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Ovarian stimulation with clomiphene and/or human menopausal gonadotropin (HMG) for in vitro fertilization (IVF) and embryo transfer (ET)

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Key words. In vitro fertilization; ovarian stimulation; clomiphene; human menopausal gonadotropin; follicle; oocyte.

The pregnancy rate in IVF-ET programs appears to be directly proportional to the number of concepti transferred[5]. Therefore, an essential prerequesite for ultimate success is the recruitment and maturation of an adequate number of oocytes. To accomplish this aforementioned goal three principal stimulation programs have evolved. 1. Clomiphene only, 2. Clomiphene plus HMG in varying modifications and 3. HMG only.

At its inception our program admitted patients with unstimulated normal menstrual cycles. Due to an unacceptably low success rate we changed to ovarian stimulation with clomiphene shortly thereafter.

As soon as the Australian group of The Royal Women's Hospital in Melbourne had reported on increasing pregnancy rates using HMG in addition to clomiphene[11] we introduced the clomiphene-HMG protocol of McBain et al.[12]. Finally, we stimulated with HMG only according to the Norfolk protocol[7] primarily in those patients who did not respond properly to clomiphene or clomiphene-HMG.

The purpose of this paper is to present our experience with the above mentioned stimulation protocols in regard to hormone parameters as well as to fertilization and pregnancy outcome.

Number of follicles, oocytes and fertilizations

McBain and co-workers found a disparity between the number of follicles seen at laparoscopy and the greater number observed ultrasonically earlier in the cycle when

Table 1. Different stimulation protocols and reproductive performance

Number of	Clomiphene only day 5–9 n = 35	Clomiphene/HMG (McBain) start day 3–4 n = 37		Clomiphene/HMG (McBain) start day 5 n = 214		HMG (Norfolk) start day 2–3 n = 27
Follicles	2.1 ± 1.1***	3.5 ± 1.9	n.s.	4.1 ± 2.1	n.s.	4.6 ± 2.8
Oocytes	1.4 ± 0.9***	2.8 ± 2.0	n.s.	3.4 ± 2.1	n.s.	4.2 ± 3.2
Fertil. oocytes	0.7 ± 0.8**	1.8 ± 1.9	n.s.	1.8 ± 1.8	n.s.	1.8 ± 2.1
Pregnancies (%)	3 (8.6)	7 (20.6)		29 (13.6)		5 (18.5)

** $p < 0.01$; *** $p < 0.001$; n = 313.

only clomiphene was used for ovarian stimulation[12]. They concluded that the smaller follicles would fail to continue their growth, due to a lack of available FSH causing these follicles to become atretic. They started to use HMG after clomiphene administration to secure the continued healthy growth of follicles which would not otherwise have been available for oocyte collection.

This addition of HMG did indeed result in an increase of the number of follicles, oocytes and pregnancies[8]. Our experience demonstrated similar results, as is shown in table 1.

An even higher number of follicles and oocytes was found when HMG only was used, following the Norfolk protocol. However, the fertilization and pregnancy rate was the same as with McBain's stimulation (table 1). Since it was the addition of HMG to clomiphene which resulted in an increase of oocytes, fertilizations and pregnancies, we wondered whether this success was merely due to the dosage of HMG. In fact, the number of follicles and oocytes as well as the fertilizations were related to the HMG dosage but, surprisingly enough, the pregnancy rate was not (table 2, a–d).

Jones et al. have demonstrated different responses of the ovaries to the same dosage of HMG; they found high, normal and low responding ovaries[9]. Our protocol is based on response modulated HMG dosage determined by daily patient monitoring of serum estradiol (E_2) and ultrasonic folliculometry. Accordingly, our low responders received a higher dosage of HMG than the normal and high responders. Nonetheless, the pregnancy rate was lower for the low responders. This supports the conclusion that the response of the ovaries is an intrinsic one in regard to a given treatment cycle. Therefore the pregnancy rate cannot be increased simply by increasing the dosage of HMG[10].

We can suggest still another explanation for the lower pregnancy rate despite higher fertilization rate if we consider that the stimulation with HMG might have been excessive in some cases.

According to our present understanding of follicular steroidogenesis, FSH causes an increase of FSH and E_2 receptors in the granulosa of the follicles while E_2 in turn causes an increase of LH receptors[13]. According to this self-priming mechanism an overdosage of FSH could possibly enhance the steroidogenesis to such extent that it might result in a premature luteinization of the granulosa. For the timely detection of such an event progesterone (P) determinations would be of some importance.

The role of progesterone determinations for the elucidation of follicular maturity and dysmaturity (pre- and postmaturity)

In figure 1 the profiles of E_2 and P of IVF conception cycles are shown. Ovulation induction has been differentiated depending upon whether the ovulation was induced exogenously by administration of human chorionic gonadotropin (HCG) or endogenously by an increase of LH. These profiles in relation to the day of HCG administration of LH increase (day 0) clearly demon-

Table 2a. HMG-Dosage and number of follicles; Stimulation clomiphene/HMG (McBain)

Number of HMG amp.	Number of treatments follicles 1–4	follicles 5–20	N
2–6	90 (78%)	26 (22%)	116
7–25	68 (59%)	48 (41%)	116

Chi^2: 9.6; $p < 0.01$.

Table 2b. HMG-Dosage and number of oocytes

Number of HMG amp.	Number of treatments oocytes 0–4	oocytes 5–20	N
2–6	92 (87%)	14 (13%)	106
7–25	70 (64%)	40 (36%)	110

Chi^2: 15.4; $p < 0.001$.

Table 2c. HMG-Dosage and fertilization rate

Number of HMG amp.	Number of treatments fertil. oocytes 0–2	fertil. oocytes 3–9	N
2–6	88 (81%)	21 (19%)	109
7–25	73 (64%)	41 (36%)	114

Chi^2: 7.7; $p < 0.01$.

Table 2d. HMG-Dosage and pregnancies

Number of HMG amp.	Total pregn.	Term. pregn.	Abortus	Extraut. pregn.	Biochem. pregn.	N
2–6	22 (20%)	13 (12%)	2 (2%)	1 (1%)	6 (5%)	110
7–25	13 (11%)	8 (7%)	3 (2%)		2 (2%)	119
					n.s.	229

Chi^2: 3.6.

strate that an endogenous LH increase is paralleled by a slight P increase and a slight E_2 decrease. On the next day P is significantly higher in LH cycles compared to HCG cycles, evidently because the LH increase was earlier than the HCG administration. If, in addition to conception cycles, non-conception cycles are included, our data more clearly demonstrate that P is significantly higher on the day of the LH surge than it is on the day of HCG administration (table 3). Both the E_2 and P profiles are quite the same as in normal cycles except that E_2 levels are higher. HCG cycles, on the other hand, are quite different in that E_2 continues to rise on the day after HCG while the rise of P is less marked.

Even before an endogenous LH increase the P base line levels correlate to a certain extent with the number of follicles. This is obviously more accurately demonstrable in patients with a large number of follicles (table 4). This table also demonstrates that those patients with few follicles whose P levels exceed the baseline levels did not conceive, suggesting that premature luteinization is inconsistent with normal implantation. Since the group of patients with increased P levels but few follicles had not received significantly more HMG than the other patients we can assume that it is primarily the response of the patients rather than the HMG dosage which is responsible for the premature luteinization.

The role of estrogen determinations for the monitoring of follicular growth and development

Johnston et al. demonstrated clearly that there is no difference between conception and non-conception cycles in the E_2 profiles of clomiphene-HMG stimulated patients[8]. Our data support this conclusion. However, the role of E_2 measurements should not be underestimated because they provide us with a daily indication of ovarian response reflecting the functional activity of the dominating follicles. Falling E_2 levels might be indicative of degeneration or atresia of follicles, warranting cancellation prior to oocyte retrieval, except during an endogenous LH increase where falling estrogens reflect a shift of steroidogenesis in the direction of P production (fig. 1).

On the other hand, the value of E_2 measurements for deciding whether or not follicles are mature enough to induce ovulation with HCG is clearly less important than is the measurement of the follicular diameter by ultrasound, because our data demonstrate that the patients with larger follicles had a greater tendency for implantation (table 8).

The role of follicular measurement by ultrasound

Initially, to determine the efficacy of folliculometry by ultrasound we measured the follicular size on the day of the LH surge in various conception cycles. For example, in patients who had undergone artificial insemination by donor sperm (AID) the dominant follicles had a mean diameter of 24.4 ± 3.0 ($n = 15$) on the day of the LH surge. Most of these patients were on clomiphene but some were unstimulated. On the other hand in IVF conception cycles after single embryo transfer the ultrasound scan on the day of ovulation induction with HCG revealed a mean diameter of 22.8 ± 1.8 mm. In contrast to the conception cycles described above, patients with single embryo transfers without implantation had a mean follicular diameter of only 20.2 ± 4.1 (table 8). Although there are reports on pregnancies after IVF-ET having resulted from immature oocytes harvested from small follicles, the overall pregnancy rate seems to be higher when mature oocytes from large follicles are used for IVF.

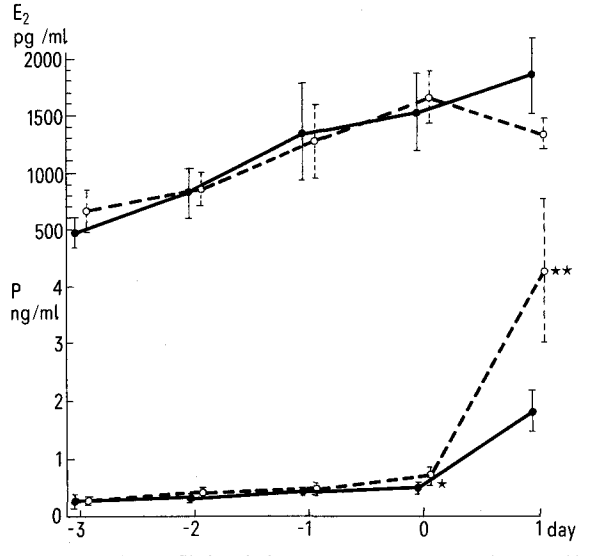

Figure 1. E_2 and P profile in relation to day of HCG or LH increase (day 0) (means ±SD); ○ – – ○, endog. LH increase; ●—●, HCG; * $p < 0.06$; ** $p < 0.025$.

Table 3. Progesterone on day of HCG or LH increase

	Number of treatments		
	Progesterone < 0.6 ng/ml	Progesterone ≫ 0.6 ng/ml	Total
LH	20	26	46
HCG	94	29	123

Chi^2: 16.5; $p < 0.001$.

Table 4. Number of follicles and progesterone on day of HCG*

Number of follicles	Number of treatments/pregnancies (%)	
	Progesterone < 0.7 ng/ml	Progesterone ⩾ 0.7 ng/ml
1–5	79/15 (17.9%)	10/0 (0%)
6–20	27/6 (18.2%)	10/3 (23.1%)

Chi^2: 4.84; $p < 0.05$; *endogen. LH increases excluded.

Table 5. Number of HMG-ampoules in relation to progesterone and number of follicles*

Number of follicles	HMG-ampoules per treatment		
	Progesterone < 0.7 ng/ml		Progesterone ⩾ 0.7 ng/ml
1–5	6.7 ± 5.2, $n = 79$, $p < 0.001$	– n.s. –	9.5 ± 10.0, $n = 10$, n.s.
6–20	12.9 ± 8.9, $n = 27$	– n.s. –	11.3 ± 6.8, $n = 10$

n.s.

*on day of HCG, endogen. LH increases excluded.

The duration of follicle and oocyte maturation

There appears to be a relative consistency of the follicular phases of the individual menstrual cycle in the vast majority of women. Accordingly we adapted the clomiphene stimulation to the patient's cycle length[12]. Women with cycles of 28 days or more started clomiphene on day 5, those with cycles of 26 days started on day 4, and those with cycles of 24 days started on day 3. Figure 2 illustrates the distribution of the induction days, i.e. days of HCG administration or endogenous LH surge subdivided into the various stimulation protocols.

In figure 3 the induction days are synchronized to the first day of stimulation. It is evident that all the protocols show a Gaussian distribution in which the majority of patients have their ovulation induction on days 9 and 10 of stimulation. It should be noted that in the Norfolk protocol the same distribution as described above was shown, notwithstanding the fact that the individual cycle length was not taken into consideration and all the Norfolk patients received their HMG on day 2 or 3 of the cycle.

When the percentage of pregnancies in relation to the various ovulation induction days is examined, it is evident that the pregnancies are distributed differently, as 2 maxima are seen. The first maximum appears on days 7 and 8 of stimulation and the second one on days 13 and 14. If we neglect the second one because of the low number of treatments which have come as far as days 13 and 14 of stimulation, it is still true that the distribution is skewed to the left and that we observe a maximum of normal term pregnancies when ovulation induction occurs on day 8 of stimulation.

These data seem to support the results of the Norfolk group[9] who found that the high responders, i.e. patients whose follicles grow rapidly after stimulation, have a higher pregnancy rate. In our study the largest group, namely the clomiphene-HMG group with clomiphene started on day 5, also shows quite clearly the above men-

Table 6. Cycle day of LH increase or HCG administration and pregnancy outcome

	Treatments/pregnancies (%)		
	Cycle day 8–13	Cycle day 14–19	Total
LH	35/3 (8.6)	34/5 (14.7)	69/8 (11.6)
HCG	159/24 (15.1)	85/12 (14.1)	244/36 (14.8)
			313/44 (14.1)

Treatment's $chi^2 = 4.7$; $p < 0.05$.

Table 7. Number of follicles on day of LH/HCG

	Number of treatments/total pregn./term pregn. (%)		
	Follicles 0–4	Follicles 5–20	Total
LH surge	56/ 6/ 4 (7.1%)	13/ 2/0 (0.0%)	69/ 8/4 (5.8%)
HCG adm.	169/21/14 (8.3%)	75/13/9 (12%)	244/34/23 (9.4%)
			313/42/27 (8.6%)

Treatment's $chi^2 = 3.8$; $p < 0.06$.

Table 8. Follicular diameter in patients with single fertilizations and transfers on day of HCG

	Not pregnant	Term pregnancy
Follic. diam. (mm)	20.2 ± 4.1	22.8 ± 1.8
n	36	10

$p < 0.06$.

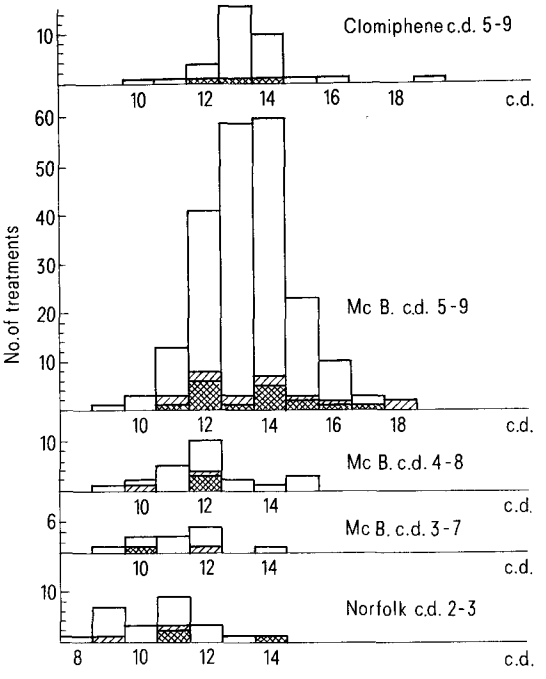

Figure 2

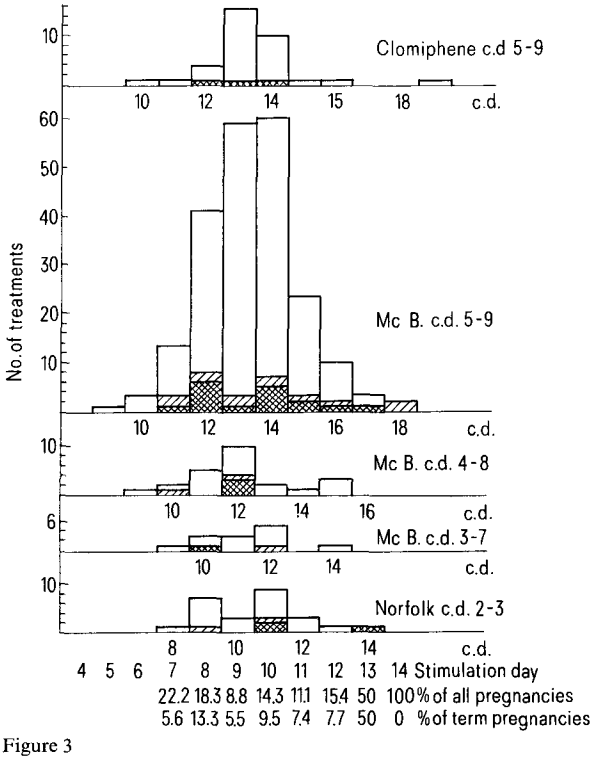

Figure 3

Figures 2 and 3. Day of HCG or LH increase and pregnancies; ⊠ = Term pregnancy; ▨ = Preterm pregnancy; Mc B., Mc Bain's stimulation (clomiphene + HMG); c.d., cycle day.

tioned two-peaked pregnancy profile in contrast to the normal distributed profile of the treatments (fig. 3). The first peak on day 8 would correspond to the high responders described by Jones et al.[9] with their higher pregnancy rate.
It seems, therefore, that the response theory is not only valid for the HMG-only stimulated patients but also for the clomiphene-HMG stimulated patients.

Ovarian stimulation and the endogenous LH increase

Edwards and Steptoe reported on a higher pregnancy rate in clomiphene-only stimulated cycles when the timing of the oocyte retrieval was performed according to the midcycle LH increase[4]. However, when that same group had combined clomiphene with HMG the pregnancy rates decreased in those cycles with an endogenous LH increase[14]. Our data support these findings as can be seen in table 6. When we examined the cycle days of LH surge and HCG administration we found that there was only a little disparity among patients who exhibited an LH increase on day 13 or before and those who exhibited the LH surge on day 14 or later. This is in contrast to the findings in the HCG group, in which $^2/_3$ of patients had received HCG before day 14 (table 6). Interestingly, the pregnancy rate was higher when the LH surge came later, whereas the pregnancy rate was slightly lower when HCG was given later.
Comparing the number of follicles with the LH and HCG groups a low number of follicles in the LH group is observed compared to the HCG group (table 7). In those 13 patients in whom the LH surge occurred in the presence of more than 5 follicles, no term pregnancy resulted. We may conclude from tables 6 and 7 that an endogenous LH surge only indicates a full maturity of follicles and a greater likelihood of pregnancy in those patients with fewer follicles (maximum 5) and whose endogenous LH surge does not occur before the usual ovulation time.
In the light of this we may postulate that ovarian stimulation by clomiphene and/or HMG may be viewed not only as a treatment but also as a provocation test for the functional integrity of follicles.

Discussion and concluding remarks

Stimulation of normal women with normal cycles with clomiphene and/or HMG increases their number of growing follicles and the number of oocytes which ovulate, but does not necessarily increase the pregnancy rate in an IVF/ET program unless certain guide-lines are followed.
Although there is a dose-response relationship between HMG and the number of fertilized oocytes, a clear-cut relationship with the pregnancy rate is missing or is even inverse (table 2d). Follicular steroid estimations after clomiphene and/or HMG stimulation have shown an increase number of follicles with hormone concentrations deviating from those of follicles of normal unstimulated cycles[15]. The most common deviations noted were increased androgen and decreased progesterone levels.
Although these findings might be suggestive of immaturity[6] some oocytes of these follicles did lead to pregnancies[15]. On the other hand we could not achieve pregnancies when there were only a few follicles, and when progesterone levels were rising without a preceding LH rise (table 4). In contrast to the normal preovulatory situation this kind of luteinization seems to reflect dysmaturity (pre- or postmaturity) rather than maturity of the follicles and oocytes. It remains to be determined whether this is the result of an overdosage of clomiphene and/or HMG, or whether this is a manifestation of primarily abnormal follicles. At the present time our data support the latter conclusions since the patients with abnormal luteinization did not receive significantly more HMG than the other patients (table 5).
While in the normal cycle the LH surge is a marker for follicular maturity, indicating impending ovulation, the role of the LH surge in stimulation cycles with many follicles is much more complex.
In our opinion it is not possible to explain the endogenous LH increase without taking into consideration the concept of ovarian inhibin[1,2]. According to this theory the granulosa produces proteinlike substances, called inhibin, which depress FSH and to a smaller extent LH levels. In parallel with luteinization and consequent progesterone production of the granulosa, inhibin decreases, thereby prompting the pituitary to release LH and FSH[3].
In the normal or clomiphene-only stimulated cycles we generally find 1, 2 or 3 dominant follicles while the rest of the follicles are very immature or atretic. In clomiphene/HMG or HMG-only stimulated cycles, however, we generally find not only more follicles but also a greater variation of follicular maturity. Thus, while the most mature follicles create a decrease of inhibin during luteinization the less mature follicles still produce enough inhibin to suppress the FSH and LH increase. Therefore it is unlikely that the overall inhibin would be low enough to allow an LH and FSH surge to occur until the luteinization of all the follicles is completed.
By this time the largest follicles and their oocytes will already have become postmature.
This is of course detrimental in terms of fertilization and embryo development, because at present we have no way of treating dysmature oocytes. However, if HCG is administered just before the most mature follicles have begun to undergo luteinization there is a possibility for fertilization and subsequent transfer for all the oocytes. This possibility even includes immature oocytes because these oocytes may effectively be brought to full maturity by preincubation[16]. This might explain why in patients with many follicles the pregnancy rate was somewhat higher when the HCG was given before day 14 of the cycle (table 6).
Successful implantation is the ultimate goal of every IVF/ET program. In order to accomplish successful implantation it is imperative to develop stimulation techniques to achieve oocytes of high quality. In this presentation we have tried to concentrate on stimulation and monitoring in regard to implantation failure and success and the final conclusions drawn can be listed as guide-lines as follows:

1. Follicular growth and development should be monitored on a daily basis by ultrasound, estrogen, progesterone and LH determinations.
2. The individual response of the patients to stimulation should be taken into consideration when determining the dosage of the stimulating agent.

3. To avoid an overdosage, only that dosage of the stimulating agent which ensures the continued growth and development of the primary cohort of follicles should be administered.
4. Caution should be exercised with endogenous LH increases. It is best to cancel treatments when an LH surge occurs before the usual ovulation time of the patient and when the dominating follicle(s) have not reached the size of 21 mm or more.
5. The treatment should be cancelled when progesterone rises without an LH surge and the follicle number is low (below 6).
6. In any cycle in which an LH surge occurs in the presence of more than 5 follicles the treatment should also be cancelled.
7. HCG should be administered when the dominating follicle is 21 mm or larger before progesterone has increased significantly.

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Extracorporeal fertilization of human oocytes and their replacement; suggested simplifications

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Introduction

Extracorporeal fertilization of human oocytes and intrauterine replacement are now accepted techniques which are offered to infertile couples all over the world[2]. The new techniques may well within a decade more or less totally replace surgery of the fallopian tubes and also be an attractive new technique to overcome certain male infertility factors[6]. For the time being, though, they are mastered by only few centers and this is surprising, taking into consideration the apparent simplicity of the techniques. In the following we would like to stress certain simplifications which can be applied without decreasing the 'success rates' in the various parts of the technique. In the following a brief inventory of the various steps of a procedure is made, finishing with a list of suggested simplifications for the in vitro fertilization–embryo transfer (IVF/ET) technique.

Natural or hormone stimulated cycles

During the last years most groups working in this field of research have tended to abandon the natural cycle in favor of stimulated cycles[7]. Clomiphene alone in oral