Programmed oocyte retrieval

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Summary: Several methods of facilitating the work schedule for in vitro fertilization programs have been proposed.

LH-RH agonists offer the possibility of manipulating the day of hCG injection and consequently the day of ovum pick-up. Moreover, after desensitization, follicles are contemporarily stimulated and a higher number of mature oocytes are retrieved.

The same objective of follicle synchronization can be obtained with progestins, which have the advantage of minor cost and less adverse effects. The use of oral contraceptives offers similar results to those obtained with progestins.

The best choice in work programming appears to be a "monitorized programmed oocyte retrieval" with the use of progestins and LH-RH agonists.

Key words: In vitro fertilization; Programming; Progestins; LH-RH agonist.

In Vitro Fertilization (IVF) and Gamete Intrafallopian Transfer (GIFT) are considered as successful and well established techniques for the treatment of infertility. Nevertheless several problems concerning the work schedule may occur in IVF clinics.

Since 1978 when Edwards and Steptoe ([1]) obtained the first success with pregnancy and delivery from an embryo grown in vitro, a precise synchronization with ovulation in ovum pick-up appeared to be a priority in assisted procreation.

A natural cycle was monitorized for that first "test-tube baby".

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In a spontaneous cycle the endogenous Luteinizing-Hormone (LH) surge and the ovulation cannot be exactly predicted and follicle aspiration must be performed only when LH increase has been demonstrated. Thus, with an endogenous LH peak, a repeated and continuous monitoring for the detection of the LH surge is necessary and ovum pick up must be scheduled between 30 and 36 hours later. This might lead to a follicle aspiration performed during the night causing inconvenience to patients, medical staff and people working in the operating theatre, particularly in district hospitals ([2]).

Immediately after these first experiences, pregnancy rates rapidly increased with the use of gonadotropins and the growth of multiple follicles. But menotropins have also been demonstrated as inhibitors of endogenous LH peak in 80% of patients undergoing Controlled Ovarian Hyperstimulation (COH) ([3]).

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Because of these reasons, human Chorionic Gonadotropin (hCG) has been administered in the periovulatory time: 1) to guarantee the presence of an LH surge, possibly inhibited by menotropins, and, at the same time; 2) to initiate a some kind of "Programmed Ooocyte Retrieval" (POR). In fact the time of hCG injection may be manipulated in order to program the follicle aspiration in a suitable moment of the day.

This method allowed the facilitation of the work schedule in operating theatres, avoiding oocyte retrievals during nights and holidays.

Since 1984 detailed studies on POR were carried out with the purpose of reducing patient stress and costs, possibly improving results in terms of pregnancy rates.

Different drugs have recently been experimented, demonstrating effectiveness and simplicity in their use. They are further reviewed.

LH-RH ANALOGS

Luteinizing-Hormone Releasing-Hormone (LH-RH) analogs are synthetic derivatives of the hypothalamic hormone with high affinity for the pituitary LH-RH receptors.

They are characterized by a very low half life and their continuous administration causes a transient increase of gonadotropins due to their release by the pituitary gland and then, after 5-6 days, determines a dramatic reduction in pituitary secretion of biologically active LH and Follicle Stimulating Hormone (FSH). This, in turn, causes a decrease in ovarian follicular activity and a decrease in estradiol (E2), androgen and progesterone (P) production ([4]).

Such effects are temporarily related to the analog administration, causing a "reversible medical hypophysectomy" by desensitization of the pituitary LH-RH receptor. The interruption of the administration restores the pituitary function.

Analogs were utilized initially in the treatment of leiomyomata uteri, endometriosis, hyperandrogenism and dysfunctional uterine bleeding to induce a transient hypogonadal state.

In assisted reproductive technologies a medical suppression of pituitary function has been used to optimize the COH in patients with repeated failures to induction of ovulation ([5, 6, 7]).

Common problems which arise during COH include: 1) a premature increase in endogenous LH secretion; 2) a follicular asynchrony, with a development of a single dominant follicle and 3) a poor E2 response to a regular exogenous gonadotropin stimulation.

In approximately 20% of women receiving gonadotropin stimulation a marked premature rise of endogenous LH secretion is demonstrated ([4]), which represents a detrimental effect on the oocytes' capacity for fertilization. The development of a dominant follicle is also an undesirable situation in induction of a multiple follicle growth, while poor estradiol response, depending on the patient's age, PCO or other unclear pathologies require higher doses of gonadotropin for the development of an adequate follicular response.

Analogs generate a dramatic reduction of the endogenous gonadotropin support, suppressing ovarian activity until menotropins are administered. The "temporary medical castration" can prevent all the above mentioned problems and moreover results demonstrate a higher success rate after desensitization and induction of ovulation with exogenous gonadotropins than by a regularly induced follicular development ([8, 9]).

The ovarian suppression and the hormonal status deriving from a total absence of endogenous gonadotropins and sexual

hormones can be protracted for an indefinite period of time and induction of ovulation with menotropins can be initiated at any time.

During the stimulation, endogenous LH and FSH remain at minimal levels insufficient to produce any effect. Multifollicular development and estradiol rise are purely the consequence of the exogenous administration of gonadotropins. When follicle diameter is considered adequate for a complete maturation of the oocyte and E2 level is consistent with the ultrasound finding, hCG is administered and ovulation occurs 34-36 hours later. The analog is maintained until hCG is given to prevent a pituitary release of LH in the periovulatory period.

These concepts are of essential importance for understanding the utilization of LH-RH analogs in a POR.

Firstly menotropins can be started independently of the day of the cycle. This would suggest the possibility of programming exactly the day of ovum pick-up by varying only the duration of the analog administration before the gonadotropin therapy, but Meldrum [6] evidenced a too wide variability in the length of stimulation between patients. So the number of days of treatment required is unforeseeable.

Secondly, and of major benefit for POR, analogs offers the possibility of manipulating the day of hCG injection and consequently the day of ovum pick-up: oocyte retrievals can be delayed by 24-48 hours to allow surgeries to be performed only on weekdays. Abdalla [10] studied the effect of such a delay on results of IVF and GIFT. Comparisons with "not-delayed" cycles demonstrated in the study group a significantly higher number of oocytes collected and a higher percentage of mature eggs.

Embryos obtained and transferred were also more numerous and, finally, pregnancy rate in the "delayed group" was 38.5% versus 16.9% in the control group. Sathanandan [5] suggested that a more prolonged stimulation in a desensitized patient would promote the maturation of a greater number of follicles. A higher fertilization rate was found in that study and the result was attributed to the recovery of more mature oocytes which generated more embryos for transfer.

Therefore the delay of hCG may be important either for a programmed cycle, to schedule ovum pick-up during weekdays, or for optimizing results of assisted conceptions.

Analogs have been also utilized by Devroey [11] to synchronize donors' and recipients' cycles in an oocyte donation program. The suppression of the donor was maintained until the first day of the recipient's substituted cycle. Synchronization in an oocyte donation program has been also achieved by Norethisterone, but in that case the occurrence of an endogenous LH peak is not completely excluded [11].

Certainly LH-RH analogs present several disadvantages mainly deriving from the ovarian suppression [4, 12]. The most commonly encountered side effects are hot flushes experienced by more than 80% of patients, headaches and fatigue [13, 14]. During COH higher doses of gonadotropin and longer regimens are generally required using analogs, resulting in higher cost and greater inconvenience for the patient [15]. Similar problems are especially noted with long term protocols. For this reason investigators are now experiencing "short protocols" with a minimum period of suppression followed by menotropins administration [10]. Thus cost and side effects would be reduced and advantages being the same as "long protocols".

In this case another disadvantage possibly arising is the persistence of a luteinic cyst from the previous cycle, with production of progesterone that has a detrimental effect for the following falliculogenesis [16].

Independently of the protocol of stimulation utilized, analogs seem to be in strict correlation with a high abortion rate probably related to poor quality embryos ([17, 18]). Moreover Van Steirteghem recently demonstrated a lower survival after the thawing of cryopreserved embryos generated during a "desensitized cycle" ([19]).

These data confirm that further studies are necessary for LH-RH analogs in POR and suggest the possibility of using other drugs for programming the cycle.

PROGESTINS

In 1984 Templeton ([20]) showed that preovulatory oocytes can be recovered during laparoscopic sterilization using a fixed schedule of ovulation induction, introducing the new concept of POR.

Cycles preceeding laparoscopy were manipulated with Oral Contraceptives (OC) or Norethindrone Acetate (NET) and then Clomiphene Citrate was administered to induce a multiple follicular development. No ultrasound monitoring was carried out.

Human Chorionic Gonadotropin was given on a fixed day of the cycle (day 16) and surgical procedures were scheduled several weeks in advance.

Results evidenced an acceptable recovery rate of preovulatory oocytes with minimal inconvenience for the patient and the medical staff.

The POR was first utilized for research purposes but the possibility of improving results and applying the regimen in assisted conceptions was prospected.

Although Messinis ([21]) in 1986 concluded for the incompatibility between IVF with ET and POR, because of the too high ovulation rate before aspiration in non-monitorized cycles; on the contrary Frydman ([22]) set his basis of programming in assisted conceptions by demonstrating that comparable pregnancy rates were achieved with embryos obtained from a POR regimen and "non-programmed" cycles.

Since 1986 several IVF clinics have been using NET for POR, but few data are available for the comprehension of the progestin mechanism of action. Recently Anderson ([23]) observed NET biological effect on the hypothalamic-pituitary-ovarian axis.

Progestin is clearly demonstrated as an inhibitory agent on gonadotropin secretion for a period as long as the NET is detected in serum, that is 48 hours after the last dose.

On the other hand NET seems not to exert any direct effect on the ovary, and particularly on steroidogenesis, as E2 and P secretions are not affected by NET administration.

Therefore, in a POR, progestin given the cycle preceeding the stimulation would reduce the follicle recruitment induced by gonadotropins during the late luteal phase: a lesser number of follicles would be stimulated and E2 would appear reduced compared to "non-programmed" COH ([23]).

It is the general experience in IVF procedures to achieve a regular multifollicular development and to obtain at the time of ovum pick-up different ranges of oocyte maturity. Immature and postmature oocytes will affect results negatively, as only the mature ones will offer major possibilities of fertilization.

The NET suppression of gonadotropin recruitment at the end of the cycle may produce, during COH, a more homogenous cohort of follicles stimulated at the same time by menotropin administration and synchronized in their maturation ([24, 25, 26]).

A premature LH surge, which has been demonstrated to be a cause of failure in IVF attempts ([27]), may occur in response to dominant follicles reaching a more advanced stage of maturity than other follicles.

The synchronization with NET in oocyte maturation seems to reduce the incidence of dominant follicles and to reduce LH levels during follicular phase ([28]), possibly avoiding the premature LH peak and allowing the recovery of more mature oocytes ([24, 25]).

Certainly similar advantages can be also obtained with LH-RH analogs, but adverse effects, e.g. hot flushes, the cost and the difficulty of administration, make their use less acceptable to the patient.

In IVF centers Norethindrone has been utilized to program the fixed day of ovum pick-up several weeks in advance possibly avoiding weekends and holidays, so reducing inconvenience to the patients and surgical team ([2, 22, 29]).

Initially a fixed regimen of follicular stimulation was adopted and menotropins were administered for the same number of days in each patient who received hCG and underwent a POR on days 11 and 13 of the cycle respectively ([22]). No ultrasound or hormonal monitoring was carried out. Results demonstrated the efficacy of POR with a fixed regimen of ovarian stimulation applied to IVF-ET. In fact there was no significant difference between "programmed" patients and the control group in terms of numbers of follicles, oocytes and embryos obtained. In this study ([22]) no premature ovulation was found and the pregnancy rate per embryo transfer was certainly acceptable (23%). Follicular maturation was not monitored daily for the great convenience of patients.

The economic and practical benefits of a protocols for POR derive from the possibility of deciding several weeks in advance the most suitable day for oocyte retrieval, according to patients' requirements, availability of operating theatres and medical staff.

This is particularly important for small IVF centers where the activity is strictly linked to the possibility of programming ovum pick-up ([2]).

Many doubts regarding the use of NET in assisted conceptions arose in 1986 when Frydman ([30]) evidenced a significantly reduced number of follicles and oocytes obtained when progestin or OC were given in the preceeding cycle compared with a control group.

However in the control group a regular monitoring of follicular growth was followed and hCG was administered only when the maturity was thought to be optimal. On the contrary, in the two study groups, NET and OC, a fixed regimen of ovarian stimulation was used and hCG was systematically given on day 11.

Therefore those results were not reliable, because the groups were differently treated.

Nevertheless it has already been observed ([23]) that NET administered during the late luteal phase reduces or abolishes the gonadotropin induced follicle recruitment, leading to a lesser number of oocytes retrieved.

Similarly Testart ([31]) in 1989 compared different regimens for COH, and the protocol with NET, Clomiphene Citrate and menotropins was found to be related to the highest number of abnormal embryos.

But this group was also the only one where hCG was administered on a fixed day, with no regard to ultrasound and hormonal parameters.

Therefore it seems likely that the negative results of NET pretreatment have been determined more by the fixed regimen of ovarian stimulation than by an adverse effect of NET per se.

According to these results, a fixed schedule for a POR offers great economic and practical benefits, but further studies are necessary before applying it routinely to IVF treatments.

Modified protocols of fixed schedule superovulation regimen have recently been proposed as alternatives to rigid protocol with no monitoring.

A single serum E2 and LH measurement on the day of hCG administration, with values of >250 pg/ml and no sign of premature LH peak, may guarantee the finding of mature oocytes at the time of laparoscopy ([22]).

Bates ([32]) suggests that E2 estimations could be important from day 8 to day 11 and, if a negative pattern is individuated, the patient should be excluded. In fact only patterns where E2 levels rise daily until hCG administration are correlated with high fertilization and pregnancy rate.

More recently a new approach to POR with NET has been proposed ([24, 26, 28]). The pretreatment with progestin was used to synchronize the menstrual cycles of patients to treat as a group in a short period of time. During COH, a regular ultrasonographic and hormonal monitoring was carried out to determine an optimal follicular maturation. The day of ovum pick-up was approximately predetermined and, even if it was minimally anticipated or delayed, a programming of ovarian stimulation was certainly possible.

Norethindrone was demonstrated as a reliable drug for cycle synchronization ([24]), as the onset of vaginal bleeding after discontinuation of NET was 2.9+0.7 days. An important result for the comparison between the fixed schedule protocol and this kind of "monitorized POR" is that the day of hCG administration has a wide variability, ranging from day 8 to day 15. This has been shown not to depend on the length of NET therapy, but on individual response to the COH.

Therefore a strict monitoring of follicular development is required to recover a high number of mature oocytes and to reduce the high ovulation rate found at the time of aspiration.

Certainly the fixed schedule protocol allows a punctual programming of surgical procedures several weeks in advance, and this is of major importance in a district hospital and in all IVF units with restricted resources; but by a "monitorized POR" the day of oocyte aspiration can be approximately predetermined, avoiding working weekends and holidays, and the retrieval of more mature oocytes can be achieved, because of the "individualized" hCG administration.

Nevertheless, even if preliminary studies have demonstrated that NET do not affect results ([22, 24, 28]), further investigations will be necessary.

ORAL CONTRACEPTIVES

The final purpose of gonadotropin suppression in POR is the cycle synchronization and this can be achieved by progestins as well as by oral contraceptives (OC).

The mechanisms of action by which OC can prevent a pregnancy and hormonal serum determinations during ingestion have been widely studied, but in programming assisted conceptions the period after discontinuation of the pill is of major importance for determining hormonal levels and their possible influence on COH.

Klein ([33]) investigated the endocrinological status of patients who received pills for a time period ranging from 6 months to 10 years. He found that the cycle immediately following tablet discontinuation was characterized by a prolongation of follicular phase. Probably the lack of FSH at the end of the luteal phase of the preceeding cycle accounts for such a delay. The first luteal phase after OC discontinuation was normal and with the second cycle no abnormalities in hormone levels were seen.

This finding has been supported by Benadiva ([34]) who reported that 50% of patients discontinuing OC, used for cycle synchronization, had a "poor response" during the following COH and in these patients serum FSH and E2 at the be-

ginning of induction of ovulation were significantly lower than in a normal responder.

Thus a certain threshold level of FSH may be important at the beginning of the cycle to guarantee a regular response to COH.

Moreover ovarian stimulation after OC has been demonstrated to be longer and associated with higher cancellation rate than COH after LH-RH analogs ([35]).

This confirms that in all the patients receiving the estro-progestinic pill for POR a profound pituitary-ovarian suppression is still evidenced during the following induction of ovulation ([34, 35]).

In a different study the initiation of menotropin stimulation was modified, considering the latent phase necessary to reestablish normal FSH levels ([12]). In fact Gonen started the induction of ovulation 7 days after OC discontinuation and, in this case, results demonstrated the great reliability of the pill in scheduling IVF cycles.

No differences were reported between study and control groups in the length of stimulation, cancellation and pregnancy rates, whereas more follicles and more oocytes were obtained with OC retreatment.

It might be possible to explain this variability of results with the fact that COH should be started only when the latent phase after OC discontinuation is finished and gonadotropins have returned to threshold levels.

The advantages offered by OC pretreatment are similar to those deriving from progestin administration and are based on the same mechanism of action. The "fixed regimen" with OC may prevent inconvenience to patients and the medical staff, avoiding repeated monitoring of follicular size or blood sampling.

The negative influences of endogenous gonadotropins during COH were similar to those present in non-programmed protocols, as in 20.2% of treatment a premature LH surge was seen ([36]).

In fact gonadotropins are not suppressed as occurred in patients desensitized with LH-RH analogs.

Finally the pregnancy rate after OC discontinuation is within an acceptable range ([12, 35, 36]).

The initial "fixed protocol" proposed by Templeton ([20]) and then indicated by Frydman ([22]), has been partially modified by Kemeters in an attempt to reduce stress and inconvenience to the patient, verifying at the same time the reliability of OC in POR ([36]).

Interestingly, investigators increased from 2 to 5 the number of days between the last estro-progestinic pill and the initiation of stimulation. At that time gonadotropin levels had returned to regular ranges and the latent phase was elapsed.

Secondly the hCG administration was individualized according to the follicle diameter so that only ultrasound monitoring was carried out to optimize the day of follicle puncture for oocyte aspiration. By this means the day of ovum pick-up was not predetermined, but is was distributed over the working days, avoiding weekends and holidays.

This regimen of "monitorized POR" demonstrated its reliability as most of the injections took place between Tuesday and Friday.

Moreover results were comparable to other standard protocols and higher fertilization and pregnancy rates were evidenced.

CONCLUSIONS

Many problems in work programming have arisen in IVF clinics because of the increased request for assisted conception. Different drugs have been used to facilitate the work schedule and their reliability and effectiveness are still being discussed.

Protocols of POR would also offer benefits in optimizing IVF results, such as ovarian suppression, independently of the drug used allowing the follicles to be at the same maturity stage at the beginning of stimulation. No great differences in diameter have been noted during COH, follicles grow as a uniform cohort and oocytes retrieved appear more fertilizable, with improvement of the pregnancy rate.

Analogs of LH-RH have been successfully used in order to achieve a suppression of endogenous gonadotropins and the possibility of being able to manipulate the day of hCG administration. The day of ovum pick-up cannot be exactly predicted, but results demonstrate that this kind of POR is effective and reliable.

Furthermore in cycles treated with analogs several problems, possibly arising during COH, e.g. a premature LH surge, the development of a dominant follicle and a poor E2 response, are prevented.

But at the same time patients receiving LH-RH analogs frequently complain of the common adverse effects, the difficulty of administration and the cost.

On the other hand progestins and OC are more convenient for their simplicity of use and the low cost, but in this case, certainly, the risk of a premature LH surge is as high as in regular stimulations with menotropins.

The fixed schedule protocol proposed to simplify the COH monitoring and to facilitate the organization of IVF units has demonstrated its conveniences as patients are not required to be in the hospital daily for ultrasound and hormonal evaluation.

Nevertheless it is the general experience that some stimulations may appear slower and others faster, depending on individual hormonal status.

Thus at least an ultrasonographic monitoring during COH does seem necessary to evaluate the follicular growth and to administer hCG only according to the size of the follicles, not concerning the day of stimulation.

Therefore the regimen of a fixed day of ovum pick-up does not appear as an effective method for optimizing results. In fact recent studies evidenced in these cases a reduced number of follicles and oocytes retrieved, associated with poor quality embryos ([30, 31]).

A better choice might be a double association of POR with a regularly monitorized cycle and progestins with LH-RH analogs, so as to take advantage and not inconvenience either of them.

Norethindrone should be given to approximately program the day of ovum pick-up. Its simple administration can be maintained for a long period and at the end, before NET is discontinued, LH-RH analog is initiated. During the ovarian stimulation a regular ultrasound monitoring is carried out to recognize the follicular maturity, while the analog administration prevents the possibility of a premature LH surge.

The hCG injection can be anticipated or delayed by 24-48 hours and surgeries are thus performed only on weekdays.

By this "monitorized POR" the work schedule is easily simplified, allowing surgical teams either to program oocyte retrievals in a predetermined period or to avoid procedures during weekends and holidays.

Patients are informed of the program 1-2 months before the treatment and the expected day of ovum pick-up could vary at most by 2-3 days, so that they can make the necessary preparations many weeks in advance, and the stress factors, always present during a treatment and dependent in part on the uncertainty about the future, are reduced. Psychological stress has already been correlated with a low pregnancy rate ([36]).

From this point of view the "monitorized POR", besides the other mechanisms of improving IVF results, might lead

patients to a better understanding of the times of the procedure, and thus higher pregnancy rates could be achieved.

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