INDUCTION OF OVULATION

by

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Failure to ovulate is a major problem in reproductive disorders. It may be the result of dysfunction at any level of a complex system including higher centres in the brain, the hypothalamic-pituitary-ovarian axis and the steroid feedback mechanism. It renders the woman infertile which is the prime reason for attempts at restoration of ovulatory cycles.

Anovulation is the basic deficit in the polycystic ovary syndrome sometimes called the Stein-Leventhal syndrome and is followed by amenorrhea, sometimes oligomenorrhea and even apparently regular cycles. Generally, anovulatory menstruation is associated with recognizable irregularity of the cycle and occurs more frequently in the menarche and in the pre-menopausal state. It is likely, that in most instances in women with regular cycles anovulation is sporadic. In primary ovarian failure ovulation has never occurred and in secondary failure ceased definitely.

Anovulation with amenorrhea may be the first and only sign of a pituitary tumor such as chromophobe adenoma. Pituitary destruction, due to various reasons such as infarct necrosis at the time of delivery or thrombosis causes anovulation and amenorrhea, but usually also other signs of grave pituitary insufficiency.

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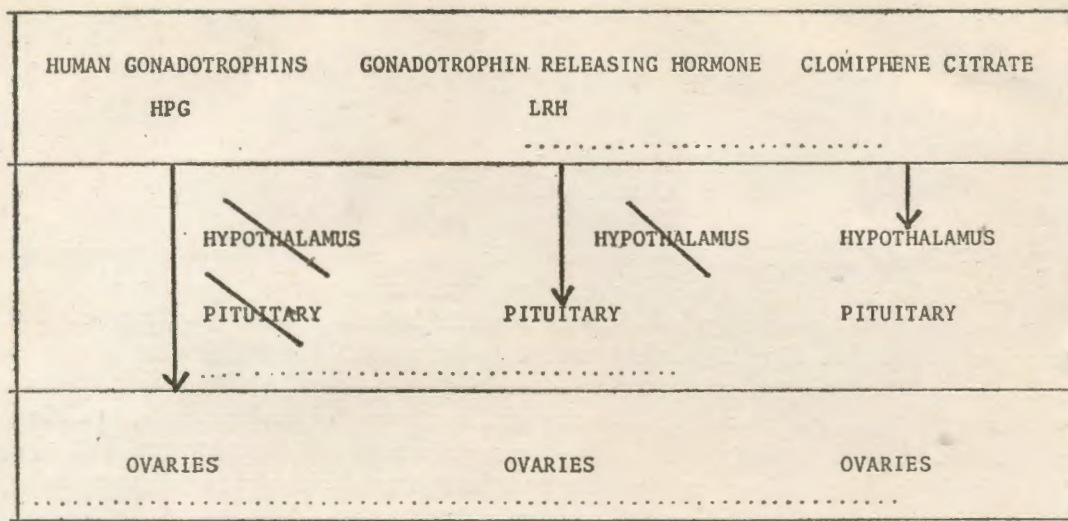
In most cases of anovulation with or without amenorrhea there are no gross organic lesions of the pituitary or the hypothalamus. Ovulation may cease in connection with the discontinuation of oral contraceptive treatment, severe dieting or emotional disturbances, but also without any obvious signs of hypothalamic dysfunction.

A pre-requisite condition for induction of ovulation in women who do not ovulate is the presence of normal ovaries with oocytes and follicles. The lack of oocytes, as in women with primary ovarian failure or with premature menopause, render a treatment impossible.

In principle, there are three approaches to ovarian stimulation for induction of ovulation, i.e. two hormonal approaches with human gonadotropins or gonadotropin releasing hormone (GnRH), and one chemical approach with clomiphene citrate. Human gonadotropins act directly on the ovaries by-passing the pituitary and the hypothalamus while clomiphene requires for its action an intact pituitary and hypothalamus (Fig. 1).

Gonadotropin releasing hormone (GnRH) has recently been isolated from the hypothalamus and synthesized (Schally et al, 1971). It reaches the anterior pituitary via a portal system and releases both FSH and LH. Consequently, it would be an ideal means of stimulating the ovaries in cases of normal pituitary and abnormal hypothalamic function. In women who do not ovulate GnRH have been shown to increase the

Three different approaches to ovarian stimulation.

Fig. 1

release of both FSH and LH from the pituitary, but not enough to stimulate sufficient follicular growth or to induce ovulation of follicles which are primed with human gonadotropins (Nillius and Wide, 1972). The reason for this is not clear. It may be that the pituitary of amenorrhoic women is not adequately adapted to GnRH and following exogenous administration of GnRH releases insufficient amounts of gonadotropins during too short a time. The amount of GnRH may not have been enough in these trials and other forms of administration of GnRH may yield better results.

Human gonadotropins seem to be the panacea for induction of ovulation as they only require normal ovaries. It is possible with human gonadotropins to induce ovulation in hypophysectomized women or in women with a pituitary adenoma or necrosis. However, the possibility of complications such as overstimulation leading to cyst formation or multiple pregnancies is a very real danger to the patient and human gonadotropins should only occasionally be used as the first means of induction of ovulation. Clomiphene citrate is relatively safe to use and when administered in daily doses of 50 to 200 mg for 5 days does not give rise to any serious side effects or complications.

It is by now well established that in order to induce ovulation in the human, gonadotropins from human sources should be used (Gemzell et al, 1958, Gemzell 1955a). They are from two sources: from human pituitaries (HPG) obtained at autopsy, or from the urine of postmenopausal women (HMG). Extracts of different potency and ratio between FSH and LH have been used, and almost all preparations give good results. A widely used preparation of HPG contains 25-30 IU (2nd IRP-HMG) of FSH activity and 20-30 IU of LH (2nd IRP-HMG) per mg with a ratio between FSH and LH of 1:1.

Further purification of this HPG preparation has yielded preparations which

based on biological units were clinically less potent while rather unpure preparations with ratios of FSH and LH of 1:6 gave rather good and consistent results. Consequently, there was little indication that HPG preparations with low LH content were superior to those with high LH contamination and that further purification of the pituitary extract was worthwhile especially when the purification procedure caused loss of biological activity. However, some preliminary results seem to indicate that in women with the polycystic ovary syndrome, who are sensitive to HPG, preparations with low LH content were to prefer rather than those with high LH contamination.

Extract from postmenopausal urine (HMG) (Donini *et al*, 1964) has been used together with HCG to stimulate the ovaries and induce ovulation and corpus luteum formation (Lunenfeld *et al*, 1960). Two commercial preparations are in common use today. They contain about 75 IU of FSH activity per ampoule with a ratio of FSH to LH of 1:1 (Pergonal[R]) or of 1:2 (Humegon[R]).

There has never been any need to use LH from the human pituitary for induction of ovulation, thanks to the luteinizing effect of HCG, which is readily extracted from pregnancy urine. Human pituitary LH does not prevent overstimulation. If it should be used instead of HCG, repeated doses have to be administered, as its biological half-life is shorter than HCG.

Several commercial preparations of HCG are available and in general have the same effect on the primed follicle.

Although pituitary and urinary FSH are chemically different, they seem to have the same clinical effect when preparations with equal FSH activity are used. The two preparations administered alternatively to the same women gave similar response as far as the number of ovulations and pregnancies were concerned and also according to the rise in total urinary estrogen excretion.

Clomiphene citrate exists as two isomers which have been separated as *cis-* and *trans-* clomiphene. Although preliminary data suggest that *cis-* clomiphene is more potent in terms of ovulation induction, the two isomers are mixed in the commercial preparation. The mechanism of action of clomiphene is not clear. Evidence is produced that estradiol uptake by the pituitary and anterior hypothalamus is reduced. It is most likely that the major stimulus for the ovarian response to clomiphene is mediated via the hypothalamus and the pituitary and acts on the release of gonadotropins. Evidence is also available that clomiphene has a direct effect on the ovaries. Administered together with HPG, it increases the sensitivity of the ovaries to HPG. Overstimulation is rarely seen even when doses of clomiphene of 50 to 200 mg were administered for periods not exceeding 5 to 10 days.

Selection of Patients

Pituitary gonadotropin failure is a state which may be seen with an apparently normal pituitary, with a pituitary that is the site of a tumour or necrosis or with a primary abnormality in the hypothalamic region. A common characteristic finding of these patients is the low levels of FSH and LH in serum although normal levels may not exclude a pituitary insufficiency (Wide *et al*, 1973). It is also a common finding of these patients that the serum level of estrogens or the excretion of total estrogens in urine is low indicating inactive ovaries. These patients, without any other symptoms of endocrine

disorders or congenital malformations, constitute the group which is suitable for treatment with human gonadotropins.

An ideal patient for treatment with human gonadotropins is under 35 with non-functioning ovaries, primary or long-lasting secondary amenorrhea, normally developed sex organs and lack of urinary gonadotropins as evidence of pituitary failure. She should be fully investigated, be complaining of infertility, have a normally fertile husband, and show no barriers to conception. Pregnancy should not be contraindicated on medical grounds and there should be no preferable alternative method (Gemzell, 1965b).

Clomiphene citrate has been proven to be an especially useful drug in patients who do not ovulate due to a hypothalamic dysfunction. It is essential that the estrogen excretion is adequate and that the serum-FSH level is within the normal range. There should be no signs or symptoms of any gross pituitary or hypothalamic changes. The only contraindication to treatment is the presence of ovarian cysts or tumours. Clomiphene should further be used in those women who ovulate only two or three times yearly, or in those women who show persistent anovulatory cycles. Women with secondary amenorrhea or oligomenorrhea attributable to a deficient stimulation by gonadotropic hormones from the pituitary or by deranged synthesis of steroid hormones with the ovaries often exhibit an estrogen production in the normal range. For these women, clomiphene is recommended (Greenblatt *et al,* 1970). Women with amenorrhea following the use of oral contraceptives and women with galactorrhea and amenorrhea belong to this group even if their endogenous estrogen production is low (Shearman 1966).

Monitoring Treatment

One of the difficulties in the treatment with human gonadotropins is the individual variation response. There is no fixed dosage schedule for all patients. It has also been apparent that the response of the same patient to the same dose may differ significantly from one cycle to the next. One woman, having aborted seven foetuses after her first treatmnt, had a single pregnancy after her second treatment without any change of dosage. There is also a very small range between a dose that will fail to stimulate the follicles at all and one which produces overstimulation. In order to make the best use of the gonadotropins, and to avoid complications the treatment should be carefully monitored. Thus, the purpose of monitoring gonadotropic therapy is to obtain an ovarian response that compares as much as possible with ovarian changes that take place during a normal spontaneous ovulation and to avoid overstimulation leading to multiple pregnancies or ovarian cyst formation.

The ovarian changes that take place during a normal ovulatory cycle can be recorded by daily determinations in blood or urine of estrogens and progesterone. The estrogens during the follicular phase reflect the maturation of the follicle, progesterone during the luteal phase the activity of the corpus luteum. By comparing changes in levels of estrogens and progesterone of normal ovulatory cycles with those obtained following stimulation with human gonadotropins it is possible to obtain information about the optimal daily dose of human gonadotropin, the number of days this dose should be administered, when HCG or LH administration should be commenced, if and when ovulation took place and the hormonal activity of the corpus luteum.

Treatment

Daily determination of total urinary estrogens (TE) has been used successfully to obtain information about the optimal daily dose of HPG and the number of days this dose should be administered (Brown *et al*, 1968). Increases in plasma progesterone will then confirm that ovulation has taken place and also the activity of the subsequent corpus luteum (Johansson and Gemzell, 1969).

Following an adequate HPG dose there is usually a latent period of 3 to 4 days without any change in TE. Thereafter, TE rises continuously during another 6 day period with a daily increase of about 50 per cent over the previous day. When a suitable level of TE is reached ovulation is induced by the administration of HCG. If the drop in urinary estrogens and the rise in plasma progesterone follow the same pattern as found after a spontaneous ovulation the risk of overstimulation is limited. If, on the other hand, no drop in TE occur and the rise in plasma progesterone is moderate, and a menstrual bleeding occurs less than 10 days after the HCG administration ovulation may be questioned. By 9 days after ovulation the detection of HCG in serum by a radioimmunoassay will confirm conception (Wide and Porath 1966).

There are other criteria such as gross appearance, sialic acid concentration, the arborization pattern and the spinnbarkeit of the cervical mucus and the ability of sperm to penetrate the cervical mucus which can be used to assess ovarian response to HPG. However, they are less reliable than the TE excretion and sometimes more difficult to evaluate. Some women display a close correlation between ovarian response and the karyopycnotic index, whereas others, mainly due to vaginitis and coitus, fail to do so.

Clomiphene therapy is much safer than treatment with human gonadotropins and may not be controlled as strictly. Pelvic examination should be performed before and after each course of treatment in order to exclude those who have enlarged ovaries and eventually rule out a pregnancy before beginning of the next course of treatment. If enlarged ovaries are detected the space of following treatment should be reconsidered. In order to obtain information about when and if ovulation takes place the woman should record her basal body temperature. Eventually, repeated determinations of plasma progesterone can reveal an insufficient corpus luteum.

It has been suggested that for a normal spontaneous ovulation a certain amount of endogenous FSH and LH is released from the pituitary and that in the same woman this amount is rather constant from one cycle to another. The same is true for most anovulatory women who are treated with HPG or HMG but there are a few exceptions. Some anovulatory women treated over periods of several years usually require more FSH with time in order to respond and other women from course to course require different amounts of FSH. As these last women usually show some ovarian activity, it is likely that the difference in requirement of HPG is due to their endogenous gonadotropins which may act against or together with the exogenous ones, or to the status of the ovaries at the beginning of the treatment.

The aim of an HPG treatment of an individual woman is to find the ideal HPG dose that will produce a change in TE and plasma progesterone which approaches as closely as possible the changes found during the normal ovulatory cycle. If this goal is achieved the chances

of a normal single conception will be good, and the risk of overstimulation is negligible.

HPG or HMG have been administered in many different ways which all more or less give good results. Divided doses (Crooke et al, 1966, Cox et al, 1966) or equal daily doses (Gemzell, 1965, 1966, 1970) of human gonadotropins usually give the same rise in urinary estrogens and the same number of conceptions; but it seems that the divided doses are more difficult to control and in general, require larger amounts of the hormones. Ovulation is induced by a rather large dose of HCG which has to be repeated after some days in order to obtain a normal length of the luteal phase. The plasma levels of progesterone should be determined at least 3 times in order to confirm ovulation and control the activity of the corpus luteum.

has not occurred and the patient is not pregnant the dose of clomiphene citrate may be increased to 100 or 200 mg. per day. If there is no response to the 200 mg. dose, treatment is abandoned and eventually, human gonadotropins is substituted. If, however, ovulation is induced following any of these course, clomiphene is continued at the dose level that gave response for another 3 to 4 times or until conception occurs.

Human gonadotropins (HPG) have been used since 1960 in Uppsala in order to induce ovulation in infertile women. Since 1968 daily estrogen determinations have been done in order to monitor the treatment. During an 8 year period (1960-1967) when 290 patients were treated, the pregnancy rate was 45 per cent, the multiple birth rate was 33 per cent and the abortion rate was 28 per cent (Table I). Clinical symptoms of overstimulation

TABLE I

	Primary and Secondary Amenorrhoea	Primary and Secondary Amenorrhoea and "Clomiphene Failures"	Primary Amenorrhoea
	1960-1967	1969-1973	1960-1971
No. of Patients	290	351	15
No. of Treatment Courses	606	759	78
No. of Pregnancies	130	143	21
No. of Singles	63	94	17
No. of Multiple Births	31	20	4
No. of Abortions	36	29	2
Pregnancy Rate	45%	41%	87%
Multiple Births Rate	33%	17%	19%
Abortion Rate	28%	20%	10%

Clomiphene citrate in doses of 60 mg. is given for 5 days starting on the fifth day of the cycle or if no cycle on the fifth day after a bleeding induced by progesterone. If after 4 weeks menstruation

leading to hospital care occurred in less than 2 per cent of the induced cycles. During a second 5 year period (1969-1973) when daily estrogen determinations were done, 351 patients were treat-

ed. The pregnancy rate was about the same or 41 per cent, but the multiple birth rate had dropped to 17 per cent and the abortion rate to 20 per cent (Table I). Only 0.5 per cent of the treatments ended with symptoms of overstimulation. It was apparent that careful monitoring of treatment with daily estrogen determinations lowered the multiple birth rate by half and probably had some effect on the abortion rate. Most of the multiple pregnancies were twins and the cases of overstimulation were mild and did not require hospital care.

The two groups included amenorrhoic women with no or low basic estrogen levels, women with primary amenorrhoea with pronounced estrogen deficiency and a few women (7) who were hypophysectomized due to a pituitary adenoma. All these women were very suitable for HPG therapy and there was no preferable alternative method. It was interesting to notice that 84 per cent of the pregnancies occurred following the first two courses with HPG and an additional 15 per cent became pregnant following the third to the fifth courses. It follows from these results that a woman might be treated 3 times with HPG after which the chance of conception is considerably smaller and an alternative treatment might be considered. It also shows that the selection of patients was of great importance.

Among the women who were treated unsuccessfully with HPG some had husbands with signs of impaired fertility such as low sperm count or increased percentage of abnormal sperms. Some were only treated once or twice and still some showed various reactions such as poor cervical mucus combined with poor sperm penetration that may explain their failure to conceive. Also included in this failure group are some women who ovulated following clomiphene therapy, but did not conceive. Following a succeeding HPG treatment only occasionally did they become pregnant.

A total number of 15 women with primary amenorrhoea were treated during a ten year period (Table I). They were all highly motivated to the treatment and no alternative method was available. All were primed with cyclic estrogen therapy before treatment with HPG. In view of the infantile form of the genital tract of these patients it was surprising to find the high rate of conception (87%) together with a low rate of abortions (10%).

In cases with the Stein-Leventhal syndrome, the treatment with HPG was not encouraging. Many of these patients were "clomiphene failures", and only a few treated with HPG conceived usually following the first or second treatment.

Women with secondary amenorrhoea and normal basic estrogen levels, women with oligomenorrhea and women with anovulatory cycles were treated with clomiphene. The daily dose varied between 50 and 200 mg. administered for 5 days. Ovulation occurred in about 80 per cent, and the pregnancy rate was about 55 per cent. In a few cases following treatment, ovarian enlargement was found, but disappeared after treatment with synthetic gestagens for 3 to 4 weeks. It was found that the same amenorrhoic women who were sensitive to clomiphene often reacted abnormally to HPG and showed symptoms of overstimulation even to small doses of HPG.

Conclusion

Induction of ovulation can be achieved by human gonadotropins (HPG) or clomiphene citrate. So far gonadotropin

releasing hormone, although highly active in releasing both FSH and LH from the pituitary, has been of little use as a therapeutic agent in the treatment of anovulatory women.

As HPG acts directly on the ovaries it has a broader therapeutice use than clomiphene which requires intact pituitary and intact hypothalamus. As HPG treatment is associated with increased risks of complications, clomiphene ought to be used as a first choice.

By careful selection of patients and strict control of treatment the best results are achieved. About 84 per cent of the pregnancies following HPG treatment occur during the first two courses of treatment. The best results were obtained in young women with quiescent ovaries who responded in a similar pattern to each treatment.

References

1. Brown, J. B., MacLeod, S. C., MacWaughton, C., Smith, M. A. and Smyth, B.: J. Endocr. 42: 5, 1968.

2. Cox, R. I. and Cox, T. L: Lancet 2: 888, 1966.

3. Crooke, A. C., Butt, W. R., Bertrand. P. V.: Acta Endocr. (Kbh.) Supp. 111, 1966.

4. Donini, P., Puzzoli, D. and Montezemolo, R.: Acta Endocr. (Kbh.) 45: 321, 1964.

5. Gemzell, C. A.: Recent Prog. Horm. Res. 21: 179, 1965a.

6. Gemzell, C. A.: Proc. 2nd International Cong. Endocr. Part II Exc. Med. Found. Amsterdam, 1965-1966.

7. Gemzell, C. A.: Int. J. Gyn. and Obst. 8: 590, 1970.

8. Gemzell, C. A., Diczfalusy, E. and Tillinger, K. G.: J. Clin. Endocr. Metab. 18: 1333, 1958.

9. Greenblatt, R. B., Gambrell, R. D., Mahouh, V. B. and Scholer, H. F. L. Nobel Symposium 15, Stockholm 1970.

10. Johansson, E. D. B. and Gemzell, C. A.: Acta Endocr. (Kbh.) 62: 89, 1969.

11. Lunenfeld, B., Menzi, A. and Volet, V. Acta Endocr. (Kbh.) Suppl. 51: 587, 1960.

12. Nillius, S. J. and Wide, L.: J. of Obst. and Gyn. Britt. Comm. 79: 874, 1972.

13. Roos, P. Acta Endocr. (Kbh.) Suppl. 131, 1968.

14. Schally, A. V., Arimcra, A., Kastin, A. J., Matsuo, H., Baba, Y., Redding, T. W., Nair, R. M. G., Debeljuk, L. and White, W. F.: Science, 173: 1036, 1971.

15. Shearman, R. P.: Lancet, 2: 1110, 1966.

16. Wide, L. and Porath, J.: Biochem. Biophys. Acta (Amst.) 130: 257, 1966.

17. Wide, L., Nillius, S. J., Gemzell, C. A. and Roos, P. Acta Endocr. (Kbh.) Suppl. 174, 1973.