Magnetic resonance imaging of the ovary: can it predict low response to empiric superovulation?

Rebecca Jane Crichton
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MAGNETIC RESONANCE IMAGING OF THE OVARY: CAN IT PREDICT LOW RESPONSE TO EMPIRIC SUPEROVULATION?

REBECCA JANE CRICHTON

Yale University

1996
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Date
Magnetic Resonance Imaging of the Ovary:
Can It Predict Low Response to Empiric Superovulation?

A Thesis Submitted to the
Yale University School of Medicine
in Partial Fulfillment of the Requirements for the
Degree of Doctor of Medicine

by
Rebecca Jane Crichton
1996
The purpose of this pilot study was to evaluate whether or not there are morphologic differences in ovarian volume, follicular size and number, as seen on Magnetic Resonance Imaging (MRI), between the ovaries of women with low response to ovulation induction and younger women with presumably normal ovarian function. Five women with an average age of 42 and a documented low response to superovulation with human menopausal gonadotropins, indicated by a peak estradiol less than 500 pg/ml, underwent MRI on the third day of their cycles. Their images were compared to the day three MRI’s of five controls with an average age of 25. There was no significant difference between the ovarian volumes in the two groups. There were, however, significant differences in follicle numbers. For follicles greater than or equal to 5 mm in diameter, there were 3.2±2.7 in the women with low response and 23.0±3.7 in the controls (p<0.0005). For follicles less than 5 mm in diameter, there were 20.2±7.0 in the low-responding women and 40.8±12.1 in the control group (p<0.01). In terms of total follicle numbers visualized on MRI, there were 23.4±7.0 in the women with low response and 63.8±14.4 in the controls (p<0.001). These results indicate that MRI is indeed capable of measuring differences between reproductive subgroups. Further studies are necessary to evaluate the full potential of MRI to prospectively assess ovarian reserve.
Acknowledgements

This research would not have been possible without the help and advice of a great many people. In the Radiology Department, Dr. Robert Troiano and the MRI technicians arrived before dawn on many an occasion in order to perform our research studies. In Pathology, Dr. Vinita Parkash provided invaluable technical advice on specimen preservation and sectioning. Most of all, I would like to thank Dr. David Keefe of the Department of Obstetrics and Gynecology and the study participants. All had an amazing belief in the process of knowledge accumulation to help people.
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INTRODUCTION

Infertility, defined as the inability to conceive after one year of regular, unprotected intercourse, affects 14% of couples and 25% of women in the United States within their reproductive lifetimes.\textsuperscript{1,2} Although there has been no increase in rates of infertility over the past decade, there has indeed been a sharp escalation in the demand for infertility services.\textsuperscript{3} Approximately 2.3% of all women of reproductive age, or 1.26 million women, now seek medical help for infertility in a given year.\textsuperscript{2,3} This increase coincides with a number of factors, foremost of which is aging of the so-called baby boom generation, born from 1946 to 1964. Ages 32 to 50, these women represent a ground-breaking generation. They were the first to exert control over their fertility with birth control and liberalized abortion and among the first to enter the workplace full-time with equal expectations. Thus, by choice or circumstance, they have tended to marry at a later age and to postpone pregnancy. The causes of their infertility are many including male factor, tubal disease, endometriosis, and ovulation dysfunction, but, of particular interest, are the women with infertility of unknown cause. Unexplained infertility patients represent 10-12% of the infertile population\textsuperscript{4,5} and 24-28% of the couples at most fertility clinics.\textsuperscript{6} The major commonality within this group has been shown to be advanced female age.\textsuperscript{7} Because the causes of their infertility remain elusive, many of these reproductively older women undergo a host of costly, time-consuming, and emotionally challenging empiric treatments in hopes of pregnancy often with little knowledge of prognosis.

It has long been recognized that fertility is compromised by advanced female age. Demographic studies have indicated that fertility peaks during the early 20s and decreases noticeably after age 30, with its greatest decrease after 35.\textsuperscript{8} Studies of women participating in donor insemination programs demonstrate two effects of aging.\textsuperscript{9} First, women over 31 years of age take twice as long as women 20 to 31 to achieve pregnancy, and, second, the probability of women over 30 having a normal pregnancy and delivery, once pregnant, decreases by 3.5% per year. These findings have resulted in the estimation that a 35 year
old woman’s chance of having a healthy baby is half that of a 25 year old’s. Other authors estimate that conception rates undergo a fourfold reduction, while rates of spontaneous abortion approximately double by age 40. Young couples having regular intercourse without contraception are expected to conceive at a rate of 20% per cycle, whereas women 40-44 conceive at a rate of 5% per cycle. A further rapid decline in fertility in the 40s is observed to the point where only 2-5% of women between 45 and 49 deliver infants. Studies of older women undergoing *in vitro* fertilization (IVF) have found that, although fertilization occurs at near normal rates, fewer oocytes are retrieved, fewer embryos are transferred, and pregnancy rates are lower in older than in younger women. Thus, age appears to disrupt oocyte developmental potential. Indeed, age is the best predictor of achieving pregnancy through IVF.

Although the above studies demonstrate that women’s ability to conceive declines steadily with age, the mechanism underlying reproductive senescence in women remains enigmatic. Two loci of reproductive senescence have been proposed: ovarian function and/or competence of the uterus to sustain pregnancy. In recent years, the development of oocyte donation protocols has provided some resolution in the debate as to whether the decreased fertility in reproductively older women arises from uterine or ovarian factors. Most studies indicate that oocyte donation abrogates the effects of age on fertility. It is also important to note that while these results support a central role for oocytes in reproductive aging, they do not rule out uterine aging, because older women receiving donated oocytes receive progesterone replacement which could correct defective uterine receptivity. Nonetheless, it appears that a central component of reproductive aging in women arises from dysfunctional oocytes, providing evidence that many older women with “unexplained infertility” may in fact have “oocyte factor infertility.”

In the 1950s, a series of cadaveric studies of accident victims were performed by Erik Block in which the number of follicles per person were counted after ovarian histologic preparation. These studies verified that, despite wide individual variation,
overall, there is an inverse relationship between female age and the number of ovarian primordial follicles. The morphology of the ovaries of the younger and older women were strikingly different, with the ovarian cortex of the young women’s ovary containing thousands of small primordial follicles and the older women’s ovary containing predominantly stroma and lacking follicles and corpora lutea.

Human ovaries are known to acquire a lifetime quota of oocytes before birth, peaking at 6-7 million oogonia at 16-20 weeks gestation. This store begins to dwindle shortly after it is established through a continual process of atresia. By puberty, the oocyte reserve is already reduced to 300,000. Ovulation explains the loss of only about 500 follicles in a woman’s reproductive lifespan. Any of these 500 ovarian follicles destined to ovulate is derived from a cohort of nearly a thousand follicles at various stages of growth and atresia. This cohort, in turn, is derived from the pool of follicles originally formed during fetal life. By menopause, only a few hundred follicles remain.

In comparing follicle numbers to menstrual history, Richardson et al. found that the mean number of primordial follicles in ovaries of women still regularly menstruating is ten times higher than in the perimenopausal women with irregular menses. In addition, follicles are virtually absent in postmenopausal ovaries. Her study reports that the rate of follicular loss accelerates dramatically in the last decade before menopause, which explains the nearly complete exhaustion of follicles at a median age of 51.3. Since menopause is the result of programmed disappearance of a limited reserve of follicles, Faddy et al. employed mathematical models based on the above total follicle counts at different ages to predict menopause. They found that follicular numbers declined bi-exponentially rather than as a simple exponential function of age, with the rate of loss greatly accelerating after follicle numbers reach a threshold of 25,000 at a median age of 37.5.

It is important to note that the rate of reproductive aging varies among individual women. Despite the menstrual irregularities occurring in the perimenopausal period, ovulation and conception may occur, albeit at reduced incidence rates and accompanied by
reduced oocyte fertility. Speroff et al. note that a 57 year and 120 day old woman conceived spontaneously. And, Faddy et al. further emphasize that menopause is a spectrum, unique to each individual. The Massachusetts Women’s Health Study finds that, for perimenopause, the median age of onset is 47.5, and the median duration is 4 years. The median age of menopausal onset which is 51.3 is reduced by 1.5 years in smokers. Even natural menopause, confirmed by increased levels of plasma gonadotropins and decreased levels of peripheral estrogen, occurs before age 40 in 1% of women. Ovarian aging, thus, does not always correspond with chronological age.

Given the spectrum of reproductive capacity at various ages, clinical tests of oocyte reserve are necessary to help women to decide whether to pursue costly therapies which depend on their own oocytes or whether to pursue alternatives such as oocyte donation or adoption. Currently, however, no accurate tests are available to diagnose oocyte infertility. The small size of the oocyte and its restricted availability for research nearly preclude the utility of direct oocyte assays.

In contrast to the oocyte, the follicle is more amenable to clinical assays. Folliculogenesis, which clearly influences oocytes’ developmental competence, has been followed both physiologically by measuring hormonal fluctuations and anatomically by visualization with imaging modalities such as transvaginal ultrasound (US). A number of endocrinologic assays and challenge tests also have been employed to study the pituitary-ovarian axis during the early follicular phase as predictors of reproductive senescence. To date, day 3 follicle stimulating hormone (FSH) level has been most studied as a marker of the perimenopausal state.

The initiation of follicle growth occurs throughout the menstrual cycle independently of gonadotropin influence. A cohort of follicles then becomes responsive to gonadotropins and is propelled into further growth as the follicles develop gonadotropin sensitivity until they develop into antral follicles. FSH acts on developing follicles to stimulate estrogen and inhibin production, which exert negative feedback on FSH secretion.
at the level of the hypothalamus and anterior pituitary gland. Diminished follicular reserve, as occurs with aging, leads to increased circulating FSH levels. As the dominant follicle becomes increasingly sensitive to FSH, it secretes increasing amounts of estradiol and inhibin. FSH level then drops below a threshold which can support less developed follicles, so they undergo atresia. This results in the emergence of a single dominant follicle. Elevated FSH levels during the early follicular phase is a highly specific but not sensitive marker of reproductive aging.

Since no specific tests identify oocyte infertility, the first line of therapy for a substantial proportion of infertile patients involves empiric superovulation with human menopausal gonadotropins (hMGs). By extending the period during which the level of FSH remains above the threshold level, multiple ovulations can be achieved with pharmacologic administration of gonadotropins. This allows additional follicles in the cohort to achieve a stage of maturity that enables them to respond to a preovulatory LH discharge and ovulate. If this fails, IVF and related technologies are tried. These treatments, however, are costly, require daily injections, and have the possible side effects of multiple gestation and ovarian hyperstimulation.

Whereas conception rates with hMG-hCG superovulation vary from a monthly high of 80% in women with hypothalamic amenorrhea, they approach zero in women above age 40. In randomized, controlled clinical trials, the monthly pregnancy rate in couples with unexplained infertility undergoing empiric superovulation with FSH agonists is 10-15% which compares favorably to their baseline pregnancy rate of only 3% per spontaneous cycle. Cumulative pregnancy rates in women over 40 were less than 8%. Individual patients respond differently in terms of peripheral E2 levels, number and size of recruited follicles, and outcome, even though they are stimulated with the same type and dosage of stimulatory medications. So-called “poor” or “low response” have peak E2 levels under 500 pg/ml, poor follicular recruitment in terms of number and size, a high cycle cancellation rate due to insufficient response, and a prior history of low response to a
standard stimulation protocol. The frequency of this condition increases as a function of age, suggesting that it may be a variety of reproductive senescence. Early identification of these patients with low response is critical, not only to individualize protocols in order to maximize chances of pregnancy, but also to counsel patients more effectively so as to give them realistic expectations and to allow for investigation of alternatives as soon as possible.\textsuperscript{40,41}

Since outcomes of IVF and ovulation induction are strongly dependent on ovarian responsiveness to exogenous stimulation, many authors have proposed methods of predicting poor ovarian response to artificial reproductive technologies in order to avoid the unnecessary risks and emotional and financial expense incurred by the patient during these treatments. However, none of these prognostic methods are universally employed. Jacobs et al. showed that with advancing age, there is, on average, a significant reduction in response to hMGs in terms of preovulatory E\textsubscript{2} level, the number of preovulatory follicles less than 15 mm, and cancellation rate. The E\textsubscript{2} level per follicle, however, remained unaltered, indicating that, perhaps, the health of the follicular apparatus remained intact.\textsuperscript{42} The authors note that the size of the follicle store and of superior response obtained with exogenous hormones varied markedly between individuals of similar age and even between siblings, and, thus, that age alone has limited predictive value for patient counselling or clinical management. Day 3 FSH has been suggested to be predictive of outcome and to provide an index of functional ovarian reserve as defined by poor gonadotropin responsiveness and pregnancy rates.\textsuperscript{43,44} However, large intercycle variability as well as large interinstitutional variability in laboratory results makes its use problematic.\textsuperscript{45,46} These same problems pertain to the FSH:LH ratio, recently found to increase before FSH alone.\textsuperscript{47} The variation in FSH and the observation that some low-responding patients have normal day 3 FSH levels led to the proposal of ovarian performance predictive tests such as the clomiphene citrate challenge test and the gonadotropin releasing hormone agonist (GnRH-a) stimulation test.\textsuperscript{48,49} While both are reasonably specific, they lack sensitivity, their results
are not immediately available, and, again, the results are dependent on individual lab standards. Theoretically, low-responding patients could be prospectively identified using a combination of the above variables, but, clinically, identification into different response groups is done retrospectively after the patient undergoes at least one cycle of IVF or ovulation induction.

Thus, there is not yet a standardized method to assess ovarian reserve or to predict outcome failure with hMGs. The relatively low efficiency of ovulation induction and IVF in producing viable pregnancies would be improved by accurate predictions of outcome that would allow termination of superovulation protocols unlikely to succeed. Since there is considerable evidence that ovarian morphology closely reflects ovarian function, an anatomic approach might be feasible. Magnetic resonance imaging (MRI), until now, has had little role in reproductive endocrinology. Using the older techniques of a body coil and conventional spin-echo sequence, ovarian morphology, as seen on MRI, was initially described as heterogeneous on T-2 weighted images. However, with new advances in MRI, like multiple phased-array coils and fast spin-echo, imaging times have decreased and soft tissue resolution and contrast have dramatically increased. Even postmenopausal ovaries can now be seen on most pelvic MRIs.

MRI has the advantages of being noninvasive and using no ionizing radiation. In addition, it provides multiplanar capacity allowing for reproducible examination of anatomy in three-dimensions and a field of view unimpeded by bone, bowel, or fat. For these reasons, MRI is quickly becoming the imaging modality of choice for the evaluation of the pelvic mass. Simple ovarian cysts can be differentiated from other lesions, and greater detail can be visualized within follicles than with other imaging modalities. MRI has been shown to be superior to transabdominal US in the visualization of ovaries in the female pelvis with distorted anatomy. Wiczyk et al. note that, in addition to showing that MRI can accurately track folliculogenesis in spontaneous and stimulated cycles, MRI demonstrates a secondary cohort of follicles, ranging in size from 0.3-0.8 cm, undetected
by transabdominal US. MRI now provides the clear delineation of individual follicles, cortex, and stroma as well as the relative distribution of follicles.

It is not yet known whether or not ovarian architecture is an indicator of a woman's fertility status, or, similarly, whether or not loss of oocyte function is reflected in this architecture. In trying to assess ovarian follicular reserve, MRI is a unique tool in that it is noninvasive, safe, and provides exquisite anatomic detail in a reproducible and quantitative fashion.

**STATEMENT OF PURPOSE**

Given the recent developments in MRI, this study is intended to assess the utility of MRI as a prognostic tool in the management of infertility. As a pilot study, its purpose is to evaluate whether or not there are morphologic differences, as viewed by MRI, between the ovaries of women with a low response to superovulation and women with presumably normal ovarian function. In doing so, ovarian volume and follicle size and number will be compared between the two distinct groups.

**METHODS**

**Part I: Validation Exercises**

In order to validate the approach of assessing ovarian size and follicular number and size with MRI, qualitative and roughly quantitative comparisons were made, first, between *in vitro* MRI images of an excised human ovary and its histologic sections and, second, between *in vivo* MRI images of an ovary prior to surgical removal and its histologic sections.

The *in vitro* examination was performed using MRI parameters developed by radiologists Dr. Robert Troiano and Dr. Shirley McCarthy that could be applicable to *in vivo* studies. These parameters were selected to obtain the smallest possible field of view (FOV) while maintaining high resolution. They included a repetition time (TR) of 4000
msec, an echo time (TE) of 90 msec, FOV of 16 cm, a section thickness of 3 mm, an intersection spacing of 0, an echo train length (ETL) of 16, number of excitations (NEX) of 4, and a matrix of 256 x 256. A general purpose circular coil was utilized because of the inherently small size of the ovary. Histologic sectioning and staining of the specimen previously imaged was performed by Dr. Harvey Kliman, a gynecologic pathologist. Sectioning was done in the approximate plane of the MR images.

The in vivo experiment was performed with the voluntary help of a 36 year old, premenopausal woman in Dr. David Keefe’s Reproductive Endocrinology Clinic with intractable pelvic pain thought to be secondary to severe endometriosis. One day prior to her scheduled total abdominal hysterectomy with right salpingo-oophrectomy, she was imaged using MRI. In the operating room, a landmarking stitch was placed on the lateral edge of the ovary prior to removal. After removal, oocytes were harvested from the distal half of the ovary for the purposes of another study. The ovary was deemed free of gross pathology and then fixed in formalin, embedded in parafin wax, and serially sectioned along the sagittal plane by Dr. Vinita Parkash of the Yale Pathology Department. The approximation of sectioning to the MR images was done by Dr. Parkash and me by lining up the anatomic landmark of a right hydrosalpinx and the placed stitch. Slice thickness was 5 microns, and every tenth slice was stained with hematoxylin-eosin stain.

Part II: MR Imaging of Controls vs. Women with Low Responses
Experimental Subjects and Study Design

The protocol for the study was approved by the Human Investigation Committee of the Yale University School of Medicine, and all subjects I recruited gave verbal and written consent as informed and willing participants.

The five patient subjects were selected because they had demonstrated a low response (i.e. peak $E_2 < 500$ pg/ml) to empiric superovulation using 3 or 4 ampules hMG daily. All patient subjects had previously undergone routine fertility screening at the Yale
Center for Reproductive Medicine and were diagnosed as having unexplained and/or age-related infertility, in the case of 4 patients, or anovulation, in the case of one patient. Their mean age was 42 years (range 36-45); they were all nulliparous and nonobese; and their mean duration of infertility was 39 months (range 29-54). The 4 patients with unexplained infertility had regular menses every 25-28 days, while the woman with a diagnosis of anovulation menstruated irregularly every 40-60 days. They had been followed at the Yale clinic for a mean follow-up time of approximately 12.6 months (range 9-20). Each woman had a documented day 3 FSH within one and one-half years of being recruited into the study of less than 12 mIU/mL. However, subsequent to having the MRI, one patient control had a basal day 3 FSH of 19.0. None of the patients noted hypoestrogenic symptoms.

For the pilot study, the first three control studies were of paid participants in a prior radiologic study. Thus, little is known of their histories, except that they were Yale-New Haven Hospital employees, were nulliparous, had regular menses (every 25-32 days), and had not taken oral contraceptive medications within the past six months. To complete the control group, two medical student volunteers were recruited. They too were nulliparous, had regular menses, and used barrier contraception. In addition, they had no known medical or gynecologic illness, nor did they take any medications. The average age of the five control subjects was 25 years (range 21-29).

I scheduled 11 study subjects for MR imaging on day 3 or 4 of their menstrual cycle before they received any stimulatory medications. I followed the three patient subjects undergoing ovulation induction the month of the MRI for peak $E_2$, follicular number and size on transvaginal US, and outcome.

**MR Imaging**

Before entering the MRI, each subject underwent a screening questionnaire to ensure that they did not have any contraindications to the procedure. For the MRI, each patient had an empty bladder and was positioned lying on the ventral side.
All images of the pelvis were obtained using a phased-array multicoil. Following a coronal localizer of each woman's ovaries, multiplanar (sagittal, coronal, and axial), fast spin-echo T-2 weighted sequences were performed. The imaging parameters were TR 5000-7500 msec, TE 117 msec, FOV 20 cm, NEX 4, matrix 256 x 256, ETL 16, bandwidth (BW) 32, and a slice thickness of 3 mm with a 0 mm gap. There was no use of gadolinium contrast.

**Image Analysis**

A cross-sectional radiologist, Dr. Robert Troiano, at Yale-New Haven Hospital and I reviewed the MR images. Measurements of ovarian diameters were made in three orthogonal dimensions from the axial, sagittal, and/or coronal planes. If simple follicular cysts were present, their diameters, too, were measured in three orthogonal dimensions. Follicle numbers and diameters were recorded for all follicles greater than or equal to 5 mm. The greatest diameter was determined by comparing the multiplanar images and taking into account the 3 mm slice thickness so as not to over or undercount. Follicles that had distinct fluid-filled antrums but were less than 5 mm in diameter were also totalled in each patient. All measurements were made using calipers and were made from basement membrane to basement membrane. Finally, examinations were assessed for pelvic pathology.

**Data Analysis**

I calculated ovarian volume using the formula for a prolate ellipse, \( V = \frac{1}{2}(A)(B)(C) \), while calculating the volumes of simple follicular cysts using the equation for a more spherical object, \( V = \frac{\pi}{6}(A)(B)(C) \), where A, B, and C are the largest diameters measured in three planes.\(^{60,58}\) To give a more accurate measure of ovarian tissue present, in the cases in which cysts were present, I calculated ovarian volume by subtracting the cystic volumes from the ovarian volume calculated above using the total ovarian dimensions. Statistical comparisons between the two subject groups for ovarian
volumes and follicle numbers were made using the Student’s t-test. P<0.05 was considered significant.

Finally, I compared the number of follicles visualized on day 3 MRI in our study to the number of follicles greater than 1 mm observed by Erik Block in his post-mortem, gold standard ovarian dissections for each of the distinct age groups studied. The mean number of follicles greater than or equal to 1 mm for two distinct age groups in Block’s study was determined by averaging the number of these follicles in each of the women he studied with an average age of death of 25 (n=12) and in each of the women with an average age of death of 42 (n=7). Again, a Student’s t-test was used to compare our means to his, p=0.05.

**RESULTS**

**Part I: Validation Exercises**

Recent advances in T-2 weighted MR imaging techniques permit delineation of the ovaries, both in vitro and in vivo. As seen in the in vitro MRI comparison to histologic sectioning of Figure 1, the fluid-filled antral follicles demonstrate high signal intensity, while the fibrous capsule appears as a low signal intensity rim. The remainder of the ovarian stroma is usually of moderate signal intensity, isointense with fat. Despite a subtle shift in the cutting plane and distortion of follicle shape by sectioning, Figure 1 provides reassurance that the MR images are consistent with actual anatomy in terms of follicular size, location, and number. The antral follicles are approximately spherical and, for the most part, are located near the ovarian surface.

The smallest structures distinguished without magnification on the in vivo MRI, seen in Figure 2, were the 0.5-1.0 mm antrums of developing or atretic follicles. In the corresponding histologic sections, these small antral follicles could likewise be seen, confirming that follicle size and number were analogous to the imaged ovarian cross-sections. Under light microscopy, the smallest structures visualized within the ovarian
tissue were the 50-55 micron primordial follicles. Lacking the hyperintense antrums, these small structures were not seen on the corresponding MRI.

Figure 1: MR image slice of an excised human ovary *in vitro* with its corresponding histologic section.

Figure 2: MR image of a human ovary *in vivo* with its corresponding histologic section, prepared one day later.
Part II: MR Images of Controls vs. Women with Low Responses

Total scan times for the multiplanar MR views of each woman’s ovaries averaged 30 minutes per subject. All MR examinations were well-tolerated by study participants.

In viewing the pelvic MRI’s, findings included the identification of an involuted corpus luteum as well as a small amount of adenomyosis in the uterus of one control subject and the presence of simple cysts in three out of five of the low-responding patient subjects. These cysts were unilocular, fluid-filled, and with diameters of approximately 2 cm. In order to compare amounts of ovarian tissue in the control versus low-responding patient groups, these cystic volumes were subtracted from the total volume within the ovarian capsule. This calculation resulted in the ovarian volumes recorded in Table 1. The differences in the mean ovarian volumes (Figure 3), with average volumes of $8.4 \text{ cm}^3 \pm 3.5 \text{ cm}^3$ in the younger control group and of $5.0 \text{ cm}^3 \pm 4.3 \text{ cm}^3$ in patients with a low response, were not significant ($p>0.1$). With cystic volumes included, the mean ovarian volume for the low-responding patients was 6.7 cm$^3$.

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Table 1: Ovarian volumes and follicle numbers less than and greater than or equal to 5mm in each control and low-responding subject, as seen on noninvasive, T-2 weighted, FSE MR images. R=right ovary; L=left ovary.
Figure 3: Mean ovarian volumes ± standard deviation, determined by measurements of MR images, in the control versus low-responding subjects. No significant difference was present.

Figure 4: Mean total number of follicles per person ± standard deviation in the control versus the low-responding patient subjects. The darker part of each column corresponds to the mean number of follicles greater than or equal to 5 mm, while the lighter part corresponds to the number of follicles
less than 5 mm. For total number of follicles, p<0.001. For follicle numbers ≥ 5 mm, p<0.0005. For follicle numbers < 5 mm, p<0.01.

Follicle numbers, visualized on day 3 MRI, within each subject's ovary are recorded in Table 1. Mean numbers of follicles per person for the the control group and low-responding patient group are divided into less than and greater than or equal to 5 mm and are represented in Figure 4. The mean total number of follicles for the controls and the low-responding patients were 63.8±14.4 and 23.4±7.0, respectively. This represented a significant difference, with p<0.001. There was likewise a significant difference (p<0.0005) between the two groups in terms of mean number of follicles greater than or equal to 5 mm. The control group had 23.0±3.7 of these, while the low-responding group had only 3.2±2.7. In terms of mean number of follicles less than 5 mm visualized in each group, there were 40.8±12.1 in the controls and 20.2±7.0 in the "low responders." This represented a significant difference, p<0.01. For the most part, the smallest follicles counted in the less than 5 mm range had antral diameters just under 1 mm. The bulk of these, however, were in the range of 1-3 mm but were difficult to measure exactly.

Lastly, a comparison is made between the number of follicles visualized on day 3 MRI and the post-mortem, histologic follicle counts done by Block in 1952 (see Figure 5). For the control group with an average age of 25, there was a mean number of 63.8±14.4 total follicles seen on MRI and a mean number of 72.7±32.5 Graafian follicles greater than 1 mm observed by Block. For the 42 year old age group, there was a mean number of 23.4±7.0 total follicles seen on MRI and a mean number of 17.0±30.0 follicles greater than 1 mm observed by Block. There was no significant difference between either of the age-group comparisons.
Comparison Between Block and Our Total Follicle Counts

<table>
<thead>
<tr>
<th>Mean Number of Follicles ≥ 1 mm</th>
<th>MRI Study 1996</th>
<th>Block 1952</th>
</tr>
</thead>
<tbody>
<tr>
<td>25 years</td>
<td>120</td>
<td>42</td>
</tr>
<tr>
<td>42 years</td>
<td>80</td>
<td>20</td>
</tr>
</tbody>
</table>

Figure 5: Comparisons between Block’s histologic assessments of mean follicle numbers greater than 1 mm in diameter ± standard deviation and the mean number of follicles visualized on MRI ± standard deviation for each of the study’s distinct age groups. Bars represent standard deviations. No significant difference between age-matched groups.

It is interesting to note that in the three low-responding patients who went on to pursue unsuccessful ovulation induction the cycle of their day 3 MRI, only a fraction of the follicles seen on MRI were picked up on the day 3 transvaginal US. In the first patient with 23 follicles less than 5 mm in diameter and a cyst of 2.5 cm diameter, 5 follicles less than 10 mm were seen on US. In the second patient with 30 follicles less than 5 mm and 3 follicles between 5 and 10 mm on MRI, no follicles were seen on US. And, for the third patient with 21 follicles less than 5 mm and 6 follicles 5-7 mm as well as 3 cysts on MRI, 6 follicles less than 10 mm and 2 cysts were recorded on transvaginal US.

DISCUSSION

With the new techniques of the phased-array multcoil and fast spin-echo, MRI provides enough anatomic detail to reliably assess antral follicle position, size, and number. This imaging modality was validated both in vitro and in vivo before pursuing comparison
between the infertility patients and controls. Not only was there good approximation
between histologic section and MR images, there was agreement between our MRI data and
Block’s post-mortem, histologic follicle counts. Both data sets indicate that the number of
antral follicles greater than 1 mm declines with advancing age. It is interesting to note that
the variances for Block’s data are much larger than our own. Perhaps, this is due to the
fact that his selection process was more random than our own process of recruiting hospital
employees and medical students with presumably normal ovarian function and infertility
patients specifically with documented low response to ovulation induction.

MRI also provided noninvasive estimation of ovarian volume both in terms of
rough histological comparison and the findings of other studies. Using MRI, Outwater and
Mitchell determined that in 38 premenopausal women the mean ovarian volume was 9.6
mL ± 10.1 mL and that in 14 postmenopausal women the mean ovarian volume was 2.5
mL ± 2.6 mL. Thus, as would be expected, our 25 year old control group have large
ovaries typical of premenopausal women, while the women with documented low response
and still menstruating show slightly smaller ovaries, though not as small as in
postmenopausal women.

The significant differences in total numbers of follicles, follicles less than 5 mm in
diameter, and follicles greater than or equal to 5 mm in diameter, observed on MRI,
between the younger control group and the older infertility patients with low response to
ovulation induction suggests that MRI is capable of documenting the differences expected
with chronological aging. How much these differences are affected by reproductive, or
biologic, aging will require the study of age-matched controls of proven fertility.
Nonetheless, there is an indication that MRI may be useful in the estimation of ovarian
reserve.

Since it remains unclear what causes decline in oocyte number and quality with
aging, most current studies of the subject, like this one, are observational in nature. They
display a correlation. These observations are potentially useful to women,
indistinguishable by traditional parameters, and physicians in that they could serve as screening mechanisms to more effectively direct patients to appropriate counselling and treatment. Large numbers of studies and correlations with outcome are necessary to establish the threshold for a new screening test. Further study is also necessary to clarify whether individual variations in the size and dynamics of the follicular pool arises from postnatal environmental influences, like the proven bad effects of cigarette smoking or ionizing radiation, or is determined during oogenesis in the fetus or even genetically, prior to conception.

Despite the unknown and likely multiple factors affecting follicle attrition and oocyte dysfunction, observations of follicular reserve have the potential, for now, to provide new insight into the management of unexplained infertility. A large part of the role of reproductive endocrinologists is, one, to speed up the time required for successful gestation, if possible, and, two, to provide realistic expectations of couples' reproductive potential. Before menopause, it seems that many women will experience a phase of subfertility lasting approximately a decade or less. Yet, during this phase, menstrual cycles will usually continue, often in a regular fashion with ovulation. As increasing numbers of women attempt childbearing between the ages of 35 and 50, it would be beneficial to be able to prospectively distinguish between outcome categories, to better assess biologic rather than chronologic age, and to predict low response to ovulation induction early on in therapy. It may be, for instance, that, although little is known about the intrinsic modulators of follicular recruitment and depletion in humans, the number of follicles available for potential recruitment into further growth and development with hMG's is proportional to the number of antral follicles 2-4 mm and just able to be seen on MRI.

The effectiveness of treatment is only one component of therapy decisions. Also important are the cost of the individual treatments, the time required to participate in therapy programs, the severity of potential side effects, and the individual characteristics of each
woman. From the point of view of cost, a thorough MRI of the pelvis is approximately $1000. This is in the context of the cost of hMG medication alone which is $1000-1500 per cycle. In addition, there are lab, physician, and US ($200 per scan) fees each cycle. The cost of the more invasive IVF procedures exceeds $10,000 per cycle, with many women attempting six or more cycles.

Despite the high cost of MRI, it has the potential, if proven to be an accurate indicator of ovarian reserve, for one complete 30-minute ovarian exam to preclude or minimize treatment costs. No other noninvasive techniques can accomplish imaging of the adnexa in as precise and reproducible a fashion as MRI. US, for example, suffers from less spatial resolution and lack of reproducibility in the plane of the transducer held by the sonographer. While fairly good at recording the mid-size, preovulatory follicles, US seems to miss the larger smaller cohort of antral follicles, seen in the early follicular phase, also observed by Block on histologic section and Wiczyk in comparing same day MRI and US images. While the spatial resolution of US is adequate in only two planes and the ovaries are not always optimally visualized, MRI has adequate spatial resolution in all planes, has unsurpassed tissue resolution, and can be used to readily locate ovaries. In general, structure is typically easier to discern on MRI than US. As yet, however, neither US nor MRI can define the primordial or preantral follicle as can histologic examination.

This study points to many exciting areas for future research. To our knowledge, no systemic study of normal ovarian anatomy in three-dimensions, as seen on MRI versus US versus histology, has been performed. With more accurate land-marking of ovarian tissues during removal, better orientation of MR and histologic sections could be achieved, making possible more detailed comparison of MR images to histology with computerized image analysis.

In order to verify that MRI reveals differences in response to superovulation and provides a marker for reproductive aging, it would be helpful to do a study comparing the low-responding patients with age-matched controls of proven fertility. MRI could then be
used to look for a correlation to the spectrum of response to various treatment protocols seen in women with unexplained infertility to empiric superovulation. As diagnostic procedures improve and understanding increases, the heterogeneous group of women with unexplained infertility may become smaller. Their diagnoses will be more subtle and specific, and their therapies will be more individualized in hopes of improving their future reproductive performance.
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