Clinical Problems

Laparoscopy and Gonadal Biopsy for Assessment of Gonadal Function in Primary Amenorrhoea

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Summary

Twenty patients with primary amenorrhoea have been investigated by laparoscopy and gonadal biopsy, endocrine and chromosomal studies. Laparoscopy and gonadal biopsy made the major contribution to the diagnosis, prognosis, and management of the patients and provided a method for study of the functional anatomy of the ovary.

Introduction

Laparoscopy and gonadal biopsy provide an opportunity for the investigation of ovarian function and for diagnosing ovarian dysfunction.[1] This procedure is particularly relevant in cases of primary amenorrhoea, secondary amenorrhoea, the polycystic ovary syndrome, and infertility. Data thus collected assist the planning of logical management, the provision of accurate prognosis, and the assessment of response to treatment. The operation may have a therapeutic effect akin to that of wedge resection of the ovaries. We present here the results of investigations on 20 patients with primary amenorrhoea and discuss the value of laparoscopy and gonadal biopsy.

Methods

Laparoscopy was performed[2,3] and a gonadal biopsy sample was taken by using Palmer biopsy forceps without coagulation. Specimens for histological examination were transported on ice without fixation. Total urinary oestrogens were measured[4] and total urinary gonadotrophins assayed by the mouse uterus test. Gonadotrophin results were expressed in human menopausal gonadotrophin (H.M.G.) units with use of the Second International Reference Preparation as a standard (normal adult range: male 5-20 units, female in the reproductive phase of life 5-30 units). Chromosomal analyses of cultures of lymphocytes and skin fibroblasts were provided by the clinical and population cytogenetics research unit, Western General Hospital, Edinburgh, or the medical genetics unit, Royal Hospital for Sick Children, Glasgow.

Histological sections were prepared in the usual way and stained with haematoxylin and eosin; Masson's trichrome stain was used for connective tissue. In normal ovaries primordial follicles are distributed along the outer layer of the cortex beneath the capsule. In adult females the distribution is patchy. Study of serial sections shows that provided a large enough arc of the circumference of the ovary is examined in a single section a reasonable estimate of the frequency of primordial follicles may be obtained. The arc must measure at least 1 cm. This method was applied to a large number of ovaries from normal women aged 18-45 years, and it was found that as age increased so the number of follicles diminished.[5]

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Results

The results of the investigation are given in Table I, the patients being divided into five groups—gonadal dysgenesis, anatomical anomaly, uterine unresponsiveness to hormones, ovaries without follicles, and ovaries with follicles.

GONADAL DYSGENESIS

All four patients in this group (Cases 1 to 4) were found to have streak gonads and a raised level of gonadotrophins. Two patients had chromosomal abnormalities. Both were of small stature, pubic and axillary hair was absent, and there was no real breast development. Case 1 was typical of Turner's syndrome. In Case 2 several minor congenital abnormalities were present. The biopsy specimen from Case 1 was scarcely recognizable as ovarian in structure. A capsule was present approximately 50 μm thick. Follicles were absent and the specialized stroma was minimal and of fibrous nature. A few tubules of possible rete type were present in the medulla and there was a small mass of hilar cells. In Case 2 the biopsy specimen was recognizable as ovarian tissue. A definite capsule was present 75 μm thick. The cortex was thin and consisted of wiry stroma, very fibrous in appearance. Follicles were absent. A few tubules were seen in the medulla but no hilar cells.

In Cases 3 and 4 the complement of chromosomes was normal. Both patients were 155 cm or over in height. Pubic and axillary hair was normal in quantity and distribution and breast development was present but poor. There were no congenital defects. In Case 3 (the heavier, taller girl with the larger uterus) definite ovarian tissue was present histologically but without follicles. The capsule was 125 μm thick and the cortex consisted of plump vacuolated stromal cells of ovarian type. In the other case no recognizable gonadal tissue existed. The specimen consisted of fibrous tissue embedded in which were two rounded masses of small basophilic cells resembling granulosa cells. Deeper in the fibrous tissue were a few rete tubules.

ANATOMICAL ANOMALY

Five patients (Cases 5 to 9) had a developmental abnormality. Two (Cases 5 and 6) were suffering from testicular feminization.

TABLE I—*Clinical Details of 20 Patients with Primary Amenorrhoea*

	Case No.	Age (Years)	Height (cm)	Weight (kg)	Chromosomes	Total Urinary Oestrogens (μg/24 hr)	Total Urinary Gonadotrophins (HMG units/24 hr)	Response to Oestrogen Therapy	Genital Tract	Secondary Sexual Development
Gonadal dysgenesis	1	18	145	48·5	45 X0		44	+	Minute 2·5-cm uterus, streak gonads	No pubic or axillary hair. Poor breast development
	2	17	130	38·1	X0/XY		35	+	Small 5-cm uterus, streak gonads	No pubic or axillary hair. No breast development
	3	22	164	58·9	46 XX		35	+	Small 5·5-cm uterus, streak gonads	Poor breast development
	4	20	155	47·6	46 XX	·5	91	+	Minute 2-cm uterus, streak gonads	Normal pubic and axillary hair. Poor breast development
Anatomical anomaly	5	18	156	46·2	46 XY		3		Short vagina. No uterus or tubes. Abdominal testes	No pubic or axillary hair. Good breast development
	6	18	157	48	46 XY		4		Adequate vagina. No uterus or tubes. Abdominal testes	No pubic or axillary hair. Good breast development
	7	31	158		46 XX		25		No vagina, uterus, or tubes. Normal ovaries	Normal female appearance
	8	19	140	39·4	46 XX				No vagina, uterus, or tubes. Normal ovaries	Normal female appearance
	9	17	152	44·4	46 XX				No uterus or vagina. Fimbrial end of tube present. Normal ovaries	Normal female appearance
Uterine unresponsiveness to hormones	10	21	150	54·4	46 XX			−	Normal 6·3-cm uterus. Ovaries normal. Extensive pelvic adhesions. No endometrium	Normal female appearance
Ovaries without follicles	11	18	163	58·9	46 XX		25		Minute 1·3-cm uterus. Small ovaries	Normal female appearance
	12	26	159	51·7	46 XX		27	+	Small 6·3-cm uterus. Small ovaries	No axillary hair. Normal pubic hair. Poor breast development
	13	23	136	38·1	46 XX		5	+	Minute uterus, tubes, and ovaries	No pubic or axillary hair. Pituitary infantilism
Ovaries with follicles	14	19	165	58·9	46 XX		3	+	Minute 2·5-cm uterus. Small ovaries	No axillary hair. Scanty pubic hair. Poor breast development
	15	26	163	53·5	46 XX	<3	1	+	Small 5·5-cm uterus. Small ovaries	No axillary hair. Scanty pubic hair. Poor breast development
	16	31	163	63·5	46 XX	<5	1	+	Small 5·5-cm uterus. Small ovaries	Normal female appearance
	17	25	166	65·3	46 XX	<5	1	+	Small 3·7-cm uterus. Small ovaries	Normal female appearance
	18	30	165	54·4	46 XX	<5	2	+	Small uterus, tubes, and ovaries	Normal female
	19	24	164	60·3	46 XX	<5	1	+	Small 5-cm uterus. Small ovaries	Scanty pubic and axillary hair. Poor breast development
	20	29	156	50·8	46 XX	<5	1	+	Small 3·7-cm uterus. Small tubes and ovaries	Normal female appearance

Both had a normal male karyotype and the gonadotrophin output was within the normal range for males. Each had a vagina but the uterus and tubes were absent. Laparoscopy showed the presence of small abdominal testes. Histologically the tissue in these two cases showed typical testicular tubules lined by Sertoli cells only, germinal cells being absent. Interstitial cells were abundant (Fig. 1).

In Cases 7, 8, and 9 Müllerian duct derivatives—vagina, uterus, and tubes—had failed to develop. Otherwise they were normal 46 XX females with well-developed breasts and sexual hair. One patient was found to have a horse-shoe kidney. Outputs of gonadotrophins were within normal limits. Normal ovaries were found at laparoscopy in each case. Microscopical examination of the biopsy specimens showed a degenerating corpus luteum in one case and a corpus albicans in both of the others. The ovaries had well-formed capsules and abundant plump stromal cells and seemed normal in every respect. The follicle counts were slightly on the low side. Some details are summarized in Table II.

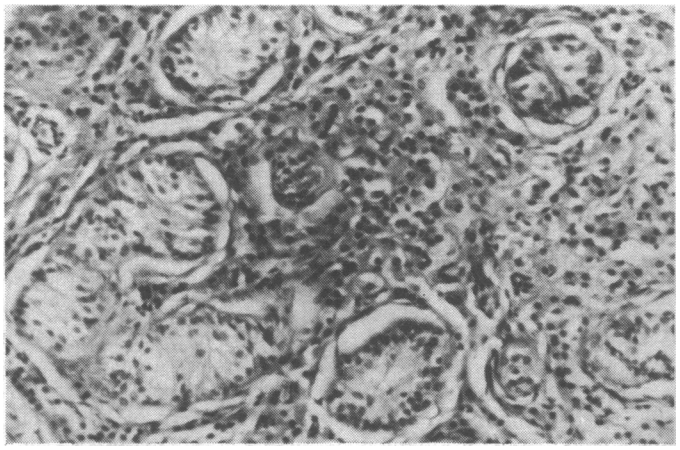

FIG. 1—Section of testicle from case of testicular feminization. The tubules are lined by Sertoli cells. Germ cells are absent. Interstitial tissue is abundant. (× 155.)

TABLE II—*Histological Details from Patients with an Anatomical Anomaly but who had Normal Ovaries*

	Case 7	Case 8	Case 9
Age (years)	31	19	17
Capsule thickness (μm)	125	75	150
Primordial follicles (No./linear mm)	0·2 (0·7)	1·4 (1·6)	0·85 (1·8)
Stroma	Active	Active	Active
Corpus luteum	−	+	−
Corpus albicans	+	−	+

(Normal values are given in parenthesis)

UTERINE UNRESPONSIVENESS TO HORMONES

Only one case of this type was examined. The patient (Case 10) was of normal female habitus, was well developed, and had normal chromosomes. The uterine cavity measured 6·3 cm but curettings were not obtained despite several attempts. A history suggestive of previous pelvic inflammation was obtained and there was a family history of tuberculosis. Laparoscopy showed extensive pelvic adhesions. Examination of the ovarian biopsy specimen showed normal structure with a plentiful supply of primordial follicles (1·2 per linear millimetre of cortex), a Graafian follicle 2 mm in diameter, a corpus luteum, and a corpus albicans. In this patient the uterus was obviously insensitive to the influence of the ovary, which was functioning normally.

OVARIES WITHOUT FOLLICLES

Three patients (Cases 11 to 13) had small ovaries but biopsy failed to show any follicular structures. Chromosomes were normal. Two patients (Cases 11 and 12) were of normal stature, and the other patient was a pituitary dwarf with growth hormone deficiency but whose thyroid and adrenal function were normal. Scanty pubic hair was present in Cases 11 and 12 but neither pubic nor axillary hair had grown in the small patient with pituitary infantilism. Breast development although poor had occurred in the taller patients but not in the dwarf. Gonadotrophin output was at the upper limit of normal in the two patients of normal statute and gave low normal values in the dwarf. Histologically the gonads had the structure of ovaries. The capsule was of normal thickness (approximately 125 μm) in all three patients. Specialized stroma was present but the cells were thin and wiry in appearance. Follicles were absent and there was no evidence of their ever having been present in any developed form.

OVARIES CONTAINING FOLLICLES

Seven patients (Cases 14 to 20) were found to have small ovaries containing follicles. Each had a small uterus and patent Fallopian tubes. Chromosome make-up was normal in each case. All were over 152 cm tall. Urinary gonadotrophin output was low, being less than 5 HMG units per 24 hours, as was the total urinary oestrogen excretion—less than 5 μg/24 hr. On histological study of the ovarian biopsy specimens these seven patients were divided into two subgroups. In Cases 16, 17, and 18 the ovarian capsule was thick, measuring 500 μm. The capsule was very fibrotic with coarse collagen fibres. Primordial follicles were fairly frequent, averaging 2 or 3 per linear mm of cortex. Many of these follicles were involved in the fibrous tissue of the capsule and some of the ova showed degenerative changes. Graafian follicles were present in all three, the largest measuring 2 mm in diameter. Corpora candidanta—that is, the remains of atretic Graafian follicles—were frequent but the formation of albicans material was minimal. There was no sign of corpora lutea or of a true corpus albicans. The specialized stroma was scanty in amount and the cells were wiry.

In Cases 14, 15, 19, and 20 the ovarian capsule was thinner, varying from 150 to 250 μm. It was less fibrotic than in the previous group. Primordial follicles were very numerous in three patients, numbering 25, 12, and 10 per linear mm of cortex, giving a rather infantile appearance to the ovary (Fig. 2). In the other patient (Case 19) there were 2 follicles per mm.

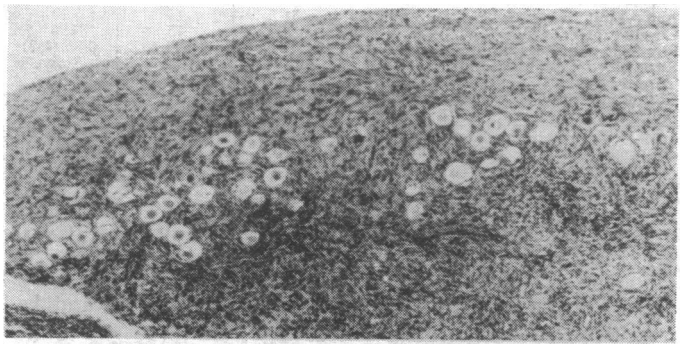

FIG. 2—Ovary of infantile appearance from patient in group having ovaries with follicles. Primordial follicles very numerous. (×62.)

Again follicles were involved in the fibrous tissues of the capsule and many ova were degenerate in appearance. Some showed pyknosis of nuclei, others were grossly vacuolated, and in many the primitive granulosa was absent (Fig. 3). Graafian follicles 1 mm in diameter and corpora candidanta were present in all four. Albicans material was fairly well formed in Case 19. No sign of a corpus luteum or of a true corpus albicans could be seen.

Only these seven patients fulfilled the criteria for gonadotrophin therapy, and Cases 15, 16, 17, and 18 received stimulation with human menopausal urinary gonadotrophins (HMG) (Pergonal) and human chorionic gonadotrophin (HCG). The menopausal gonadotrophin dose was selected by predetermining individual patient sensitivity.[6 7] Each patient produced ovulatory responses and two patients became pregnant (Case 18 and Case 20).

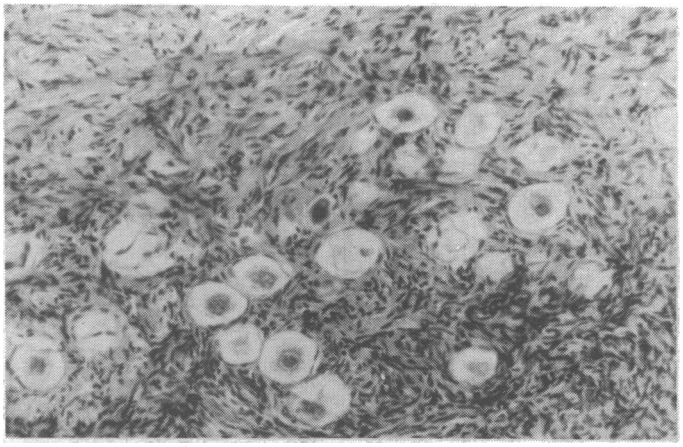

FIG. 3—High-power view of follicles in Fig. 2. Primitive granulosa layer is deficient or absent in some. Degenerative changes are obvious in the ova. (×155.)

Discussion

There can be no doubt about the value of laparoscopy and gonadal biopsy in the diagnosis, prognosis, and future management of patients with primary amenorrhoea. Dysgenetic gonads can be investigated and any necessary supportive hormone therapy given with confidence. Gonadal tumours can be excluded or gonadectomy indicated. Removal of abdominal testes can be postponed in young patients with testicular feminization although this may be necessary later. Normal ovarian function can be confirmed when anatomical anomalies or defects interdict menstruation or pregnancy. Gynaecography,[8 9] although a safe and simple procedure, cannot be as accurate as visual inspection, nor does it permit biopsy sampling.

Chromosome studies are an accepted ancillary investigation of primary amenorrhoea. Nevertheless, all the patients with definitive ovaries histologically, with or without follicles, had normal chromosomes. Only in patients with dysgenetic gonads did chromosomal analysis help to elucidate the problem of diagnosis. Estimation of total urinary gonadotrophins was of limited value. High values usually indicated undeveloped ovaries (Cases 1 to 4). Normal values indicated normal functioning ovaries or inactive ovaries without follicles (Cases 11, 12, and 13). Low values suggested a deficiency of endogenous production and the possibility of potentially normal ovaries (Cases 14 to 20). High values, however, have been reported in primary amenorrhoea associated with an apparently normal follicular apparatus.[10]

Nevertheless, endocrine studies can contribute important information,[11] especially to the complete assessment and ultimate management of patients having ovaries without follicles and of those having ovaries containing follicles. The assessment of ovarian function by stimulation with heterologous gonadotrophins[12 13] has been superseded by stimulation with homologous gonadotrophins.[14] Such a test becomes infinitely more valuable with foreknowledge of the gonadal status and histological findings, and the two investigations are a necessary part of the clinical management of patients undergoing induction of ovulation with gonadotrophin. Similarly information from the use of clomiphene to test pituitary reserve of luteinizing hormone[15] could be augmented by previous laparoscopy and ovarian biopsy in patients in whom ovarian response to gonadotrophin is relevant to their future treatment.

So far as function and specific therapy are concerned the main interest centres on the patients without follicles but having an otherwise well-formed gonad, and those with ovaries containing follicles. The histological pattern in the former group (Cases 11 to 13) suggests a congenital deficiency in the number of follicles and the possibility that the follicles have been used up in the slow but constant partial maturation of follicles in childhood. It has been shown that the number of ova diminishes from 2×10^6 at birth to 3×10^5 at age 7,[16] and maturing follicles have been reported at all ages of childhood.[17] The fertility of this group is suspect but a firm opinion would require the assessment of ovarian response to gonadotrophin stimulation and repeat laparoscopic biopsy to detect any ovarian tissue response and to ascertain whether follicular formation can be promoted. So far there has not been an opportunity to investigate these patients in this way.

There was failure of maturation of follicles in the second group of patients (Cases 14 to 20). The size of the adult ovary is in part due to an increase in the stromal compartment. This increase is thought to be the result of new formation associated with repeated follicular maturation.[18] The smallness of the ovaries in these patients might therefore have been due to infrequent and poor maturation of follicles. In cases 14, 15, 19, and 20 the increase in the number of follicles could have been more apparent than real and due to crowding within a small ovary. Nevertheless, the resemblance to children's ovaries was striking. Further observation will be necessary to determine whether these seven patients really form two distinct subgroups. Gonadotrophin therapy to induce ovulation is obviously indicated but the presence of severe degeneration of ova in some of these cases may indicate a process other than mere lack of pituitary gonadotrophins. Four patients stimulated with HMG and HCG produced ovulatory responses and two patients became pregnant. There is a lack of information regarding the functional anatomy of the ovary. Many of the changes noted in these ovaries give rise to problems relating to capsular thickness and structure, quality of cortical and medullary stroma, the frequency of primordial follicles, and the mechanism and timing of follicular maturation. Laparoscopy supplies a method whereby some of these questions may be answered.

Laparoscopy and gonadal biopsy made the major contribution to the diagnosis, prognosis, and management of patients with primary amenorrhoea. Endocrine and chromosomal investigations provided additional information and helped in the selection of patients for induction of ovulation with gonadotrophins. This approach to the investigation of amenorrhoea provides a method for study of the functional anatomy of the ovary about which few data are available.

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Computers in Medicine

Computer Simulation Model of an *X*-ray Department

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Summary

A computer simulation model has been designed to predict the effects of changes in the work load and resources of an *x*-ray department. The model has been used to produce histograms of patient waiting times and to show the effect on these of introducing changes in the speed of processing films and in the numbers of cubicles and radiographers available. The predicted benefit of using a faster film processor has been confirmed in practice.

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Introduction

The demands made on *x*-ray departments change over a period of time, and it has been suggested that the work load of individual departments should be assessed and analysed at intervals so that information is available about the sources and incidence of these demands.[1] The consequences of changes made in a department to meet altering demands may be difficult to estimate, and the technique of simulation is one way of assessing an operational change before adopting it.[2]

Computer simulation models have been used to examine the effects of making changes in outpatient appointment systems,[3 4] and models simulating inpatient medical care[5] and the effects of varying usage of maternity wards[6] have been described.