Letters to the Editor

Laparoscopic management of ovarian cysts in a Hungarian county hospital

Dear Sir,

We read with special interest, the paper by Chapron et al. (1998), about the diagnostic methods in the management of ovarian cysts, with special reference to the application of frozen sections.

In our practice, a high portion of adnexal masses is managed by laparoscopy. Consecutive steps of the diagnostic approach comprise bimanual pelvic palpation, transvaginal ultrasound, CA-125 antigen value and diagnostic laparoscopy. Cases suspected of malignancy are managed by laparotomy. These include immobile masses that have nodular surface, non-homogeneous echo-structure on ultrasound scan with solid parts or endovegetation, cases with CA-125 concentrations of >35 µg/ml and masses assessed by laparoscopy to have abnormal vessels and/or uneven rough capsular surface.

We do not routinely consider cystic masses, a priori, to be malignant if they are detected in menopause, are bilateral or are >5 cm in diameter. We have attempted laparoscopic cystectomy or adnexitomy with success in several of those cases as well. During the period October 1, 1995, to December 31, 1997, we carried out laparoscopy due to cystic adnexal mass in 39 cases. In seven cases, we returned to laparotomy or mini-laparotomy because of bowel adhesions and/or adherence. In 32 cases, the cysts were removed via laparoscopy; histological tests demonstrated benign changes in all cases. One endometriosis, eight parovarial cysts, and 32 simplex cyst were diagnosed (both cyst types were present in one patient at the same time). No frozen sections were made.

Although our numbers are small, we conclude that, even with wider criteria of benignity, malignant masses did not occur.

References


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Dear Sir,

It was with considerable interest that we read the comments by Varga et al. (1998) following the publication of our paper on the place of frozen section in the laparoscopic management of organic ovarian cysts (Chapron et al., 1998). We entirely agree with Varga et al., when they underline that the menopausal status of the patient, or the size or bilateral character of the cyst, or the heterogeneous appearance of the adnexal mass at ultrasound, or a raised concentration of CA-125 are insufficient grounds to indicate malignancy for an ovarian mass. Taken separately, none of these parameters is a formal indication to carry out laparotomy.

In daily practice, the problem we encounter when a patient presents with an adnexal mass, is being sure of selecting those with a malignant pathology so that they will be operated by laparotomy and not by laparoscopy.

In this context, although the information provided by the pre-operative workup and the diagnostic phase of laparoscopy can seem a reliable basis for suspicions of malignancy (Chapron et al., 1996), analysis of the literature indicates that cases of ovarian cancer have gone undetected (Nezhat et al., 1992; Blanc et al., 1993; Trimbos and Hacker, 1993). This is why, in certain carefully selected cases (patients with clinical or paraclinical suspicion of an ovarian tumour limited to the ovary), and provided that very experienced anatomo-pathologists are available, we recommend using frozen sections to optimize the results of the diagnostic phase of laparoscopy. In the opposite case of patients undergoing laparotomy because of a suspicion of malignant pathology, the final histology results sometimes indicate a benign lesion (Chapron et al., 1997). In these situations, the use of frozen sections could perhaps have avoided laparotomy.

These results concerning the advantages and place of frozen sections for the management of adnexal masses are only preliminary. Above all, they do not mean that frozen sections should be used systematically for all patients presenting with an adnexal mass. In this context, larger series are essential to pinpoint the indications for frozen sections.

References


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Letters to the Editor


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Cigarette smoking and infertility

Dear Sir,

We read with great interest the article by Augood et al. (1998) in which they performed a systematic review of the literature to determine whether there is an association between smoking and risk of infertility in women of reproductive age. Using meta-analysis they concluded that smoking might have a role to play in intraovarian regulation disturbances resulting in granulosa cell dysfunction that lead to CLI.

Bódis et al. (1992, 1993) observed an anticyclical behaviour of progesterone and oestradiol; certain effectors raise production while lowering oestradiol synthesis. On the basis of these results, we speculate that certain substances modulate a pathway switch in steroidogenesis from progesterone to further metabolized steroids. A possible candidate could be the enzyme steroid 17-α hydroxylase processing progesterone to 17-α hydroxy progesterone, a precursor of oestrogen synthesis. It seems possible that some compounds, e.g. nicotine bitartrate, may be unable to operate the switch to allow the metabolic pathway to run down to oestradiol.

Nicotine is one of the most abundant organic compounds found in cigarette smoke. Out of the 5–15 mg of nicotine contained, as much as 1 mg can be absorbed by smoking a single cigarette (Barbieri et al., 1986). These authors also conclude that nicotine and the other nicotinic alkaloids are likely to act in an additive mechanism to inhibit aromatization in possibly many or all sites of steroid aromatization.

In this sense, cigarette smoking means a specific attack on critical control mechanisms of intra-ovarian processes which are responsible for normal luteal function.

References


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Long distance transport ICSI

Dear Sir,

In 1987, the Queensland Fertility Group pioneered the conduct of satellite in-vitro fertilization (IVF) clinics in Australia in Mackay (Hennessey et al., 1989) and later in Townsville. These clinics provided access to assisted reproduction treatments for country patients without the expense and inconvenience of travelling >1000 km to Brisbane and yielded pregnancy rates similar to those of the city clinics (Harrison et al., 1994). Satellite IVF programmes evolved in the USA in 1984 (Talbert et al., 1991) with treatment at a central facility after patient preparation
References


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The use of twinning rates as a reproductive health monitor in England and Wales 1988–1996

Dear Sir,

The twinning rate in women aged <20 years increased in England and Wales during the years 1988–1996. Assuming that assisted reproduction techniques (ART) are not used by these women, it may be inferred that those hazards monitored by twinning rates have abated.

The ‘natural’ dizygotic (DZ) twinning rate is coming to be seen as the least problematic reproductive health indicator (James, 1997). In recent years, ART has boosted twinning rates; so the problem is to estimate rates of twinning among conceptions which did not have the benefit of medical supervision. Nationwide data on such conceptions are not available so indirect methods have to be used.

I have shown (James, 1995) that age-specific total twinning rates for England and Wales declined from 1960 to about 1980, and thereafter increased until 1992 (the last date for which data were then available). In the present note, data are added for 1993–1996.

Table I shows the data from 1976 onwards. The latest figures contrast with earlier data in an interesting manner. Previously (prior to 1992) in all maternal age categories except the youngest, there had been a higher twinning rate outside than inside marriage. This feature has now been exactly reversed. I should like to suggest why. Firstly, one may offer a hormonal explanation for the interaction between age and marital status on twinning in previous years. The suggestion is that in general (what used to be called) illicit sexual intercourse is the occasion of erotic arousal and high female gonadotrophin concentrations (and hence increased risk of DZ twins). By exception, in young women such intercourse was an occasion of emotional turmoil but (because of anxiety) erotic deficit and low gonadotrophin levels, and hence reduced risk of DZ twins. Secondly, in recent years, conditions have changed in two ways: (i) in general a greater percentage of married than unmarried conceptions has been preceded by medical assistance and hence by the greater possibility of iatrogenic twinning; and (ii) the social climate has changed (as exemplified by the official change of nomenclature from ‘illegitimate’ to ‘outside marriage’) so that, *ex hypothesi*, intercourse in young unmarried women is now no longer so frequently attended by anxiety and its inhibiting influence on twin conception. Finally, during the years 1988–1996 there has been a rapid change in the marital status of women giving birth before the age of 20. In 1988, 24% of them were married: in 1996, only 12% of them were. So in principle it is possible that part of the recent increase in the (notes continued) reported twinning rate in young unmarried women was caused by the reluctant fathers cancelling belated weddings on learning (after a pregnancy

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No. of multiple maternities in England and Wales, 1976–1996

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scan) that twins (rather than a singleton) were expected. Such dishonourable conduct could not, however, explain the overall increase in twinning in women of this age group (which is the main point of this letter).

I have argued that if ART may be assumed to be rarely exercised on women aged <20 years, then the ‘natural’ twinning rate had increased in them since about 1980 (James, 1995). The present data add weight to that judgement. The twinning rate within marriage showed a non-significant increase during 1988–1996; and the twinning rate outside marriage significantly increased in that maternal age group across those years ($\chi^2 = 8.0, P < 0.005$).

We may provisionally suppose that any reproductive hazard should have no effect on older women if it has none on younger women. If we make this assumption, then there should at present be no anxiety concerning those reproductive hazards monitored by natural DZ twinning rates. To strengthen this assurance, it would be useful to get information: (i) on the proportion of their patients aged <20 years, e.g. from assisted reproduction clinics, and (ii) on the proportion who had used ART, e.g. from a survey of twin-bearers aged <20 years. However, even if the threat is no longer posed, the question remains: ‘What did cause the decline in DZ twinning rates in the US in 1930–1960 and elsewhere in 1960–1980?’

References

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Integrins and endometriosis: fact or artefact?
Dear Sir,

The surprising data recently published by Hii and Rogers (1998) underscores the importance of the dialogue and the peer review process that occurs in the scientific literature. These authors report that expression of the endometrial $\alpha_v\beta_3$ integrin is present in glandular epithelium throughout the menstrual cycle, and that $\alpha_v\beta_3$ expression is not diminished in women with endometriosis. Unfortunately, their findings can be attributed entirely to artefact.

Both ourselves and others have described the temporal and spatial relationship of endometrial integrin expression throughout the menstrual cycle (Lessey et al., 1992, 1994a; Tabibzadeh, 1992). As integrin antibodies are notoriously unreliable in formalin fixed tissues, all of our published work on endometrial integrins has been performed on cryopreserved tissues. Hii and Rogers used formalin fixed paraffin embedded endometrium. Few, if any, of the 40 or so papers in the literature that have used the LM609 antibody have utilized this integrin antibody in fixed tissue. The antibody specifica-

iions from Chemicon (the supplier) and communications with Dr Cheresh (who initially produced the LM609 clone at Scripps Research Institute, La Jolla, CA, USA) indicate that LM609 antibody is not suitable for use in formalin-fixed tissues. As shown in Figure 1A, we were able to reproduce the artefact of apparent immunostaining using proliferative phase endometrium that had been formalin-fixed and paraffin embedded but found no indication of positive $\alpha_v\beta_3$ staining in the same sample that had been snap-frozen and examined using the accepted immunohistochemical methods for cryopreserved tissues (Figure 1B). Glandular structures (asterisk) in formalin-fixed sections of human bronchus, known to be negative for the $\alpha_v\beta_3$ integrin (Damjanovich et al., 1992; Albelda, University of Pennsylvania, PA, USA, personal communication) also stain positive using LM609 (Figure 1C) illustrating that the artefact is not confined to endometrial glands. Smooth muscle cells (arrow) which have been shown to express $\alpha_v\beta_3$ in bronchus are negative when formalin-fixed tissue is used with this antibody.

Over the past 5 years we have examined >2500 human endometrial samples for $\alpha_v\beta_3$. We consistently find that proliferative and early secretory endometrium lack epithelial staining for the $\alpha_v\beta_3$ integrin. This integrin appears at ~cycle day 20 coincident with the opening of the window of implantation and is delayed in women with luteal phase defects (Lessey et al., 1992). The staining pattern obtained using a $\beta_3$ subunit-specific antibody SSA6 has always provided comparable results to the LM609 in frozen samples. This pattern of $\alpha_v\beta_3$ immunostaining was independently confirmed by Rai et al. (1996). To be certain of our initial results, we performed Western blots on proliferative and secretory phase endometrium using a known positive control from platelet extract which contains the integrin subunit, $\beta_3$. We demonstrated this subunit was present and regulated in a cycle dependent manner on the isolated glandular epithelial elements (Lessey et al., 1992). We now have data using Northern blots demonstrating cycle dependent expression of the $\alpha_v\beta_3$ integrin message in the endometrium (unpublished data).

In contrast to the findings in the study by Hii and Rogers (1998), we have documented that the $\alpha_v\beta_3$ integrin is missing in a subset of women with endometriosis and the assay for the endometrial $\alpha_v\beta_3$ integrin has a high positive predictive value for the diagnosis of endometriosis (Lessey et al., 1994b). Women whose endometrium is missing this integrin have reduced cycle fecundity compared with those with endometriosis who normally express this integrin during the window of implantation. This integrin may also be missing in the endometrium of some women with hydrosalpinges (Meyer et al., 1997), reinforcing the potential usefulness of this marker for detecting defects in uterine receptivity.

Thus, we respectfully express our significant scepticism regarding the findings in the present study that shows no difference in glandular epithelial $\alpha_v\beta_3$ expression throughout the menstrual cycle and a lack of relationship between integrin expression and endometriosis. As appropriate negative controls were not utilized and the technique for using LM609 in fixed tissue was not carefully validated, we would further stress that all of the conclusions of Hii and Rogers should be reconsidered.
Their results which we have reproduced are due to artefact and yield misleading immunohistochemical information.

References


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Dear Sir,

The statement by Bruce Lessey and Arthur Castlebaum that our immunohistochemical findings on α,β3 integrin expression in human endometrium are entirely attributable to artefact is a simple, but unsatisfactory, explanation for the differences in published data between the two groups.

In their letter, Lessey and Castlebaum raise several issues that require response. It is incorrect to state that ‘appropriate negative controls were not utilized and the technique for using LM609 in fixed tissue was not carefully validated’. In the absence of α,β3 protein to pre-absorb the LM609 antibody, the best negative control is matching type and concentration of mouse immunoglobulin (Ig)G. This was used as stated in our methods (Hii and Rogers, 1998). More importantly, a number of frozen and fixed biopsies from the same subjects were utilized to confirm similar immunostaining patterns between frozen and fixed tissues. We believe that this adequately validates the fixed tissue protocol. We were well aware that our data contradicted that of Lessey et al. (1994), and spent considerable energy in carefully validating our methods before submitting the paper for publication.

In their letter, Lessey and Castlebaum present very limited data (without any detail regarding methods) to support their argument that LM609 immunostaining on fixed sections is artefact. These results cannot be compared with those in our paper for several reasons. As far as methods are concerned, our bank of human endometrial tissue is only lightly fixed (4–6 h in 10% buffered formalin), and we used a specific antigen retrieval step with prolonged blocking and primary antibody incubations. With our protocol, cells known to express α,β3, e.g. endothelium and vascular smooth muscle cells, were positive (Lessey and Castlebaum were unable to show positive staining in bronchial smooth muscle cells).

There are other possible reasons for the discrepancy in results between Lessey and ourselves, and we have listed these in the discussion section of our paper (Hii and Rogers, 1998). Briefly, we are the first group to publish detailed endometrial work using the antibody LM609. Nearly all of Lessey’s work uses SSA6, a β3 integrin subunit-specific antibody. It is also important to note that Bridges et al. (1994), using the BB10 antibody to β3, published results that are different again to both Lessey and ourselves.

Figure 1. (A) Immunohistochemistry performed using the monoclonal antibody LM609 revealed reactivity in proliferative phase formalin-fixed endometrium, especially in the glandular structures. (B) In the same tissue that had been snap frozen in liquid nitrogen and cryosectioned, immunohistochemistry showed no specific immunostaining, consistent with previous experience (Lessey et al., 1992, 1994a). (C) In formalin-fixed glandular structures surround human bronchus, a tissue which does not exhibit artefactual positive staining was also noted (asterisk). The smooth muscle which has been shown to express α,β3 integrin (Damjanovich et al., 1992) lacks specific immunostaining when LM609 is used in formalin-fixed tissue (arrows).
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Other evidence which should be considered in this debate comes from Sillem et al. (1997), who showed that endometrial epithelial cells collected in the proliferative phase (days 8–12 of the cycle) expressed $\beta_3$ integrin in vitro and that this expression was independent of oestrogen or progestosterone treatment.

In summary, at the present time, we stand by our endometrial $\alpha_v\beta_3$ LM609 data and acknowledge that it contradicts Lessey and Castlebaum’s endometrial SSA6 $\beta_3$ data. There is major disagreement in the literature on endometrial expression of $\beta_3$ and $\alpha_v\beta_3$, and clearly further work on this issue is required.

Finally, from a biological point of view, we agree with the views put forward by Aplin et al. (1996) who stated ‘it is unlikely that attachment is wholly dependent on one recognition event’. We look forward to more scientific investigation of these issues.

References

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