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Comparison of dysplasia profiles in stimulated ovaries and in those with a genetic risk for ovarian cancer

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ABSTRACT

Aim: Ovarian epithelial dysplasia (OED) was first described after prophylactic oophorectomy for genetic risk of ovarian cancer. In light of Fathalla's incessant ovulation theory, this study was set up to describe the presence of ovarian abnormalities (dysplasia) after ovulation induction and to compare dysplasia profiles in stimulated and genetic risk ovaries.

Methods: One-hundred and twenty-four patients who had undergone salpingo-oophorectomies or ovarian cystectomies between 1990 and 2005 were reviewed. They were divided into three groups: (1) previous *in vitro* fertilisation ($n = 35$); (2) prophylactic oophorectomies for genetic risk ($n = 27$) and (3) fertile non-cancerous controls ($n = 62$). Eleven cytological and architectural epithelial features were defined and a dysplasia score was calculated to quantify ovarian epithelial abnormalities.

Results: Mean dysplasia score was significantly higher in the genetic risk and stimulated ovary groups than in controls (9.55 versus 3.62, $p < 0.0001$; 7.51 versus 3.62, $p < 0.0002$, respectively). Cytological and architectural abnormalities were more frequent in the genetic risk group, while the profile of abnormalities was different in the genetic risk and stimulated groups.

Conclusions: These findings support a possible relationship between OED and the use of ovulation-stimulating drugs. The increased dysplasia score in stimulated and genetic risk ovaries might be consistent with progression towards neoplastic transformation, and may justify the use of the term dysplasia or intraepithelial ovarian neoplasia. The observation of dysplasia in the stimulated group may differentiate women at risk. Conversely, the fact that the dysplasia profile after stimulation differs from that in genetic risk ovaries suggests that ovarian stimulation may predispose to a different evolution.

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1. Introduction

Ovarian serous carcinoma is the most threatening type of gynaecological cancer: 70% of patients die from the disease within 5 years, essentially because of late diagnosis at the advanced peritoneal carcinomatosis stage (stages III and IV).[1] The prognosis is excellent in the early stages of the disease, but the lack of a specific pattern of symptoms, poor anatomical accessibility of the ovaries and incomplete knowledge of the growth kinetics of this type of tumour and of a possible clinical latency phase impede efficient screening, which is currently not advocated.[2]

The identification of precancerous lesions of the ovary would be an important advance in our understanding of ovarian cancer formation and, as for other gynaecological cancers, might allow the development of effective screening and prevention programmes.[3]

In addition to genetic predisposition (BRCA mutations) or family history of ovarian neoplasia, epidemiological studies show that long periods of ovulation and uninterrupted ovulation are risk factors for ovarian cancer.[4,5] These findings led Fathalla[6] to advance the incessant ovulation hypothesis whereby repair of the ovulation scar at the surface of the epithelium could trigger carcinogenesis. Following on from this hypothesis, the possible role of ovarian hyperstimulation has also been addressed, but remains controversial.

Histopathological examination of material from prophylactic salpingo-oophorectomies performed in patients at genetic risk has revealed frequent abnormalities often interpreted as possible pre-cancerous 'ovarian dysplasia' lesions.[7] We sought to identify similar lesions in excised material from salpingo-oophorectomies performed for benign disease after ovarian hyperstimulation as part of sterility treatment, and to compare them with the dysplastic lesions found in ovaries at genetic risk.

2. Materials and methods

2.1. Study population

One-hundred and twenty-four patients were selected out of a total of 5500 patients who had undergone adnexectomies (salpingo-oophorectomies) and/or ovarian cystectomies between January 1990 and December 2005. These patients were divided into three groups: (1) Group A or control group: 62 patients who underwent adnexectomy. Patients who had no personal family history of gynaecological neoplasia (breast, ovary or endometrium), who had undergone an adnexectomy and/or cystectomy for a benign disorder and whose final histopathological report showed non-cancerous ovaries were selected first. This cohort was then age-matched to Group B (see below). To reduce the confounding and protective effect of oral contraceptives, patients who had opted for an intrauterine contraceptive device were selected. Finally, to obtain better statistical power, two randomly selected controls were chosen for each at-risk patient. (2) Group B: 33 patients, including two who underwent surgery twice, providing 35 samples of excised material. Patients in this group underwent an adnexectomy and/or ovarian cystectomy and had previously undergone ovarian hyperstimulation for *in vitro* fertilisation. None of the patients in this group had an ovarian cyst identified by echography during the initial sterility examination. A few years after hyperstimulation these patients underwent an adnexectomy and/or ovarian cystectomy. This group of patients was obtained by crossing the MAP records (4500 patients) with the surgical records for adnexectomies (5500 adnexectomies and/or ovarian cystectomies) from January 1990 to December 2005. Group B consisted of two subpopulations defined according to the time interval between stimulation and cystectomy/ovariectomy: Group B1 ($n = 14$) with a time gap <7 years, and Group B2 ($n = 21$) with a time gap >7 years. (3) Group C: patients who had undergone bilateral prophylactic salpingo-oophorectomies for confirmed genetic predisposition (BRCA 1 or 2 gene mutation) or had a strong family history of gynaecological cancer (breast and/or ovarian), with no further pregnancy desired and aged > 35 years. Twenty-seven patients were identified who had undergone a prophylactic adnexectomy after detection of the BRCA 1 (12 patients) or BRCA 2 (two patients) gene mutation, or because of a notable family history of ovarian and/or breast neoplasia (13 patients). Group C therefore consisted of two subpopulations: Group C1: patients ($n = 13$) with no BRCA mutation, and Group C2: patients ($n = 14$) with a confirmed gene mutation.

2.2. Histopathological criteria

Our definition of ovarian atypia was based on previous studies of ovarian dysplasia, in particular those described by Nieto and colleagues,[8] to which we added four further criteria. In order for the method to be reproducible, 11 histopathological criteria were selected: epithelial multilayering, tufting, surface papillomatosis, nuclear chromatin irregularity, nuclear contour irregularity, cellular pleomorphism, nuclear size (increased or not), epithelial inclusion cysts, deep epithelial cortical invaginations, psammoma and stromal hyperplasia.

In each case the abnormal areas were scored from 0 to 2 (0 = normal, 1 = moderately abnormal, 2 = severely abnormal), whether they were located at the surface or located in an inclusion cyst. A dysplasia score for each patient was obtained by adding the scores for the 11 items (total score range, 0–22).

The histopathological slides of the 35 stimulated ovaries, 27 at-risk ovaries and 62 controls were all read blindly by four pathologists who were experts in onco-gynaecology. The average number of sections available per patient from the three groups was seven (range, 5–11) and the slide with the highest dysplasia score for each patient was retained. When there were major disagreements between pathologists, the slides were read again to reach a consensus.

2.3. Statistical analysis

The main measurement was the dysplasia score, with the working hypothesis that Group C ovaries (those at genetic risk) would have a higher dysplasia score than control ovaries.

A comparison of the mean dysplasia scores for the three groups was carried out using Student's *t*-test. All groups were age-matched.

3. Results

3.1. Epidemiological data

The epidemiological data for the patients (parity, duration of exposure to oral contraceptives, surgical indication and age at oophorectomy) are given in Table 1.

In Group B, the females were infertile in 61% of cases (ovarian dysovulation 15%, tubal pathology 45% and endometriosis 40%) and the male partners in 18%. The cause of infertility was unknown in 21% of cases.

The average number of stimulation cycles was three, with different protocols used. The potential impact of the various drugs could not be studied because treatments were often combined and the numbers were low.

All patients in Group C, by definition, presented with a family history of cancer; 82% had a family history of breast cancer at an early age (mean, 44 years). Personal and family history prompted screening for BRCA 1 or 2 mutations, which were found in 53.5% of cases.

3.2. Cytological and architectural abnormalities

Cytological and architectural abnormalities of the ovarian epithelium were rare in controls (Group A), frequent in the stimulated ovaries group (Group B) and even more frequent in the genetic risk group (Group C).

From these data, a mean dysplasia score was calculated for each group. The mean dysplasia scores of Groups A, B and C were 3.62, 7.51 and 9.55, respectively.

There was a statistically significant difference between dysplasia scores for Group B and controls (7.51 versus 3.62, $p < 0.0002$), and between Group C and controls (9.55 versus 3.62, $p < 0.0001$), but not between Group B and Group C ($p = 0.1$).

Abnormalities were more marked in women with proven BRCA mutations (Group C2: dysplasia score = 11.14) than in women with only a family history of ovarian or breast cancer (Group C1, dysplasia score = 8.1), although this difference was not statistically significant.

Finally, a time-effect was noted in stimulated women: abnormalities were rare within 7 years of stimulation (Group B1, dysplasia score = 1.28), but appeared more frequently after this time period (Group B2, dysplasia score = 11.66); this difference was statistically significant ($p < 0.0001$).

These results are summarised in Table 2, which shows that the number of abnormalities seems to increase with the level of risk.

3.3. Histopathological characteristics of excised material

The histopathological features of the excised tissue from Group A were always benign (Table 3). Likewise, the histopathological features of excised material from Group B were always benign cysts (33 cysts detected by ultrasonography) (Table 3).

Histopathological abnormalities were most often absent in the control group (Group A), except for inclusion cysts which were present in approximately 50% (Table 2). Inclusion cysts were often observed in the stimulated ovaries (Group B) and prophylactic ovariectomies (Group C) groups (Table 2).

The abnormalities most often found in Group B included: epithelial multilayering (66%), tufting (66%), inclusion cysts (63%) and cellular pleomorphism (60%). The rarest patterns were psammoma (8.5%) and stromal hyperplasia (20%).

Finally, in Group C, the most representative histopathological abnormalities were: inclusion cysts (92.5%), tufting (85%), stromal hyperplasia (74%) and epithelial multilayering (67%). According to these differences, a 'profile of dysplasia' can be drawn for each group (Table 2 and Fig. 1).

4. Discussion

The first histopathological observation of ovarian dysplasia was reported by Gusberg and Deligdish in 1984.[9] These authors examined excised material from prophylactic adnexectomies carried out on three women whose monozygotic twins had invasive cancer of the ovary. In all three cases, the ovaries were macroscopically normal. However, microscopic analysis revealed various cytological and architectural anomalies such as surface papillomatosis, inclusion cysts, nuclear pleomorphism, epithelial multilayering and epithelial invaginations. By analogy with other precancerous lesions of the genital tract, the association of these various abnormalities was designated ovarian dysplasia or ovarian precancerous lesions.

Ovarian dysplasia is thus defined by the presence of cytological and architectural changes which are potentially precancerous: multilayering, tufting, papillomatosis, nuclear chromatin irregularity, cellular pleomorphism, nuclear size and loss of polarity. Inclusion cysts, deep epithelial cortical invaginations, psammoma bodies and stromal hyperplasia are histological changes that are often associated with dysplasia and are possibly seen in a 'preneoplastic phenotype'.[9,10] Several histopathological scores have been devised based on abnormalities found in areas adjacent to stage 1

Table 1 – Demographic characteristics of the study population at the time of surgery.

Variable	Control Group A ($n = 62$)	Stimulated Group B ($n = 35$)	Genetic risk Group C ($n = 27$)
Age (years) (range)	42.1 (32–51)	40.7 (26–53)	46.3 (37–62)
Body mass index	22.6	23.4	23.5
Nulliparity	0	7	0
Parity	2.7 (1–4)	1.1 (1–3)	1.9 (1–4)
Use of oral contraception	55 (89%)	28 (80%)	13 (46.5%)
Duration of exposure to oral contraception (months) (range)	58.2 (20–180)	41.2 (4–96)	86.7 (0–240)

Table 2 – Comparison of ovarian dysplasia profiles in the three groups.

	Control Group A (n = 62)	Stimulated Group B (n = 35)	Genetic risk Group C (n = 27)	P*
Epithelial multilayering	27.5	66	67	0.9902
Tufting	37	66	85	0.0657
Surface papillomatosis	24	43	41	0.9051
Nuclear chromatin irregularity	29	46	52	0.7584
Nuclear contour irregularity	19.5	57	63	0.6987
Cellular pleomorphism	34	60	48	0.3582
Increased nuclear size	21	60	11	<0.0001
Epithelial inclusion cysts	50	63	92.5	0.0077
Psammoma	6.5	8.5	44	0.008
Cortical invagination	17.5	31.5	48	0.2854
Stromal hyperplasia	16	20	74	<0.0001

All results shown are percentages.
* P: prophylactic oophorectomy versus stimulated group.

Table 3 – Histopathological features of excised material.

	Group A (n = 62)	Group B (n = 35)	Group C (n = 27)
Pyosalpinx	5	2	0
Endometrioma	12	9	1
Serous cystadenoma	5	6	4
Mucinous cystadenoma	6	2	3
Ovarian fibroma	5	0	1
Follicular cyst	9	10	6
Haemorrhagic cyst	17	5	2
Torsion/ischaemia of adnexum	3	1	0
Normal ovaries	0	0	10

ovarian carcinoma,[11,12] in the contralateral ovary of women with stage 1 ovarian carcinoma[13,14] and in prophylactic oophorectomies for BRCA mutations.[10,15–19]

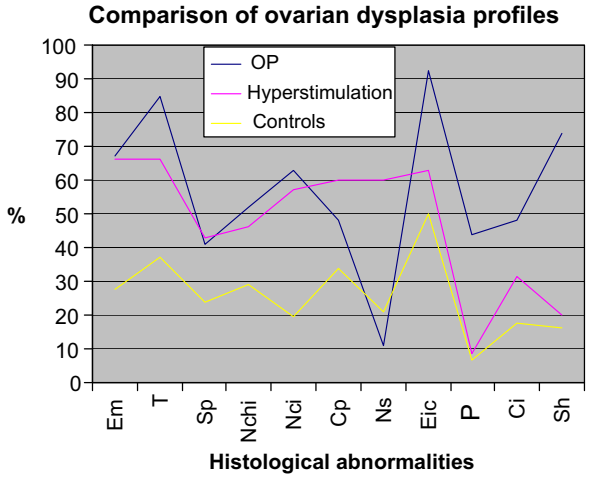

Fig. 1 – Comparison of ovarian dysplasia profiles. *Legend:* Em, Epithelial multilayering; T, Tufting; Sp, Surface papillomatosis; Nchi, Nuclear chromatin irregularity; Nci, Nuclear contour irregularity; Cp, Cellular pleomorphism; Ns, Nuclear size (increased or not); Eic, Epithelial inclusion cysts; P, Psammoma; Ci, Cortical invaginations; Sh, Stromal hyperplasia.

Further evidence for ovarian dysplasia being distinct from cancerous and normal ovarian epithelium was provided by morphometry and nuclear texture analysis.[20,21]

In 1971, Fathalla[6] suggested from the higher frequency of ovarian cancer in nulliparous women that ovulation might be implicated in malignant modification of the ovarian epithelium. Every ovulation breaches the epithelial surface. During healing of the invaginations of the surface epithelium (Photos 1 and 2), cells in the stroma can form inclusion cysts (Photo 3) in a micro-environment in which the epithelial cells are subjected to a paracrine influence (hormonal influence through oestrogens and gonadotrophins), or to the action of cytokines such as interleukins 1 and 6, or to the influence of growth factors stimulating cell proliferation.[22–24]

The prevalence of inclusion cysts in dysplastic ovaries ranges from 62 to 75%.[13–15] This prevalence seems to be directly linked to the total duration of ovulation periods. Mittal and colleagues[14] found two to three times more cases of tubal metaplasia in cortical inclusion cysts in ovaries contralateral to ovarian carcinoma than in normal ovaries.

Conversely, inclusion cysts are rarer in normal ovaries, with a prevalence not exceeding 40%.[25] Although these cysts occur in normal ovaries they may not have the same activity potential. A statistically significant increase in Mib 1 (equivalent to Ki67) has been reported in the dysplastic epithelium of ovaries from patients at risk.[19] Coraksi and colleagues[26] found elevated Ki67 expression (between 17 and 25%) in stimulated

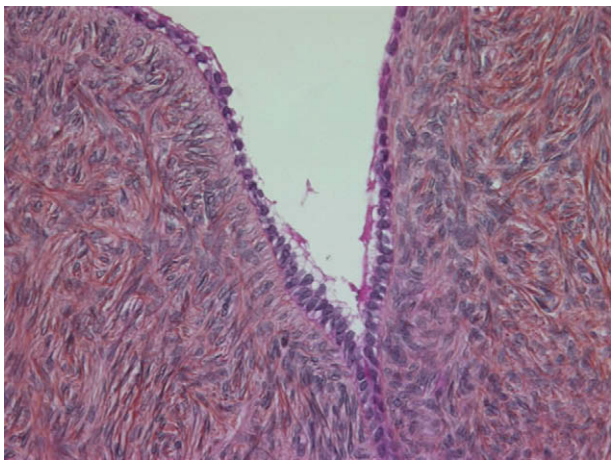

Photo 1 – Cortical invagination and stromal hyperplasia, HES × 20.

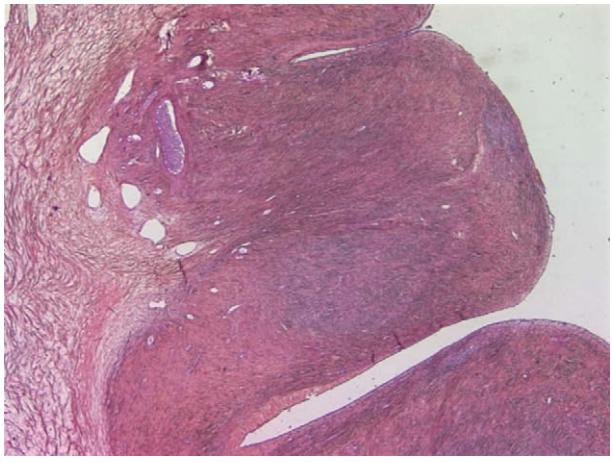

Photo 2 – Cortical invagination, HES × 2.5.

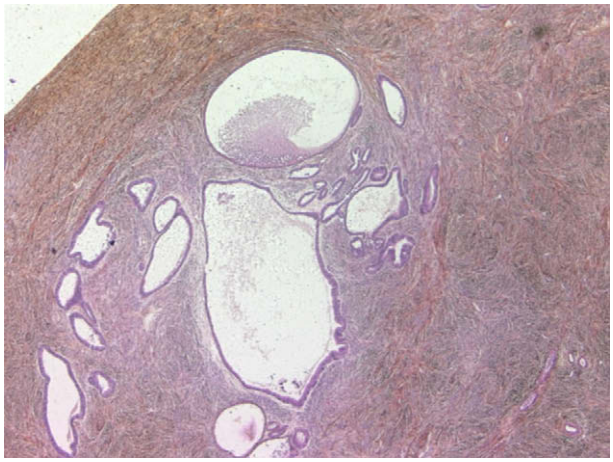

Photo 3 – Epithelial inclusion cysts, HES × 2.5.

and dysplastic ovaries from rats. Likewise, cortical invaginations have been observed in 41–85% of ovarian dysplasias[13–15] and in up to 21% of normal ovaries.[25]

The incessant ovulation theory is consistent with several epidemiological and experimental studies and, as use of the oral contraceptive pill was confirmed to be protective,[4] ovarian hyperstimulation was thought to increase the risk of ovarian cancer. Although these findings were confirmed by several groups,[27–29] they are nevertheless controversial. Our objective was to examine ovaries removed after hyperstimulation to determine whether histopathological abnormalities are present and are comparable to the dysplastic lesions described in ovaries known to be at high risk (contralateral or genetic).

A similar study was undertaken by Nieto and colleagues and their score system (designed for stimulated ovaries)[8] was therefore used in this study, with additional cytological criteria (inclusion cysts, cortical invaginations, psammoma and stroma hyperplasia) also described in a previous study.[18] This was used to develop a scoring system for excised material from prophylactic ovariectomies performed because of genetic risk.

Our results were comparable to those of Nieto, with a significant presence of abnormalities in stimulated ovaries (7.90 versus 5.7 for Nieto, 7.51 versus. 3.62 here).[30] However, the average dysplasia score was lower than that observed in the genetic risk group.

Our findings raise a number of issues. When only patients with the BRCA 1 or 2 mutation were considered, the prophylactic salpingo-oophorectomy score rose to 11.14. This was significantly higher than the score in patients with a family history of ovarian/breast cancer without proven mutation (score 8.1). This finding is of considerable interest, particularly as patients with BRCA mutations in our series had an exposure to protective oral contraception that was nine times longer than patients with a family history only. Ovarian dysplasia in patients with BRCA mutations may be a precancerous lesion phenotype in the light of recent studies which classify ovarian serous carcinoma into low-grade and high-grade[31–33]: this classification considers endometriosis and borderline serous and mucinous tumours to be precursors of low-grade ovarian carcinoma, whereas precursors of high-grade carcinoma include ovarian dysplasias and, as mentioned recently by some authors, dysplasias of the fimbriated portion of the fallopian tube.[34,35]

By separating mutated BRCA patients from non-mutated family history patients in the prophylactic ovariectomy group, the following gradation of ovarian dysplasia score was obtained, which increases according to the supposed increased level of risk:

$$\text{Score}^{\text{exposed}}(7.51) < \text{Score}^{\text{OP with ATCD}}(8.1) < \text{Score}^{\text{OP mutated}}(11.14).$$

This gradation, which parallels that of risk, suggests that these lesions may progress towards neoplastic transformation. The dysplasia profile was different in Groups B and C. Tufting, inclusion cysts, psammoma, cortical invaginations and stromal hyperplasia were more frequent and more severe in genetic risk ovaries. Conversely, cellular pleomorphism and increased nucleus/cytoplasm ratio were more frequent in the stimulated ovaries group. Madhavi and colleagues[36] recently proposed a critical reappraisal of the

possible causal link between induction of ovulation and ovarian cancer. Their conclusion, supported by others,[28,37–40] was that there was a significant association between stimulation of ovulation and borderline tumours. These results can be explained by high 'oestrogen receptor' expression in borderline ovaries (oestrogens stimulate cell proliferation in cells containing oestrogen receptors and gonadotrophin receptors can also be detected in experimentally induced ovarian tumours).[36,40–42]

The different profiles of dysplasia (Fig. 1) observed in genetic risk ovaries and stimulated ovaries could partly explain this difference: hyperstimulation may favour a different evolution (perhaps towards borderline tumours), while ovaries at genetic risk may be more prone to invasive cancers.

Clearly, our results should be interpreted with caution as only patients who had undergone ovarian adnexectomy or cystectomy after hyperstimulation were studied. There were no data concerning other stimulated patients and hence the true prevalence of ovarian dysplasia and cancer after stimulation is unknown. It cannot be concluded therefore that hyperstimulation truly increases the risk of neoplasia. However, our observations are consistent with Fathalla's theory. This makes it difficult to estimate risk factors. In addition, our study is purely observational. A series of stimulated ovaries were found that presented dysplastic lesions, but the progression of these lesions cannot be predicted. The profile of these lesions was different from that observed in prophylactic oophorectomies. Further follow-up studies are therefore necessary to assess the progression of these abnormalities.

In conclusion, the results of this study, based on a retrospective analysis of 35 ovaries or ovarian cysts after stimulation compared with 62 controls and 27 prophylactic ovariectomies performed for genetic risk, confirm the presence of a significant number of histopathological abnormalities after stimulation.

Gradation according to risk level (hyperstimulation <family history of breast or ovarian cancer <BRCA 1 or 2 gene mutation) is consistent with progression of this process towards cancer formation, fully justifying the term 'ovarian dysplasia' or 'ovarian intraepithelial neoplasia' for this group of precancerous abnormalities.[43] Immunohistochemical and molecular studies could offer even more insight into early ovarian carcinogenesis and these studies are currently in progress in our institution.[44]

The fact that the dysplasia profile is slightly different between stimulated ovaries and ovaries at genetic risk is also consistent with previous evidence that hyperstimulation might favour a different evolution (possibly towards borderline tumours).

Finally, from a practical point of view, these findings are an alarm signal: their implications are serious enough to justify an interventional attitude in women presenting with clinical or ultrasound ovarian abnormalities several years after ovarian hyperstimulation.

Conflict of interest statement

None declared.

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