Follicular stimulation for high tech pregnancies: are we playing it safe?

S Fishel, P Jackson

The techniques for in vitro fertilisation have changed and improved at a great rate. The finding that the incidence of pregnancy increases in proportion to the numbers of conceptuses replaced, up to at least three (table I), generated widespread interest in the use of follicular stimulants in the hope of obtaining many follicles and many conceptuses[2] (or, as in the case of some units, oocytes for anonymous donation). Improvements in the efficiency of freezing techniques led to the more general use of these potent follicular stimulants, with the aim of having a stock of frozen conceptuses preserved for the couple.

Almost invariably the minimum "blanket" follicle stimulation is now provided by gonadotrophins (menotrophin), with or without an antioestrogen (clomiphene or tamoxifen). Women with ovulatory problems require more potent follicular stimulants. An outline of the development of such follicle stimulant treatments is shown in the box.

Historical use of follicular stimulants for human in vitro fertilisation

Natural cycle

Antioestrogens (clomiphene citrate, tamoxifen)

Menopausal gonadotrophins (human menopausal gonadotrophin—luteinising hormone : follicle stimulating hormone, 1:1)
- alone
- in conjunction with antioestrogens, or

Pure follicle stimulating hormone
- alone
- in conjunction with human menopausal gonadotrophin

Gonadotrophin releasing hormone agonist (pituitary densensitisation)
- with human menopausal gonadotrophin alone or
- with follicle stimulating hormone + human menopausal gonadotrophin

Department of Obstetrics and Gynaecology, University of Nottingham, Queen's Medical Centre, Nottingham NG7 2UH
S Fishel, PHD, *senior lecturer*
P Jackson, DMLT

Correspondence to:
Dr Fishel.

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Problems with follicle stimulation

The spontaneous surge of luteinising hormone that occurs during the follicular phase of ovulation causes two problems. Firstly, this surge is generally triggered by a single dominant follicle, and at this stage the presence of intraovarian inhibitory factors ensures that there will be fewer mature follicles-oocytes and thus fewer available conceptuses. Secondly, the spontaneous surge of luteinising hormone often triggers ovulation outside "normal" working hours or at weekends, forcing many clinics to abort the treatment cycle. Thus it is often the practice to give the clinician full control of the follicular phase, using gonadotrophin releasing hormone agonists. Super agonists, such as buserelin, are used over a long period (about 14 days) to induce desensitisation of the pituitary gonadotrophes. This effectively reduces basal concentrations of luteinising hormone and inhibits its spontaneous surge for as long as the agonist is given. Ovulation must be induced by using human chorionic gonadotrophin. When such analogues are used, however, high doses of gonadotrophins are required, and extremely potent regimens such as the use of pure follicle stimulating hormone (urofollitrophin) have been introduced to achieve an "adequate" follicular response.

TABLE I—*Incidence of term pregnancies in unstimulated and stimulated cycles after in vitro fertilisation*

Ovarian stimulation	No of patients having replacement	Percentage of term pregnancies per conceptus replaced			
		1	2	3	Combined
Natural cycle[1]	91	10	–	–	–
Clomiphene citrate[1]	387	9	19	16	14
Clomiphene citrate and human menopausal gonadotrophin*	388	14	27	35	30

*data in preparation.

TABLE II—*Examples of ovarian stimulation and ovarian response*

Ovarian stimulants*	No of patients	Mean (SEM) No of ampoules of human menopausal gonadotrophin	Mean (SEM) No of follicles	Mean (SEM) concentration of plasma oestradiol 17β (pmol/l)
Natural cycle	91	0	1	1114 (234)
Clomiphene citrate	387	0	2·4 (0·3)	2191 (73)
Clomiphene citrate + human menopausal gonadotrophin	388	10·0 (0·16)	6·5 (0·15)	5446 (112)
Gonadotrophin releasing hormone agonist + follicle stimulating hormone + human menopausal gonadotrophin	136	15·9 (0·74)	9·0 (0·63)	4352 (22)

*Assuming the induction of ovulation with human chorionic gonadotrophin.

Potential risk of cancer from excessive ovarian stimulation

Although this approach is clearly successful in some practitioners' hands,[3] in many patients the plasma oestrogen concentration and the number of ovulating follicles arising in a single stimulated cycle may be equivalent to the product of up to two years of normal ovulation during the natural menstrual cycle (table II). Excessive oestrogen secretion has been implicated in ovarian, endometrial, and breast carcinoma,[4] and excessive gonadotrophin secretion has been implicated in ovarian cancer. Should we be concerned about such exorbitant ovarian stimulation?

There are three main hypotheses on the aetiology of ovarian cancer. Firstly, epithelial ovarian cancer may be caused by repeated ovulations disrupting the ovarian epithelium and leading to malignant transformation.[5,6] Secondly, persistent stimulation of the ovary by gonadotrophins may have a direct carcinogenic effect or may act through or in conjuction with raised

concentrations of oestrogens.[7,8] Henderson *et al* recently stated that "neoplasia is the consequence of excessive hormonal stimulation of a particular target organ, the normal growth of which is under hormonal control"[4]; oestrogen increases granulosa cell proliferation and hence the frequency of mitotic activity, which may lead to malignant phenotypes.[4,9] Consistent with these two theories are the many observations that increased parity, early onset of menopause, and, to a lesser extent, late menarche and the contraceptive pill, have a protective effect.[10]

The third hypothesis is concerned with the metabolism of chemical carcinogens in the local ovarian environment. Ovarian tumours have been induced in mice by administering carcinogenic polycyclic aromatic hydrocarbons—for example, 7, 12-dimethylbenz (a) anthracene (DMBA),[11] which undergoes hormone dependent metabolism to reactive intermediates in the rat ovary.[12] Bengtsson *et al* recently showed that mono-oxygenase activity in proliferating human granulosa cells caused DMBA to metabolise under the influence of gonadotrophins and raised steroid concentrations.[13] In vivo DMBA and mono-oxygenase activity in human granulosa tissue was increased fivefold in stimulated cycles compared with the activity in other types of cells isolated from human ovarian tissue, whereas granulosa cells obtained during natural cycles had very low values of both. The results of these studies suggest that when granulosa cells are in the phase of rapid growth under gonadotrophin and oestrogen stimulation microsomal cytochrome P450 dependent hydroxylase systems convert DMBA to reactive epoxide intermediates, forming covalent linkages to protein and DNA which may induce follicular cell destruction or transformation to cancer cells.[13]

Even in the most successful centres (as judged by numbers of patients delivering per treatment cycle or per replacement) more than four fifths of patients will not deliver a baby at their first attempt. Most patients make many attempts. In previous generations follicular stimulants (mainly milder stimulants than those available to in vitro fertilisation clinics today) were given to oligomenorrhoeic or amenorrhoeic women—that is, women with low gonadotrophin or oestrogen secretion who ovulate infrequently if at all. Most women now attending high tech centres for in vitro fertilisation, gamete intrafallopian transfer, etc, have normal or regular menstrual cycles between follicular stimulation cycles. Excessive gonadotrophin administration, persistently raised oestrogen concentrations, or many multiple ovulatory cycles may make such women more prone to changes in ovarian tissue than those with ovulatory disorders.

As it is not until the fourth decade that the incidence of ovarian carcinoma can be fully assessed, and since the current methods of potent follicular stimulation have been available and widely used only in the past three years, perhaps a cautious approach with individual stimulation regimens may be wiser than a "blanket" approach, particularly since reports of ovarian carcinoma developing during treatment have already been published.

Reports of ovarian carcinoma during treatment

There are at least three reports of ovarian carcinoma in women treated with follicular stimulants.[14-16] Two of these date from 1982. In the first a 26 year old nulliparous woman had been repeatedly treated for primary infertility with 700 mg clomiphene citrate per cycle, together with human chorionic gonadotrophin. After a treatment cycle she presented with abdominal distension and pain due to ascites and cystic masses in the pelvis.[14] She had severe hyperstimulation induced by clomiphene with subsequent persistent ovarian cysts. After exploratory laparotomy some weeks later, when a more solid abdominal mass was detected, the results of histology showed borderline serous papillary cystadenocarcinoma in both ovaries. Cells in free ascitic fluid showed pleomorphism which suggested malignancy.

In the second report from 1982 a 32 year old woman with primary infertility and oligomenorrhoea was given 1125 IU of follicle stimulating hormone and luteinising hormone per cycle on eight occasions in the first year of treatment.[15] After a two year lapse, treatment was started again using the same dosage for three cycles. During the last cycle the left ovary was noted to be slightly enlarged. One month later a palpable cyst, 4 cm big, was found in the left ovary, and in a further three weeks the patient presented with sudden lower abdominal pain and a tender mass rising to the umbilicus. Examination of the excised ovarian mass showed an endometrial tumour of the left ovary with encroachment of the serosal coat. The uterus showed endometrial hyperplasia and changes associated with a well differentiated adenocarcinoma which extended to the superior parts of the endocervical canal. The tumour had invaded the inner third of the myometrium.

In the third report, in 1987, a 25 year old woman with primary infertility was referred for in vitro fertilisation.[16] She was given 500 mg clomiphene citrate and 225 IU follicle stimulating hormone and luteinising hormone for follicular stimulation. Five days after laparoscopy, at which no oocytes were collected, the patient presented with a non-tender pelvic mass resembling 14 weeks' gestation and persistent lower abdominal pain. At laparoscopy biopsy specimens were taken from the left tube because of the finding of caseous adhesions over a thick walled hydrosalpinx. Histology showed papillary serous adenocarcinoma with extensive infiltration of the stroma. The patient was readmitted for laparotomy and a partially necrotic tumour mass was found affecting both ovaries, the uterus, and the bladder, and filling the pelvis.

Although these cases do not prove a causal link between ovarian stimulation and genital cancer, gonadotrophins (or other associated factors) may be an added stimulus, and ovarian hyperactivity may accelerate ovarian neoplasia. Women who attend infertility clinics because they are nulliparous, infertile, and relatively old are perhaps more prone to develop ovarian tumours,[10] and previously established neoplastic changes may flare up in response to repeated (high dose) follicular stimulation. In all three cases the tumours developed rapidly.

Individualised approach to treatment

In view of these concerns there are several approaches that may be taken to minimise risk. Firstly, if an abnormality is found on a pretreatment scan further investigations should be done before starting follicular stimulation. Then if long term stimulation is required endogenous gonadotrophins and oestrogens could be reduced in the intervening cycles, by using either the contraceptive pill or buserelin. In any year the type of stimulation can be varied so that more potent follicular stimulants are used perhaps twice or three times, with a return to the natural cycle or with antioestrogens alone in the interim.

It is possible to suggest some guidelines on individual suitability for this type of treatment. We assessed the response to stimulation with clomiphene citrate and human menopausal gonadotrophin in 406 patients by weight and age (table III). Fifty seven patients (14%) had an excellent response producing more than eight ovulatory follicles, and these women were significantly younger and weighed less than the 33 patients (8%)

TABLE III—*Age and weight of patient in relation to follicular response*

	No response	No of ovulatory follicles <5	5-8	>8
No of patients	33	122	194	57
Mean (SE) age (years)	34·9 (0·87)*	33·6 (0·42)†	33·2 (0·3)	31·7 (0·57)
Mean (SE) weight (kg)	63·6 (1·43)‡§‖	60·4 (0·75)¶**	58·2 (0·58)	57·2 (1·11)

*p=0·004 compared with >8 follicles.
†p=0·008 compared with >8 follicles.
‡p=0·002 compared with >8 follicles.
§p=0·049 compared with <5 follicles.
‖p=0·002 compared with 5-8 follicles.
¶p=0·02 compared with 5-8 follicles.
**p=0·02 compared with >8 follicles.

who did not respond to stimulation. The degree of response was also related to age and weight. A total of 122 patients had fewer than five follicles. These women were older and weighed more than those with more than eight follicles. There was an inverse relation between the number of ovulatory follicles and weight. An initial analysis of patients who received more potent follicular stimulants shows a similar trend (data not shown). Follicular stimulants for in vitro fertilisation should therefore be given in the first instance at least according to age and weight. Patients should also be assessed individually on their previous response to stimulation and on their endocrinological findings.

Finally, if a cause or link between prolonged severe stimulation and ovarian carcinoma were to be confirmed certain patients who have had many years' stimulation treatment could be offered bilateral oophorectomy once they were in the menopause.

Conclusion

We emphasise that there is no direct evidence to support our hypothesis. We would, however, consider a cautious and individual approach to follicular stimulation, rather than a blanket approach, to be in the patient's best interest. At our clinic patients are carefully assessed before a particular stimulation regimen is decided on.

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MATERIA PARAMEDICA

The first recorded death from asthma? Pliny the Elder and sulphur dioxide

Many asthmatics are highly sensitive to traces of sulphur dioxide in inspired air. As little as one part per million can induce an attack within minutes.[1] In clinical practice this usually occurs during or after ingestion of food or beverages containing permissible concentrations of sulphur dioxide that are harmless to non-asthmatics. Sulphur dioxide is widely used in the food and drinks industries for its properties as a preservative and antioxidant. It has antibacterial and antifungal properties and is used to inhibit oxidative processes associated with food spoilage.[2] When food or beverages containing this gas are being ingested it volatilises and is inhaled.

Variable amounts of sulphur dioxide are present in volcanic emanations. During increased volcanic activity the concentration in the ambient air will depend on distance from the crater, wind direction, and various other meteorological features. Anyone who has stood at the crater's edge of Mount Etna on one of that volcano's livelier days will retain a memory of choking sulphurous gases. In the 1960s one member of a party on Stromboli died from the effects of sulphur dioxide while the others were unaffected (A T Huntington, personal communication). A survey of analyses of volcanic emanations showed that sulphur dioxide exceeded 20% of total active gases in nearly one third of samples.[3]

There is an implied warning here to asthmatics travelling to the vicinity of volcanoes. Presumably the peasantry that densely populates the fertile foothills of many volcanoes has eliminated the genetically predisposed to asthma by a process of Darwinian selection.

Gaius Plinius Secundus (AD 23-79) was one of the great polymaths. He wrote a 37 volume encyclopaedia comprising all then known knowledge, and much else. He played an active role in administration and at the time of his death was commander of the Roman fleet at Misenum at the northern tip of the Bay of Naples. He had adopted the son of his widowed sister, and they are accordingly known to us as Pliny the Elder and the Younger respectively. The two letters of Pliny the Younger (AD 61-113) to Cornelius Tacitus describing the eruption of Mount Vesuvius that destroyed Pompeii in AD 79, and the account therein of his uncle's death, make compulsive reading.[4] The clear and detailed description suggests that Pliny the Elder died in an attack of asthma from inhalation of volcanic sulphur dioxide. The following is adapted from Betty Radice's translation.

My uncle . . . gave orders for the warships to be launched . . . with the intention of bringing help, . . . for this lovely stretch of coast was thickly populated. . . . Ashes were already falling, hotter and thicker. [He told the helmsman] to make for Stabiae [where] he was not yet in danger. [On arrival at Stabiae and] after his bath he lay down and dined. . . . Then he went to rest and certainly slept, for as he was a stout man his breathing was rather loud and heavy and could be heard by people coming and going outside his door. [Because of the worsening situation] he was awakened . . . and joined the rest of the household. . . . My uncle decided to go down to the shore and investigate the possibility of escape . . . he repeatedly asked for cold water to drink. Then the flames and smell of sulphur . . . roused him to stand up. He stood leaning on two slaves and then suddenly collapsed, I imagine because the dense fumes choked his breathing by blocking his airways which were constitutionally weak and narrow and often inflamed. . . . His body was found intact and uninjured.

The Younger Pliny and his uncle both lived at Misenum, and he would have been familiar with his uncle's pattern of sleeping. That his breathing was "loud and heavy" suggests nocturnal wheezing. Snoring is excluded by the absence of any mention thereof (L *sterto*). An idea of how noisy his breathing was on the night of the eruption is suggested by its audibility to people outside the bedroom door. That "his airways were constitutionally weak and narrow and often inflamed" is a remarkably perceptive statement from a 17 year old, and fits in well with modern ideas of long standing recurrent bouts of asthma.—BERNARD J FREEDMAN

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