms, of the uterus and adenomyosis, i.e. a gynaecological disorder characterized by the presence of heterotopic endometrial glands and stroma in the myometrium, may alter the peristaltic activity, which could contribute to fertility problems. Adenomyosis can be presented in its most common appearance, i.e. a diffuse form, or the involvement can be focal, surrounded by a pseudocapsule. The latter is also known as an adenomyoma [3, 7, 11, 12]. Third, it is assumed that the characteristics of the blood vessels and the thickness of the junctional zone affect the success rate of implantation of the embryo in the uterus [7, 13, 14]. To enable the implantation of an embryo, the uterus needs to adapt. This process, which is called decidualization, already starts during the menstrual cycle. There are several lines of evidence that suggest that the junctional zone is subjected to profound remodelling before and during pregnancy. The junctional spiral arteries undergo morphological changes during the menstrual

cycle, independent of trophoblast migration. Without these changes, the migration of the developing trophoblast into the junctional spiral arteries cannot occur. This migration is necessary for the transformation of the junctional spiral arteries into low-resistance, high capacity uteroplacental vessels, which are required for deep placentation during the pregnancy. All changes in the vasculature of the junctional zone have an influence on the uterine blood flow. Dysregulation of the uterine blood flow is linked with pregnancy problems and other menstrual disorders such as dysmenorrhea and menorrhagia. A proportion of women with unexplained infertility might thus have decreased uterine perfusion [13, 15, 16]. Lesny et al. (1999) evaluated the differences in junctional zone thickness between conception and non-conception *in vitro* fertilization/embryo transfer cycles by means of ultrasonography (US) [14]. The junctional zone of the women who conceived after fertilization was significantly thinner on day 8 of ovulation induction and significantly thicker at embryo transfer.

Due to its availability, US is a commonly used screening technique for many uterine and ovarian problems. However, its significant operator dependence and relative inability to determine tissue characterization limits its diagnostic value [17]. MRI is a non-invasive imaging technique using magnetic fields. It is characterized by good soft tissue contrast resolution, direct multidirectional imaging and good image quality. By means of MRI, Hricak et al. (1983) were the first to describe the uterine zonal anatomy [18]. On T_2-weighted images, the endometrium is presented as a high signal-intensity zone, the junctional zone as a low signal-intensity zone and the outer myometrium as a medium signal-intensity zone (Figure 2).

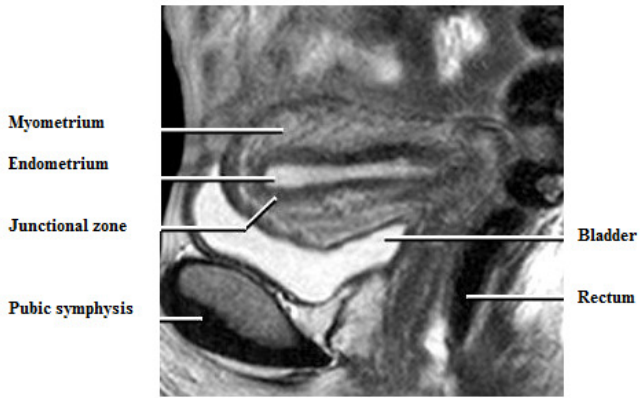

Figure 2: The uterine zonal anatomy on MRI

Sagittal T_2-weigthed MR image of the uterus showing the typical zonal anatomy, with a high signal endometrium, a low signal junctional zone and an intermediate signal myometrium [19].

Morphometric studies have revealed the origin of the difference in intensity between the inner and outer muscle layer of the myometrium, including a threefold increase in nuclear area per unit area, a lower water content and a higher vascular density in the junctional zone [20-22]. Nowadays, MRI devices are improved, providing more detail, thus allowing better measurements of the uterus.

In literature, a normal junctional zone is commonly agreed to be equal or less than 5 mm in thickness [23]. Using MRI, Hauth et al. (2007) defined the thickness of the junctional zone as a function of age in 100 healthy women between 21 and 73 years old [24]. The thickness was shown to significantly increase until women are between 41-50 years old and then to decrease again. MRI findings in 21 women in different phases of the menstrual cycle, nine women using oral contraceptives and twelve not, demonstrated that the zone is significantly smaller in the pill using group in each menstrual phase compared to the non pill group [25]. Both studies showed that there are no significant differences between the follicular and luteal (secretory) phase of the menstrual cycle. Mitchell et al. (1990) also found no differences in junctional zone thickness between these two menstrual phases when examining 12 female volunteers (20-33 years) [26]. This is in contrast with the results of Haynor et al. (1986), who evaluated the changes in appearance of the uterus on MR images obtained during the menstrual cycle in 6 healthy women (23-33 years) [27]. The thickness of the myometrium was shown to increase rapidly during the follicular phase and to increase at a slower rate during the luteal phase. Wiczyk et al. (1988) came to the same conclusion when evaluating 5 women between 22-40 years of age with normal ovulatory cycles [28]. It is clear that there is no consensus so far about the influence of the menstrual cycle on the normal junctional zone thickness.

Data about abnormal thickness of the junctional zone, which are mainly based on women with adenomyosis, are also divergent. According to Mark et al. (1987), a junctional zone of more than 5 mm in thickness is abnormal and allows a confident diagnosis of adenomyosis [29]. Kang et al. (1995), however, suggest that a uterus with a maximum junctional zone thickness of 8 mm or less can easily be normal, while Reinhold et al. (1996) propose an optimal junctional zone value of 12 mm or more for the diagnosis of adenomyosis [30, 31]. The latter study group also evaluated the junctional zone to total myometrium thickness ratio in patients with and without adenomyosis [31]. Althought it was hypothesized that this ratio might be a better predictor for the presence of adenomyosis, it did not improve the accuracy of diagnosing this uterine disorder. The results mentioned above indicate that there is no overall

agreement about the thickness of an abnormal junctional zone. Moreover, until now there are no results about the thickness of the junctional zone in infertile women.

The main goal of this study was to determine whether substantial differences exist in the thickness of the uterine junctional zone between fertile and infertile women during the menstrual cycle. To test the hypothesis that infertility might be linked to abnormalities in the thickness of the uterine junctional zone, a threshold for the normal thickness of this zone during the menstrual cycle was first determined. Subsequently, the thickness at each stage of the menstrual cycle was compared between fertile and infertile women. Significant differences were described as abnormal thickness values correlating to infertile women. By making a subdivision in each group between women who use contraceptives and those who do not, the effect of contraceptives was determined. In addition, the best uterine segment and the best menstrual phase for the thickness measurements were assessed. The thickness of the junctional zone was evaluated by means of MRI, taking the epidemiological influencing factors age and uterus volume into account. Data were obtained from nullipara women (who have no children), primipara women (who obtained a MRI scan between 6-12 months after delivery of their first child) and infertile women between 19 and 35 years of age. The first two groups had no medical history of infertility and only women, from whom the cause of infertility had not been determined, were included in the infertile group.

2. Materials and methods

2.1 Study population

Over a recruitment period of 28 months (21/12/2007 – 29/04/2010), 30 nullipara women, 17 primipara women and 22 infertile women with an age ranging between 19 and 33 years (mean age = 28.1) were included in the study after given informed consent. Inclusion criteria were women between 19-35 years of age, nullipara and primipara women with no medical history of infertility and infertile women with an unknown cause of infertility. Furthermore, MRI was only performed on primipara women who delivered their first child 6-12 months before their cooperation in the study [32]. Exclusion criteria were women with a pacemaker, clips or other MRI-incompatible implanted devices and pregnant women. Women with congenital or acquired uterine abnormalities were also excluded. The nullipara women were recruited through personal contacts, while the primipara and infertile women were engaged through cooperation with the gynaecologists of the Ziekenhuis Oost-Limburg (Genk, Belgium).

An epidemiological study was performed to obtain information about the women's use of contraceptives, their age and their menstrual cycle (length, start last menstruation) (Table 1). The primipara women also had to fill in the date of delivery. 10 nullipara and 22 infertile women did not use contraceptives. The other 20 nullipara women, as well as the 17 primipara women, did use contraceptives. In the contraceptive using group (n = 37), 30 women took a combination pill, 3 primipara women used a progesterone containing pill, 3 nullipara women had a vaginal ring (oestrogens and progesterone) and 1 nullipara woman had a progesterone containing subdermal implant.

Table 1: Epidemiological information about the study population

	Nullipara women (n= 30)		Primipara women (n= 17)		Infertile women (n= 22)	
Contraceptive	- (n= 10)	+ (n= 20)	- (n= 0)	+ (n=17)	- (n= 22)	+ (n= 0)
Age (years)	19-32 (26.7)	20-33 (26.0)	/	22-33 (28.4)	26-33 (30.5)	/
Length menstrual cycle (days)	24-30 (28.3)	28-30 (28.1)	/	27-28 (27.9)	26-56 (33.3)	/
Months postpartum	/	/	/	6-12 (8.4)	/	/

Blood laboratory tests were performed to obtain information about the women's human chorionic gonadotrophin (hCG), creatinine, follicle-stimulating hormone (FSH), luteinizing hormone (LH), oestradiol and progesterone levels. Based on these hormone levels and the information of their menstrual cycle, the phase of the menstrual cycle was determined (Supplement section A).

2.2 MRI

In the nullipara group, a MRI scan was performed three times during the menstrual cycle, more specifically during the follicular phase (day 6-13 of the menstrual cycle, with the start of the menstruation taken as day 1), the ovulatory phase (day 14-16) and the luteal phase (day 17-28). The primipara and infertile group only got one MRI scan per cycle. MRI examinations were performed randomly during the menstrual cycle.

MRI scans were performed on a 1.5 Tesla (T) MR imaging system (Siemens Magnetom SymphonyTim, Siemens, Erlangen, Germany; Syngo MR B15). An intravenous catheter was placed in the elbow crease. Study patients were positioned on the table of the scanner in head first - supine position and an 8 channel pelvic coil was placed on the pelvis of the patient.

The MRI examination started with a localizer sequence and T_2-weighted turbo spin echo (T_2 TSE) sequences in three different planes (transversal, coronal and sagittal). After manual injection of the abdominal-specific antispasmodic buscopan (1 ml, 20 mg/ml, Boehringer Ingelheim, Germany), diluted in sodium chloride (10 ml, 0.09%, Baxter, Lessines, Belgium), T_2 TSE sagittal images with and without fat suppression were obtained (Supplement section B). The parameters of the different sequences are listed in Table 2.

Table 2: MRI-specific parameters

Parameters/Sequence	Localizer	T_2 TSE	T_2 TSE	T_2 TSE	T_2 TSE: buscopan	T_2 TSE: buscopan
Orientation	Sagittal	Transversal	Coronal	Sagittal	Sagittal	Sagittal
Repetition time (msec)	20	5100	5000	5000	5610	4060
Time to echo (msec)	5	88	89	89	89	93
Field of view (mm)	400	370	320	340	360	360
Slice thickness (mm)	10	5	5	5	4	4
Flip angle (degree)	40	180	180	180	180	180
Voxel size (mm x mm x mm)	3.1 x 1.6 x 10	1.1 x 0.8 x 5	0.8 x 0.6 x 5	0.9 x 0.7 x 5	0.9 x 0.7 x 4	0.9 x 0.7 x 4
Acquisition time (min.sec)	0.14	1.03	2.52	2.02	2.22	3.12
Fat suppression	-	-	-	-	+	-

2.3 Image analysis: thickness

The T_2 TSE images obtained in the three anatomical planes were used to localize the uterus in the pelvis and to ensure that the uterus was within normal limits (no congenital or acquired uterine abnormalities, Supplement section C).

All images acquired for thickness determination were analyzed independently by two observers, a professional radiologist and a student, on a specialized workstation (MMWP, Syngo MMWP VE36A). The T_2 TSE sagittal images after buscopan injection and without fat suppression were used to measure the following parameters: corpus and cervix length, antero-posterior (AP) size, junctional zone and outer myometrium thickness at the level of the cervix, the isthmus, the middle and the fundus of the uterine anterior and posterior wall (Figure 3). The corpus and cervix sizes laterolateral (LL) were measured on the coronal images obtained before buscopan injection. All measurements were performed using the measuring cursor included in the workstation's software.

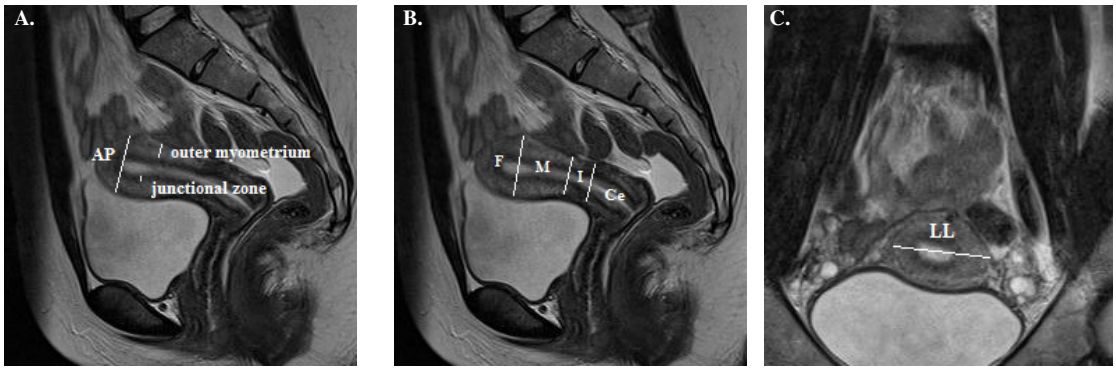

Figure 3: Uterine measurement parameters

On the T_2 TSE sagittal images after buscopan injection and without fat suppression, the junctional zone thickness and the thickness of the outer myometrium (A) were measured at the level of the cervix (Ce), the isthmus (I), the middle (M) and the fundus (F) of the uterine anterior and posterior wall (B). On these images, the corpus and cervix antero-posterior (AP) size was also determined. The corpus and cervix sizes laterolateral (LL) were measured on coronal images obtained before buscopan injection (C).

2.4 Statistics

The volume of the corpus and cervix was calculated using the formula:

length x height (AP) x width (LL) x 0.523

The sum of both volumes corresponds to the total volume of the uterus.

To determine the normal thickness of the junctional zone during the menstrual phase and the effect of the menstrual phase and the use of contraceptives on the junctional zone thickness in the nullipara women, a mixed model was used:

$$Y_{ijk} = \mu + \beta_1 f_i + \beta_2 s_j + \beta_3 (fs)_{ij} + b_k + \varepsilon_{ijk}$$

with: $i = 1,2$ (1 = no contraceptives, 2 = contraceptives)

$j = 1,2,3$ (1= follicular phase, 2 = ovulatory phase, 3 = luteal phase)

$k = 1, \ldots, 30$ (number of women)

Y_{ijk} = thickness *k-th* women for the *i-th* factor and the *j-th* phase

μ = overall mean when all factors are at baseline (= 1)

$\beta_1 f_i$ = effect of the *i-th* factor (contraceptives), reference = contraceptive users

$\beta_2 s_j$ = effect of the *j-th* phase (baseline = luteal phase)

$\beta_3 (fs)_{ij}$ = interaction between factor and phase

b_k = random intercept (random women effect)

ε_{ijk} = error term, assumed to follow N $(0,\Sigma)$

The mixed model used for the assessment of the junctional zone values and the effect of pregnancy on the thickness of the zone in the primipara women was:

$$Y_{ijkl} = \mu + \beta_1 g_i + \beta_2 h_k + \beta_3 (gh)_{ik} + \beta_4 (gm)_{ij} + b_l + \varepsilon_{ijkl}$$

with: $i = 1,2$ (1 = nullipara women, 2 = primipara women)

$j = 1,2$ (1= no contraceptives, 2= contraceptives)

$k = 1,2,3$ (1= follicular phase, 2 = ovulatory phase, 3 = luteal phase)

$l = 1, ..., 37$ (number of women)

Y_{ijkl} = thickness *l-th* women of the *i-th* group for the *j-th* factor at the *k-th* phase

μ = overall mean when all factors are at baseline (= 1)

$\beta_1 g_i$ = effect of *i-th* group (reference = primipara women)

$\beta_2 h_k$ = effect of *k-th* phase (baseline = luteal phase)

$\beta_3 (gh)_{ik}$ = interaction between group and phase

$\beta_4 (gm)_{ij}$ = interaction between group and contraceptive use

b_l = random intercept (random women effect)

ε_{ijkl} = error term, assumed to follow N $(0,\Sigma)$

The mixed model for the primipara group, excluding the k-th factor, was used to determine the junctional zone thickness values in infertile women and to evaluate possible differences in junctional zone thickness between fertile and infertile women.

The above-mentioned models were also used to analyse the total thickness of the myometrium, i.e. the sum of the junctional zone thickness and the outer myometrium thickness.

A correction factor was added to the models when an influencing effect of age and/or uterus volume on the thickness was found:

Nullipara group:

βa_{ijk} = age of the women and the contraceptive factor (i), the stage (j) in the *k-th* women

βv_{ijk} = volume of the uterus and the contraceptive factor (i), the stage (j) in the *k-th* women

Primipara group:

βa_{ijkl} = age of the women in the i-th group and the contraceptive factor (j), the stage (k) in the *l-th* women

βv_{ijkl} = volume of the uterus in the i-th group and the contraceptive factor (j), the stage (k) in the *l-th* women

Infertile women:

βa_{ijl} = age of the women in the i-th group and the contraceptive factor (j) in the *l-th* women

βv_{ijl} = volume of the uterus in the i-th group and the contraceptive factor (j) in the *l-th* women

All statistical analyses were performed by means of the computer program SAS (Proc Mixed) and/or SPSS (Procedure Mixed). A p-value of less than 0.05 was considered to indicate a statistically significant difference. Inter-observer statistics was performed for the consistency in measurements by means of the informal Bland-Altman test [33].

3. Results

The first part of the result section addresses the thickness of the junctional zone. The thickness of the total myometrium is discussed in the second part.

3.1 Thickness of the junctional zone

3.1.1 Normal thickness of the junctional zone

The normal thickness values of the junctional zone were determined in 10 nullipara women, who did not use contraceptives. In 8 of the 10 women, data were obtained during the three different phases of the menstrual cycle. The other two women cancelled their last MRI appointment due to personal reasons. For these women, data were only collected during the ovulatory and luteal phase of the menstrual cycle.

The thickness of the junctional zone was measured at eight different locations in the uterus: in the cervix, the isthmus, the middle and the fundus of the anterior and the posterior wall of the uterus (Table 3). By combining the different measurement locations, an overall uterine thickness of the junctional zone was calculated.

Table 3: Normal junctional zone thickness threshold values (in cm) and standard error (se) determined in nullipara women who did not use contraceptives at each of the uterine measurement locations during the menstrual cycle

Location / Phase	Follicular (n=8)	Ovulatory (n=10)	Luteal (n=10)
Isthmus anterior	0.28 (0.03)	0.27 (0.03)	0.33 (0.03)
Mid anterior	0.28 (0.04)	0.25 (0.04)	0.32 (0.04)
Fundus anterior	0.32 (0.05)	0.28 (0.05)	0.32 (0.04)
Isthmus posterior	0.34 (0.02)	0.29 (0.02)	0.31 (0.02)
Mid posterior	0.34 (0.04)	0.29 (0.05)	0.33 (0.04)
Fundus posterior	0.39 (0.05)	0.29 (0.06)	0.33 (0.03)
Cervix anterior	0.31 (0.04)	0.28 (0.03)	0.34 (0.03)
Cervix posterior	0.28 (0.04)	0.26 (0.03)	0.33 (0.04)
Overall uterine thickness	0.32 (0.02)	0.28 (0.02)	0.33 (0.02)

At each of the eight measurements sites, the thickness of the junctional zone decreases towards the ovulation and increases during the luteal phase (Figure 4). The decrease towards the ovulation (p=0.0227) and the subsequent increase (p=0.027) are, however, only significant when the different measurement locations are combined.

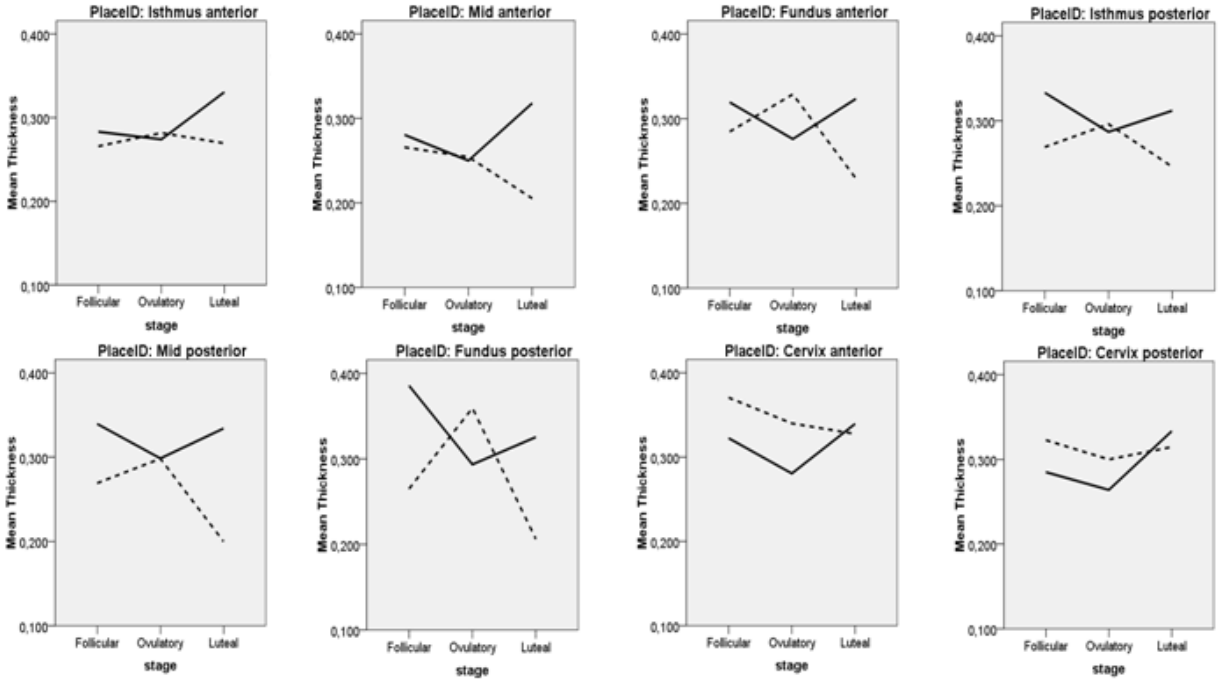

Figure 4: Progress of the mean thickness of the junctional zone during the menstrual phase

For the eight uterine measurement locations, the progress of the mean thickness of the junctional zone during the menstrual cycle is shown. The mean thickness is expressed in cm. — = nullipara women who did not use contraceptives, --- = nullipara women who did use contraceptives.

3.1.2 Effect of contraceptives on the thickness of the junctional zone

To determine the effect of contraceptives on the thickness of the junctional zone, MRI measurements were performed in 20 nullipara women who used contraceptives. Thirteen women were scanned during the three different phases of the menstrual cycle. Six women were examined twice and one woman got only one scan. The reason for cancellation by the latter seven women varied from personal reasons to inconveniences sensed by the subject at previous examination while injecting buscopan (or contrast agent). The thickness of the junctional zone was measured at the eight different locations of the uterus (Table 4).

Table 4: Mean thickness (in cm, ± se) of the junctional zone in nullipara women who did use contraceptives

Location / Phase	Follicular (n=18)	Ovulatory (n=17)	Luteal (n=17)
Isthmus anterior	0.27 (0.02)	0.28 (0.02)	0.27 (0.02)
Mid anterior	0.26 (0.03)	0.25 (0.03)	0.21 (0.03)
Fundus anterior	0.28 (0.03)	0.32 (0.04)	0.23 (0.03)
Isthmus posterior	0.27 (0.01)	0.29 (0.02)	0.24 (0.02)
Mid posterior	0.27 (0.03)	0.29 (0.04)	0.20 (0.03)
Fundus posterior	0.26 (0.03)	0.36 (0.05)	0.21 (0.02)
Cervix anterior	0.37 (0.03)	0.34 (0.02)	0.33 (0.02)
Cervix posterior	0.32 (0.03)	0.30 (0.02)	0.32 (0.03)
Overall uterine thickness	0.28 (0.02)	0.31 (0.02)	0.25 (0.02)

The junctional zone thickness in the contraceptive using women increases towards the ovulation, followed by a decrease during the luteal phase at each measurement location, except for the middle of the anterior wall and the cervix (Figure 4). The increase in thickness towards the ovulation is only significant at the fundus of the posterior wall ($p=0.0156$), while the decrease during the luteal phase is significant at the isthmus, the middle and the fundus of the posterior wall and the fundus of the anterior wall ($p<0.05$). In general, there is no difference in overall uterine thickness of the junctional zone between the follicular and ovulatory phase ($p=0.062$), but the thickness decreases significantly during the luteal phase ($p<0.0001$).

A comparison of the thickness of the junctional zone at the eight different locations in the uterus between the nullipara women who did and did not use contraceptives (Table 5), shows that the junctional zone in the corpus of the uterus is thicker in the second group during the follicular phase of the menstrual cycle. The junctional zone of the cervix, however, is thinner in the non contraceptive users. Only at the isthmus in the posterior wall of the uterus, the junctional zone is significantly different between both groups ($p= 0.0116$).

Table 5: Mean difference (in cm, ± se) in the junctional zone thickness between non contraceptive using and contraceptive using nullipara women at each of the uterine measurement locations during the three phases of the menstrual cycle

Location / Phase	Follicular	Ovulatory	Luteal
	No users – users	No users – users	No users – users
Isthmus anterior	0.015 (0.033)	- 0.008 (0.032)	0.061 (0.032)
Mid anterior	0.015 (0.050)	- 0.004 (0.047)	0.107* (0.048)
Fundus anterior	0.034 (0.060)	- 0.049 (0.058)	0.093 (0.058)
Isthmus posterior	0.071* (0.030)	- 0.011 (0.028)	0.068* (0.028)
Mid posterior	0.076 (0.055)	0.0003 (0.053)	0.134* (0.053)
Fundus posterior	0.127 (0.064)	- 0.067 (0.063)	0.113 (0.062)
Cervix anterior	- 0.050 (0.045)	- 0.063 (0.042)	0.009 (0.043)
Cervix posterior	- 0.039 (0.045)	- 0.036 (0.042)	0.015 (0.043)
Overall uterine difference	0.035 (0.028)	-0.030 (0.027)	0.075* (0.027)

***** Significant at 5% level.

During the ovulatory phase, the junctional zone is thinner in the non contraceptive using nullipara women at all locations except for the middle of the corpus posterior wall, but this difference is not statistically significant ($p>0.05$). In the luteal phase, the junctional zone is thicker in the women who did not use contraceptives, but only at the middle of the anterior and the posterior corpus wall and the isthmus of the posterior corpus wall this difference is statistically significant ($p<0.05$). In general, the results show that contraceptives have no significant effect on the thickness of the junctional zone during the follicular ($p=0.2165$) and the ovulatory phase ($p=0.2796$). During the luteal phase, however, the junctional zone is significantly thicker in nullipara women who did not use contraceptives, compared to those who did ($p=0.0063$). At the isthmus of the posterior wall, the junctional zone was found to be significantly thicker during the follicular and luteal phase of the menstrual cycle in nullipara women who did not use contraceptives. The largest difference in junctional zone thickness between the two groups was found at the middle of the posterior uterine wall during the luteal phase.

3.1.3 Effect of pregnancy on the thickness of the junctional zone

To get an impression about the possible influence of a pregnancy on the thickness of the junctional zone, MRI scans were performed in 17 primipara women. These women did use contraceptives and were only examined once during their menstrual cycle, due to the care for their baby. Thirteen women were scanned in the follicular phase, one was scanned during ovulation and the other three women were in their luteal phase. First, the mean thickness of the junctional zone was determined at the eight different locations of the uterus (Table 6).

Table 6: Mean thickness (in cm, ± se) of the junctional zone in primipara women who did use contraceptives

Location / Phase	Follicular (n=13)	Ovulatory (n=1)	Luteal (n=3)
Isthmus anterior	0.38 (0.02)	0.25 (0.09)	0.47 (0.05)
Mid anterior	0.61 (0.04)	0.17 (0.16)	0.62 (0.09)
Fundus anterior	0.63 (0.05)	0.19 (0.20)	0.68 (0.11)
Isthmus posterior	0.35 (0.02)	0.24 (0.07)	0.43 (0.04)
Mid posterior	0.64 (0.05)	0.19 (0.17)	0.76 (0.10)
Fundus posterior	0.62 (0.05)	0.19 (0.20)	0.66 (0.11)
Cervix anterior	0.26 (0.02)	0.20 (0.10)	0.34 (0.05)
Cervix posterior	0.26 (0.02)	0.17 (0.10)	0.31 (0.06)
Overall uterine thickness	0.47 (0.02)	0.20 (0.10)	0.53 (0.06)

Subsequently, the difference in thickness between the primipara and nullipara women who did use contraceptives was calculated to determine the effect of pregnancy on the junctional zone (Table 7). This comparison was only performed for the follicular phase, due to the small population of primipara women in the ovulatory and luteal phase.

Table 7: Mean difference (in cm, ± se) in the junctional zone thickness between primipara and nullipara women who did use contraceptives during the follicular phase of the menstrual cycle

Location / Phase	Follicular
	Primipara - Nullipara
Isthmus anterior	0.12* (0.03)
Mid anterior	0.36* (0.05)
Fundus anterior	0.35* (0.07)
Isthmus posterior	0.08* (0.02)
Mid posterior	0.37* (0.06)
Fundus posterior	0.34* (0.07)
Cervix anterior	-0.10* (0.03)
Cervix posterior	-0.04 (0.03)
Overall uterine difference	0.18* (0.03)

* Significant at 5% level.

During the follicular phase, the junctional zone of contraceptive using primipara women is significantly thicker than that of contraceptive using nullipara women in the uterine corpus ($p<0.05$). In the cervix, the junctional zone is thinner in the first group. This observation is, however, only significant at the anterior wall of the cervix. In general, there is a significant difference in junctional zone thickness between the two groups, with the thickest junctional zone seen in the primipara women.

3.1.4 Thickness of the junctional zone in infertile women

The 22 infertile women participating in this study did not use contraceptives at the time of their examination. However, they all were on hormone therapy. Consequently, the determination of the menstrual phases was impossible. Therefore, a mean junctional zone thickness, representative for the whole cycle, was assessed (Table 8). This was done for the eight different uterine locations.

Table 8: Mean thickness (in cm, ± se) of the junctional zone in infertile women

Location	Whole menstrual cycle (n=22)
Isthmus anterior	0.37 (0.02)
Mid anterior	0.39 (0.04)
Fundus anterior	0.36 (0.04)
Isthmus posterior	0.36 (0.02)
Mid posterior	0.41 (0.04)
Fundus posterior	0.37 (0.04)
Cervix anterior	0.37 (0.02)
Cervix posterior	0.35 (0.02)
Overall uterine thickness	0.38 (0.02)

Results were compared with those obtained in the nullipara women who did not use contraceptives to determine the effect of infertility on the junctional zone thickness (Table 9).

Table 9: Mean difference (in cm, ± se) in the junctional zone thickness for the whole menstrual cycle between infertile and nullipara women who did not use contraceptives

Location	Whole menstrual cycle
	Infertile - Nullipara
Isthmus anterior	-0.08 (0.04)
Mid anterior	-0.11 (0.06)
Fundus anterior	-0.06 (0.07)
Isthmus posterior	-0.05 (0.03)
Mid posterior	-0.09 (0.07)
Fundus posterior	-0.04 (0.06)
Cervix anterior	-0.06 (0.04)
Cervix posterior	-0.06 (0.04)
Overall uterine difference	-0.07 (0.03)

The junctional zone was found to be non-significantly thinner in the infertile women at each of the uterine measurement locations.

3.2 Thickness of the total myometrium

3.2.1 Normal thickness of the total myometrium

The normal thickness threshold of the total myometrium was assessed in the same population as described in section 3.1.1 (10 non contraceptive using nullipara women). The thickness of the total myometrium was calculated by adding the thickness value of the junctional zone to that of the outer myometrium for the 8 different locations in the uterus and at each phase of the menstrual cycle (Table 10).

The total myometrium thickness was found to be proportional to the uterine volume: the larger the uterine volume, the thicker the myometrium. Because of this influencing effect of uterus volume on the total myometrium thickness ($p<0.0001$), a correction factor was included in the statistical analysis programs used for both the non contraceptive using and the contraceptive using nullipara women.

Table 10: Normal total myometrium thickness threshold values (in cm, ± se) determined in nullipara women who did not use contraceptives after correction for uterus volume

Location / Phase	Follicular (n=8)	Ovulatory (n=10)	Luteal (n=10)
Isthmus anterior	0.64 (0.10)	0.62 (0.11)	0.72 (0.11)
Mid anterior	0.60 (0.08)	0.50 (0.09)	0.60 (0.09)
Fundus anterior	0.63 (0.10)	0.45 (0.11)	0.60 (0.11)
Isthmus posterior	0.56 (0.09)	0.41 (0.09)	0.51 (0.10)
Mid posterior	0.67 (0.10)	0.47 (0.10)	0.51 (0.10)
Fundus posterior	0.68 (0.08)	0.55 (0.08)	0.61 (0.08)
Cervix anterior	0.63 (0.11)	0.61 (0.11)	0.61 (0.11)
Cervix posterior	0.64 (0.09)	0.64 (0.09)	0.59 (0.09)
Overall uterine thickness	0.64 (0.06)	0.52 (0.06)	0.59 (0.06)

In the nullipara non contraceptive using group, the volume-corrected thickness of the total myometrium decreases towards the ovulation and increases during the luteal phase at each of the six measurement locations of the uterine corpus. The decrease is significant at the posterior wall and at the fundus of the anterior wall ($p<0.05$). The increase during the luteal phase is only significant at the fundus of the anterior wall ($p=0.038$). At the anterior wall of the cervix, the myometrium thickness decreases towards the ovulation and then stabilizes during the luteal phase. At the posterior wall of the cervix, a stabilization is found during the follicular phase, followed by a decrease after ovulation. The overall uterine mean thickness of

the myometrium decreases significantly towards the ovulation (p=0.007), followed by a non-significant increase during the luteal phase (p>0.05).

3.2.2 Effect of contraceptives on the thickness of the total myometrium

The total myometrium thickness, corrected for uterine volume, was determined in the same contraceptive using nullipara group as described in section 3.1.2 (Table 11).

Table 11: Mean thickness (in cm, ± se) of the total myometrium in nullipara women who did use contraceptives after correction for uterus volume

Location / Phase	Follicular (n=18)	Ovulatory (n=17)	Luteal (n=17)
Isthmus anterior	0.61 (0.08)	0.64 (0.08)	0.68 (0.08)
Mid anterior	0.55 (0.07)	0.50 (0.06)	0.51 (0.07)
Fundus anterior	0.65 (0.08)	0.63 (0.08)	0.70 (0.09)
Isthmus posterior	0.67 (0.07)	0.59 (0.07)	0.63 (0.08)
Mid posterior	0.65 (0.08)	0.69 (0.07)	0.64 (0.08)
Fundus posterior	0.69 (0.06)	0.71 (0.06)	0.75 (0.07)
Cervix anterior	0.59 (0.09)	0.59 (0.08)	0.58 (0.09)
Cervix posterior	0.62 (0.07)	0.65 (0.07)	0.65 (0.07)
Overall uterine thickness	0.62 (0.05)	0.62 (0.04)	0.63 (0.05)

In the nullipara contraceptive using group, the volume-corrected total myometrium thickness follows divergent progresses during the menstrual cycle. The overall myometrium thickness, however, is stable during the follicular phase, followed by a slight increase after ovulation, which is not statistically significant (p>0.05).

To assess the effect of contraceptives on the thickness of the total myometrium, the mean difference of the total myometrium thickness between non contraceptive and contraceptive using nullipara women was determined (Table 12). The effect of uterine volume on the thickness of the total myometrium was taken into account.

Table 12: Mean difference (in cm, ± se) in the total myometrium thickness (corrected for uterus volume) between non contraceptive using and contraceptive using nullipara women

Location / Phase	Follicular	Ovulatory	Luteal
	No users – users	No users – users	No users – users
Isthmus anterior	0.04 (0.08)	- 0.02 (0.08)	0.05 (0.08)
Mid anterior	0.06 (0.07)	0.002 (0.06)	0.09 (0.06)
Fundus anterior	- 0.008 (0.08)	- 0.17* (0.08)	- 0.09 (0.08)
Isthmus posterior	- 0.09 (0.07)	- 0.16* (0.07)	- 0.09 (0.07)
Mid posterior	0.03 (0.08)	- 0.17* (0.07)	- 0.11 (0.08)
Fundus posterior	0.005 (0.07)	- 0.14* (0.06)	- 0.12 (0.06)
Cervix anterior	0.04 (0.09)	0.02 (0.08)	0.04 (0.08)
Cervix posterior	0.02 (0.07)	- 0.006 (0.07)	- 0.05 (0.07)
Overall uterine difference	0.02 (0.05)	- 0.08 (0.04)	- 0.03 (0.04)

* Significant at 5% level.

During the follicular phase, the total myometrium is thicker at most uterine locations in the nullipara women who did not use contraceptives. In the ovulatory and luteal phase, the total myometrium is, in general, thinner in this group. There are, however, no statistically significant differences in thickness between the two groups at the three menstrual phases.

3.2.3 Effect of pregnancy on the thickness of the total myometrium

The total myometrium thickness was determined at the eight different uterine locations during the menstrual cycle in the primipara population described in section 3.1.3 (Table 13). In this group of women, the volume of the uterus had a significant proportional effect on the thickness of the total myometrium ($p<0.0001$). The results shown here are therefore volume-corrected data.

Table 13: Mean volume-corrected thickness (in cm, ± se) of the total myometrium in primipara women who did use contraceptives

Location / Phase	Follicular (n=13)	Ovulatory (n=1)	Luteal (n=3)
Isthmus anterior	0.73 (0.08)	0.68 (0.22)	0.69 (0.12)
Mid anterior	0.73 (0.08)	0.21 (0.22)	0.77 (0.12)
Fundus anterior	0.66 (0.07)	0.22 (0.21)	0.77 (0.12)
Isthmus posterior	0.59 (0.07)	0.36 (0.18)	0.61 (0.10)
Mid posterior	0.73 (0.09)	0.14 (0.23)	0.64 (0.13)
Fundus posterior	0.73 (0.06)	0.12 (0.17)	0.64 (0.09)
Cervix anterior	0.79 (0.09)	0.81 (0.24)	0.81 (0.13)
Cervix posterior	0.71 (0.07)	0.72 (0.19)	0.75 (0.11)
Overall uterine thickness	0.70 (0.04)	0.39 (0.12)	0.70 (0.07)

To determine the possible influence of a pregnancy on total myometrium thickness, a comparison was made between the mean thickness calculated in the contraceptive using primipara and the contraceptive using nullipara women (Table 14). This comparison was only performed for the follicular phase, due to the small population of primipara women in the ovulatory and luteal phase.

Table 14: Mean difference (in cm, ± se) in the volume-corrected thickness of the total myometrium between primipara and nullipara women who did use contraceptives

Location / Phase	Follicular
	Primipara - Nullipara
Isthmus anterior	0.13* (0.06)
Mid anterior	0.01 (0.06)
Fundus anterior	-0.03 (0.06)
Isthmus posterior	-0.01 (0.05)
Mid posterior	-0.004 (0.07)
Fundus posterior	0.02 (0.05)
Cervix anterior	0.18* (0.07)
Cervix posterior	0.05 (0.05)
Overall uterine difference	0.03 (0.03)

***** Significant at 5% level.

The total myometrium thickness in primipara women who use contraceptives does not differ significantly from that in the contraceptive using nullipara women.

3.2.4 Thickness of the total myometrium in infertile women

The total myometrium thickness was determined in the same infertile population as described in section 3.1.4. First, a mean myometrium thickness representative for the whole menstrual cycle was calculated at each of the eight uterine locations (Table 15). Because the volume of the uterus had a significant influence on the thickness of the total myometrium ($p<0.0001$), the data presented for the infertile women are volume-corrected.

Table 15: Mean volume-corrected thickness (in cm, ± se) of the total myometrium in infertile women who did not use contraceptives

Location	Whole menstrual cycle (n=22)
Isthmus anterior	0.70 (0.07)
Mid anterior	0.68 (0.07)
Fundus anterior	0.79 (0.07)
Isthmus posterior	0.58 (0.05)
Mid posterior	0.59 (0.07)
Fundus posterior	0.74 (0.07)
Cervix anterior	0.74 (0.08)
Cervix posterior	0.74 (0.06)
Overall uterine thickness	0.67 (0.04)

A comparison was made between the total myometrium thickness values obtained in the non contraceptive using infertile and nullipara women (Table 16).

Table 16: Mean difference (in cm, ± se) in the volume-corrected thickness of the total myometrium for the whole menstrual cycle between non contraceptive using infertile and non contraceptive using nullipara women

Location	Whole menstrual cycle
	Infertile - Nullipara
Isthmus anterior	0.06 (0.06)
Mid anterior	0.07 (0.06)
Fundus anterior	0.07 (0.06)
Isthmus posterior	0.08 (0.04)
Mid posterior	0.12 (0.06)
Fundus posterior	0.12 (0.06)
Cervix anterior	-0.04 (0.07)
Cervix posterior	-0.08 (0.06)
Overall uterine difference	0.05 (0.03)

In the corpus of the uterus, the total myometrium is thicker in the infertile women. The opposite is true for the cervix. In general, however, there is no significant difference in total myometrium thickness between infertile and fertile women who did not use contraceptives.

4. Discussion

The uterine junctional zone has been shown to be responsible for uterine peristalsis, which is involved in sperm transport and implantation [8, 9]. The characteristics of the blood vessels and the thickness of the junctional zone are assumed to affect the success rate of embryo implantation in the uterus [13, 14]. In addition, junctional zone disorders, such as uterine leiomyomas and adenomyosis, are linked with infertility [3, 7, 10]. Despite of the various links of the junctional zone with (in)fertility, it has still not been precisely described which role exactly the zone plays in (in)fertility and at what stage of the menstrual cycle. Moreover, a comparative MRI study between fertile and infertile women concerning the uterine junctional zone has never been performed so far to our knowledge. The main goal of this study was to determine, by means of MRI, whether substantial differences exist between the junctional zone thickness of fertile and infertile women. Detection of junctional zone abnormalities in infertile women using MRI may lead to a non-invasive, objective way of diagnosing infertility and may yield a possible target for infertility treatment in affected women.

In literature, it is commonly agreed that the normal junctional zone thickness is equal or less than 5 mm [23]. A common agreement about the influence of the menstrual cycle on the junctional zone thickness, however, does not yet exist. A possible reason for the opposite findings described in literature [24-28] might be the fact that the thickness was measured at different locations in the uterus. Hauth et al. (2007), for example, measured the junctional zone thickness at the location where the zone reached its maximum thickness, while McCarthy et al. (1986) determined the maximum thickness at the midaxis of the organ [24, 25]. In addition, previous studies showed that various (epidemiological) factors, such as age and use of contraceptives, may have an effect on the uterine characteristics [24, 25]. Excluding these factors during data analysis may yield conflicting data.

In this study, eight different measurement locations in the uterus were selected. The thickness of the junctional zone was measured at the level of the cervix, the isthmus, the middle and the fundus of the anterior and posterior wall of the uterus. The possible influence of age, use of contraceptives and uterus volume was determined and data were corrected for these factors when necessary. Age and uterus volume were found to have no significant effect on the thickness of the junctional zone in any of the three study populations. Concerning the total

myometrium thickness, a significant effect of the uterus volume ($p<0.0001$) was found for all three study groups: the larger the uterine volume, the thicker the total myometrium. This finding is not surprising as the largest part of the uterus is comprised by the middle layer of the uterus, i.e. the myometrium, which thus accounts for the main part of the uterus volume. All total myometrium thickness data shown in this report are volume-corrected.

In a first step, a normal threshold for the thickness of the junctional zone during the menstrual cycle was determined in nullipara women who did not use contraceptives (Table 3). The normal threshold values calculated for the thickness of the junctional zone are consistent with the normal junctional zone value described in literature (equal or less than 5 mm) [23]. In addition, the non-significant differences between the three phases of the menstrual cycle at each of the eight uterine locations are consistent with the findings of McCarthy et al. (1986), Mitchell et al. (1990) and Hauth et al. (2007) [24-26]. However, the data are in contrast to the findings of Haynor et al. (1986) and Wiczyk et al. (1988), who found significant differences in junctional zone thickness between the menstrual phases [27, 28]. It is worthwhile mentioning that these groups measured the thickness at particular days in the menstrual cycle, while in our study measurements were performed at random days during the follicular phase and luteal phase. Nevertheless, when the eight different uterine locations were combined, a significant difference was also found between the menstrual phases ($p<0.05$). The difference in significance between the individual and combined uterine locations might be due to measurements errors. The junctional zone thickness was found to decrease towards the ovulation and to increase during the luteal phase at each of the eight uterine measurement locations (Figure 4). These findings are in line with the evolution of the junctional zone thickness in women who conceived after *in vitro* fertilization [14]. The decrease towards ovulation is not surprising given the fact that fertility problems have been linked to a hypertrophic junctional zone [23]. A hypertrophic junctional zone during ovulation generates more peristaltic waves which might be detrimental for the implantation of the trophoblast. The decrease in junctional zone thickness towards ovulation seen in our study population might result from mechanical compression by the rapidly growing endometrium or a negative effect of increasing oestradiol concentrations on the junctional zone thickness.

In nullipara women who did use contraceptives, the overall uterine thickness of the junctional zone followed the opposite progress as the one observed in the non contraceptive using nullipara group: an increase during the follicular phase followed by a decrease during the

luteal phase (Table 4, Figure 4). Because a hypertrophic junctional zone is thought to be pernicious for fertility and the goal of contraceptives is to cause artificial infertility, this reversed progress is not surprising. Only the decrease in junctional zone thickness during the luteal phase was found to be statistically significant. One should note that it is difficult to determine the menstrual cycle in women who take contraceptives as the actual cycle and ovulation are compressed by the contraceptive hormones. As such, one cannot speak of a real menstrual cycle. However, this difficulty was overcome by the determination of the hormone levels in the blood and by the use of a questionnaire in which the study person had to fill in the start of her last menstruation.

By comparing the results of the nullipara women who did not use contraceptives with those who did, the effect of contraceptives on the junctional zone thickness could be assessed. A significant effect was only found during the luteal phase (Table 5). During this phase, the junctional zone is significantly thinner in the contraceptive using women ($p=0.0063$). A thinner junctional zone in contraceptive using women was previously described by McCarthy et al. (1986) and can be explained by an inhibition of a positive effect of oestrogens on the junctional zone. Contraceptive hormones inhibit the release of gonadotrophins from the pituitary gland, the follicle growth and the ovulation. Consequently, the normal increase in oestradiol levels is prevented. Lower oestradiol levels lead to a thinner junctional zone.

In literature, there is no standard uterine location for uterine zone thickness measurements, even though this could be a confounding factor in uterine analysis. Therefore, the best location in the uterus and the best menstrual phase for junctional zone thickness measurements, defined as the location/phase with the largest difference in junctional zone thickness between women who did and did not use contraceptives, was assessed in this study. The largest difference between the two nullipara groups was found at the middle of the posterior wall of the uterus during the luteal phase. Kunz et al. (2005) have shown earlier that the posterior wall of the uterus is the best location to diagnose adenomyosis, a disorder that affects the thickness of the junctional zone [10]. The luteal phase as optimal time point for thickness measurements could be explained by the fact that decidualization of the maternal tissue, which is required for successful pregnancy, already starts in the late secretory phase of the menstrual cycle [13].

The possible effect of a pregnancy on the thickness of the junctional zone was determined in primipara women who gave birth for the first time 6 to 12 months before their cooperation in the study. The selection of primipara women instead of pregnant women is due to the fact that the uterine zonal anatomy becomes indistinct during pregnancy and to the prohibition of exposing pregnant women to a magnetic field without clinical indications. The uterine zonal anatomy gradually reappears within 6 months of delivery [32]. Although more objective results would be obtained in non contraceptive using women, the 17 primipara women participating in this study all used contraceptives. The population of non contraceptive using primipara women (n= 4) was too small to yield statistically significant results. The 17 primipara women included in the study only got one MRI scan. Due to personal practical reasons (nursing their child), it was too difficult for these women to undergo three examinations. In one woman, the MRI scan was performed during the ovulatory phase, in three women during the luteal phase and in 13 women during the follicular phase. Due to the low amount of data collected during the ovulatory and luteal phase, the mean thickness of the junctional zone obtained for these phases cannot be taken as a real standard (Table 6). Results obtained in the women during the follicular phase (n = 13), however, may yield a first impression of the possible effect of a pregnancy on the junctional zone thickness.

The results of the contraceptive using primipara women were compared with the results obtained in the follicular phase of the contraceptive using nullipara women (Table 7). The junctional zone appears to be significantly thicker in the uterine corpus of the first group ($p<0.05$). Lesny et al. (1999) described a significant thicker junctional zone at embryo transfer in women who conceived after *in vitro* fertilization [14]. The thicker junctional zone corresponds with the appearance of the endometrium during pregnancy. The thickness of the endometrium, a mucosal layer responsible for nourishment of the foetus [4, 13], increases in preparation for the possible implantation of a fertilized ovum during the follicular phase. The nutrients for the foetus are mainly supplied by the uterine arteries. Decidualization of junctional spiral arteries is characterized by initial swelling and separation of the musculoelastic layers. Without these changes, the migration of the developing trophoblast into the junctional spiral arteries cannot occur. This migration is necessary for the transformation of the junctional spiral arteries into low-resistance, high capacity uteroplacental vessels, which are required for deep placentation during the pregnancy [13]. Regarding these transformations of the junctional zone spiral arteries, it is not surprising that the junctional zone is and should be thickened during pregnancy. These findings correspond with the results

of the nullipara women, which suggest that the junctional zone should not be too thin at the start of decidualization. In this way, the difference in thickness between the start of decidualization and the thickness required for pregnancy is not too large to overcome.

After the determination of the junctional zone thickness values in fertile women (nullipara and primipara), a comparison was made between non contraceptive using nullipara and infertile women to find out whether substantial differences exist in the junctional zone thickness between these two groups. Since the infertile women were on hormone therapy, a differentiation between the three menstrual phases could not be made. Therefore, a mean juntional zone thickness, representative for the whole menstrual cycle, was calculated (Table 8). These thickness values were then compared with the junctional zone thickness values of the non contraceptive using nullipara women (Table 9). There was no significant difference in junctional zone thickness between the fertile and infertile women over the whole menstrual cycle, although the zone was in global 0.7 mm thinner in the latter group ($p>0.05$). This is in line with our findings so far, namely that the juntional zone should not be too thin in preparation to pregnancy.

The second part of the study focussed on the thickness of the total myometrium and its relation to the junctional zone thickness. The normal total myometrium thickness threshold values, the effect of contraceptives and pregnancy on the total myometrium thickness and the difference in thickness between fertile and infertile women were investigated in the same study groups as described for the junctional zone measurements. In addition, the total myometrium thickness, i.e. the sum of the junctional zone thickness and the thickness of the outer myometrium, was analysed at the same eight uterine measurement locations as described for the junctional zone.

The overall uterine total myometrium thickness was found to follow the same progress as the junctional zone thickness in the non contraceptive using nullipara women: a decrease in thickness towards the ovulation, followed by an increase during the luteal phase (Table 10). In the contraceptive using nullipara women, the overall uterine thickness of the outer myometrium responded differently to oestrogens and progesterone than the junctional zone. While a significant decrease in junctional zone thickness was observed during the luteal phase, no significant differences in total myometrium thickness were found between the three menstrual phases (Table 11). The total myometrium was found to be thicker in the

contraceptive using group. While the junctional zone was significantly thicker during pregnancy, the total myometrium thickness did not differ significantly between contraceptive using primipara and nullipara women (Table 14). This might indicate that the outer myometrium thickness decreases during pregnancy. In the non contraceptive using infertile women, the total myometrium was slightly, but not statistically significant, thicker than in the non contraceptive using nullipara women over the whole menstrual cycle (Table 16). These results indicate that total myometrium thickness results should not be generalized to the junctional zone thickness and that conclusions about the junctional zone should be based on measurements of the junctional zone alone.

5. Conclusion and synthesis

The main goal of this study was to determine whether substantial differences exist in junctional zone thickness between fertile and infertile women. Detection of junctional zone abnormalities on MRI in infertile women may lead to a non-invasive, objective way of diagnosing infertility and the abnormalities may become a target for infertility treatment in affected women. Normal junctional zone thickness values were determined and compared with the junctional zone thickness values obtained in infertile women. In addition, the effect of the menstrual cycle, the use of contraceptives and pregnancy on the junctional zone thickness was assessed. Data were obtained, by means of MRI, from nullipara, primipara and infertile women between 19-33 years of age. The junctional zone thickness was determined at eight different uterine locations and a correction for the possible influence of age and uterus volume was made when necessary.

The normal junctional zone thickness values obtained in this study are consistent with the normal junctional zone value described in literature: equal or less than 5 mm. However, this study did not allow to determine the effect of oestrogens, and thus of the menstrual cycle, on the junctional zone thickness. During the luteal phase, a significant difference was found between the contraceptive and non contraceptive using nullipara women, with a thinner junctional zone in the first group. This might suggest that the luteal phase, i.e. the phase at which the uterine adaptations in preparation to implantation starts, plays an important role in fertility and that the junctional zone should not be too thin during this phase. This assumption was further supported by the findings in the primipara women. The junctional zone was significantly thicker in the contraceptive using primipara women, compared to the contraceptive using nullipara women. A possible hypothesis would be that the difference in junctional zone thickness between the start of decidualization and pregnancy might be too large to overcome if the junctional zone would be too thin during the luteal phase. Although the difference between the junctional zone thickness in nullipara women and infertile women was not statistically significant, the thinner junctional zone found in infertile women is also in line with the proposed influence of the junctional zone thickness on fertility. In general, this study shows that the thickness of the junctional zone appears to play a role in (in)fertility, mainly during the luteal phase of the menstrual cycle, although it was not possible to define standard junctional zone thickness threshold values by mean of MRI for the diagnosis of infertility.

In addition, this study indicates that total myometrium thickness results should not be generalized to junctional zone thickness results. Conclusions about the junctional zone should be based on measurements of the junctional zone alone.

This study might be an onset for further investigation regarding differences in other uterine characteristics between fertile and infertile women. Possible interesting characteristics to compare are the perfusion of the junctional zone, i.e. resistance of the junctional zone arteries, and its peristaltic waves. The perfusion of the junctional zone was already addressed during my internship at Ziekenhuis Oost-Limburg (Genk). Despite testing of different methods to evaluate the uterine perfusion, an optimal method was not yet found. Nevertheless, the spiral arteries of the junctional zone have to transform into low-resistance uteroplacental vessels for deep placentation and a lower perfusion, i.e. a higher resistance of the junctional zone arteries, is expected to be found in infertile women.

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Supplemental information

Supplement section A

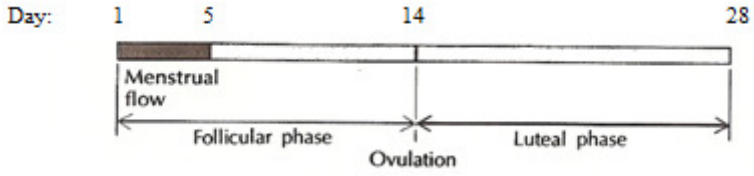

Figure A.1: The different phases of the menstrual cycle

The menstrual cycle generally consists of 28 days with the first day of menstruation taken as day 1. The first phase is the follicular phase starting with the menstruation (day 1 – day 13). After the ovulation (day 14), the luteal phase (day 15 – day 28) starts. [4].

Table A.1: Hormone levels per menstrual phase and post-menopausal

Hormone / Menstrual phase	Follicular	Ovulatory	Luteal	Post-menopausal
FSH (U/L)	3.5 – 12.5	4.7 – 21.5	1.7 – 7.7	25.8 – 134.8
LH (U/L)	2.4 – 12.6	14.0 – 95.6	1.0 – 11.4	7.7 – 58.5
Oestradiol (pg/ml)	12.5 – 166.0	85.8 – 498.0	43.8 – 211.0	<5.0 – 54.7
Progesterone (ng/ml)	0.2 – 1.5	0.8 – 3.0	1.7 – 27.0	0.1 – 0.8

FSH, LH, oestradiol and progesterone levels fluctuate during the menstrual cycle. Based on these hormone levels, the menstrual phase can be determined. FSH and LH are expressed in units/litre (U/L, U = enzyme activity) [34].

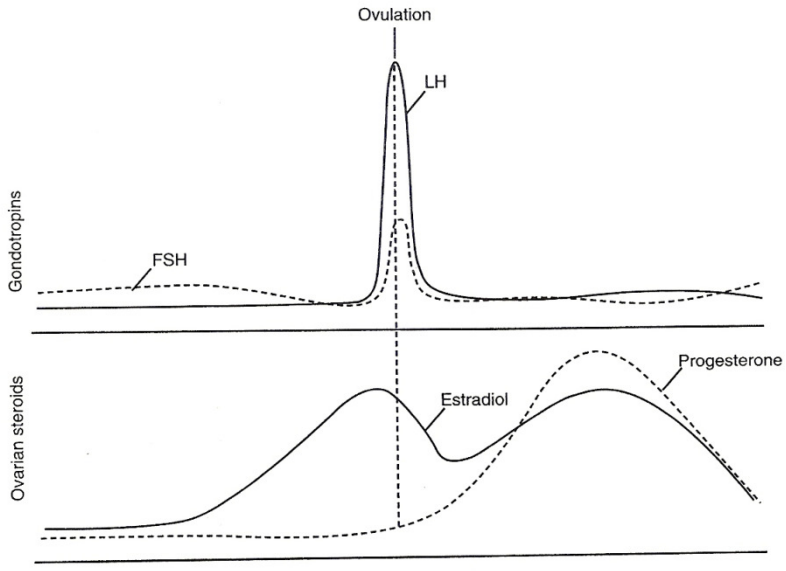

Figure A.2: The evolution of FSH, LH, oestradiol and progesterone levels during the menstrual cycle [11]

Supplement section B

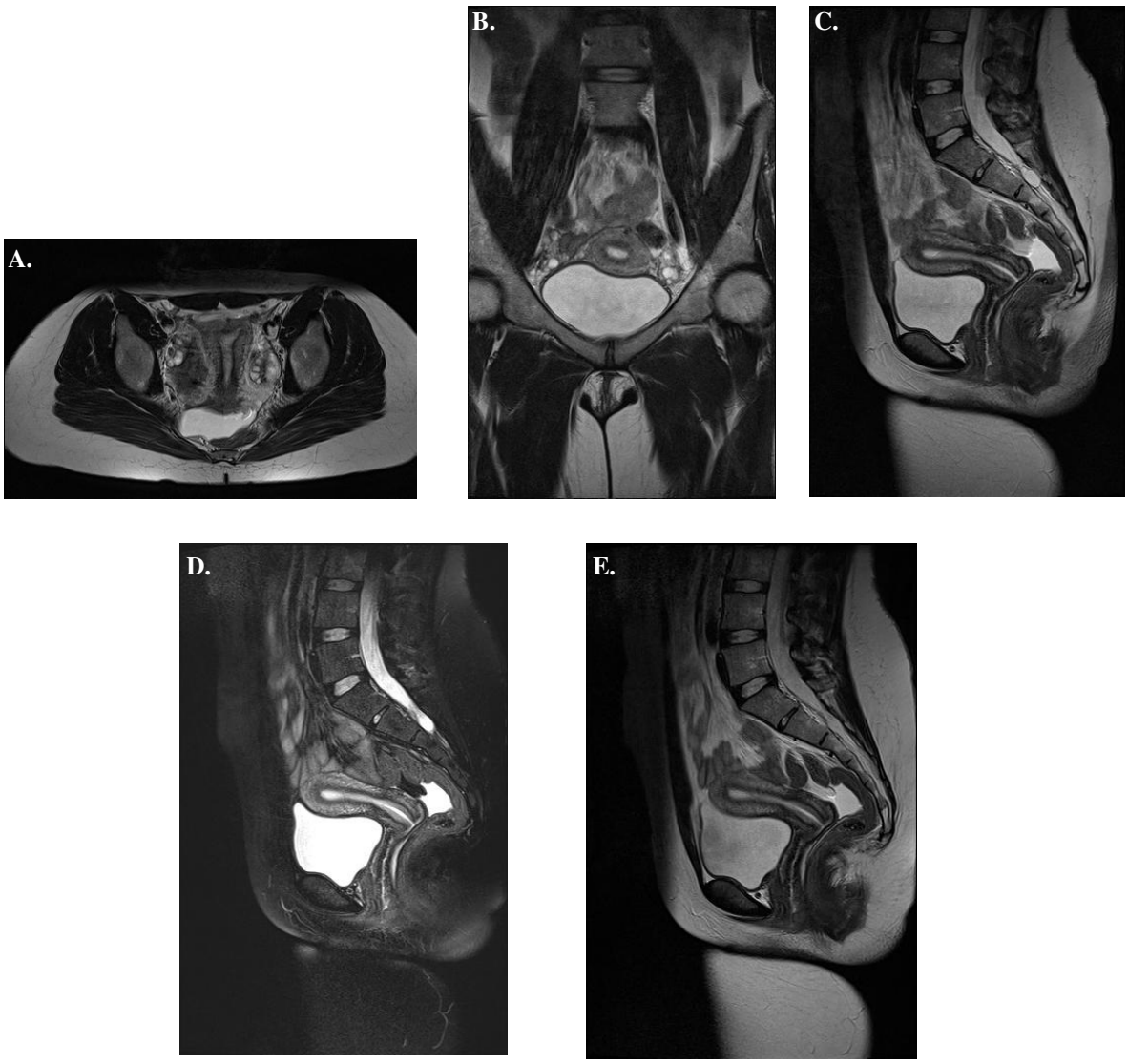

Figure B.1: Examples of T_2 TSE images of the uterus

The MRI examination started with T_2-weighted turbo spin echo (T_2 TSE) sequences in three different planes: transversal (A), coronal (B) and sagittal (C). After injection of buscopan, T_2 TSE sagittal images with (D) and without (E) fat suppression were obtained. Images were obtained in a 25 year old nullipara woman who did not use contraceptives.

Supplement section C

Table C.1: Classification of congenital and acquired uterine abnormalities [3]

Class		Description / cause
Congenital Mullerian duct anomalies		
I	Uterine agenesis	Failure of normal development of both mullerian ducts (uterus absence)
II	Unicornuate uterus	Unilateral mullerian duct agenesis
III	Uterus Didelphys	Both mullerian ducts develop but fail to fuse
IV	Bicornuate uterus	Partial fusion of the mullerian ducts
V	Septate uterus	Failure of resorption of the septum between the mullerian ducts
VI	Diethylstilbestrol-related anomalies	Diethylstilbestrol is a synthetic hormone that was used in the 1950's and 1960's to prevent miscarriage. Patients who used this hormone often have abnormal uterine morphology.
Acquired uterine abnormalities		
	Leiomyomas	Benign smooth muscle neoplasm of the uterus
	Adenomyosis	Disorder characterized by the presence of heterotopic endometrial glands and stroma in the myomerium

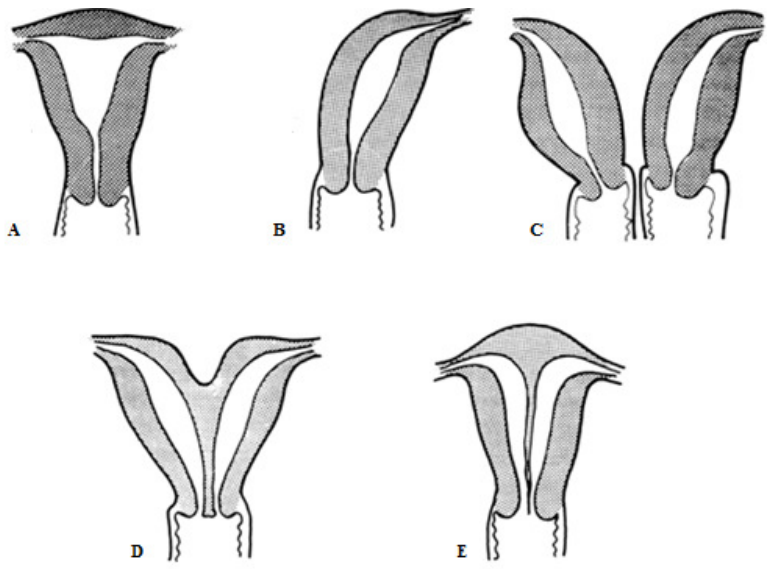

Figure C.1: Congenital uterine abnormalities

(A) Normal uterus, (B) Unicornuate uterus (class II), (C) Uterus didelphys (class III), (D) Bicornuate uterus (class IV), (E) Septate uterus (class V) [35]

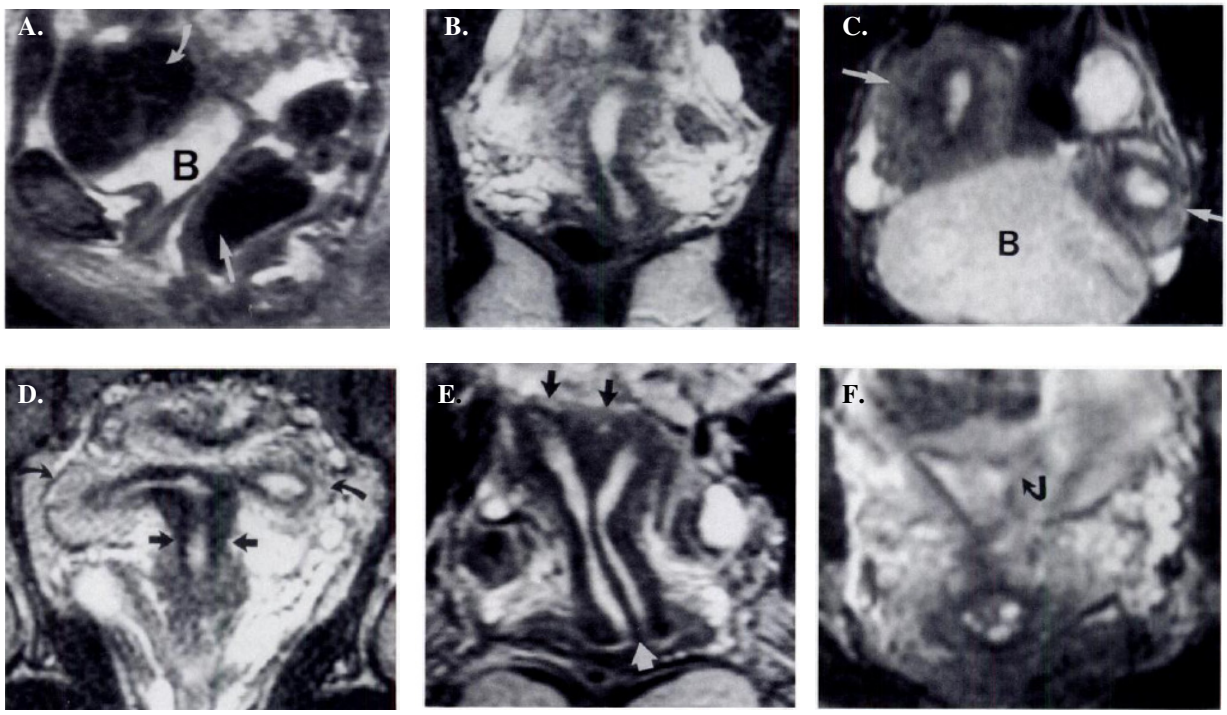

Figure C.2: MR images of the six classes of congenital uterine abnormalities

(A) Mid sagittal T_2-weighted image showing the total absence of the uterus: uterine agenesis. (B) Coronal T_2-weighted image demonstrating a banana-shaped uterine cavity with a single fallopian tube: unicornuate uterus. (C) Coronal T_2-weighted image demonstrating two separate uteri: uterus didelphys. (D) Axial T_2-weighted image showing widely separated uterine horns merging into a single cervix: uterus bicornis unicollis. (E) Coronal T_2-weighted image of a septate uterus with a thin fibrous septum. (F) Coronal T_2-weighted image of the uterus demonstrating a distorted hypoplastic left uterine horn: diethylstilbestrol-related anomaly [3].

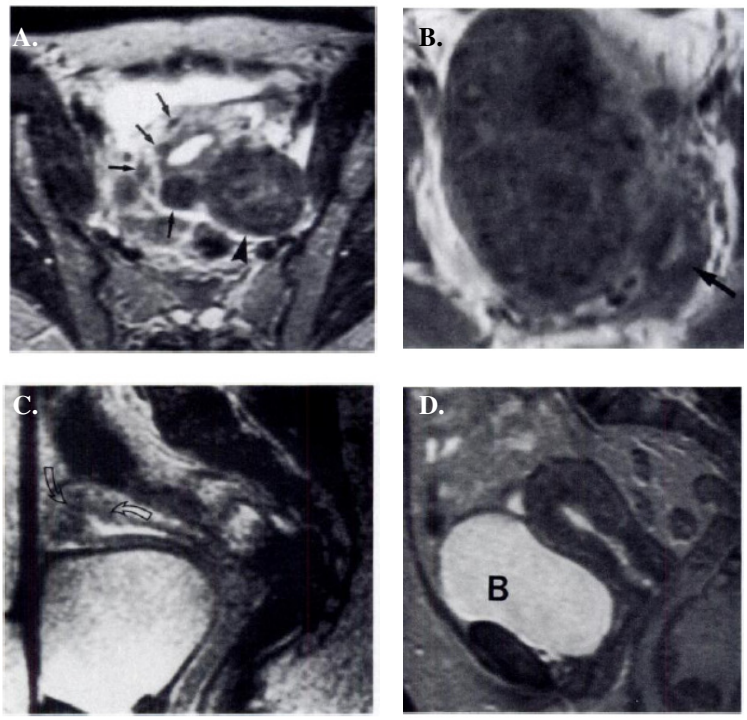

Figure C.3: MR images of the acquired uterine abnormalities

(A) Axial T_2-weighted image revealing several small fibroids (arrows) in addition to a large one (arrowhead): multiple leiomyomas. (B) Axial T_2-weighted image showing a large leiomyoma. (C) Sagittal T_2-weighted image demonstrating focal adenomyosis. (D) Sagittal T_2-weighted image of diffuse adenomyosis [3].

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Richting: **master in de biomedische wetenschappen-klinische moleculaire wetenschappen**
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