Progesterone immunosuppressive levels and luteal steroid profiles in the cycles induced with Clomiphene Citrate

F. SCARPELLINI (*) - L. SCARPELLINI (*)
N. DINO (**) - P. BENVENUTO (*)

Summary: Several studies have focused their attention on the possible interferences of the endocrine with the immunitary system; these interrelations have been summoned to explain some aspects of the implantation. It has already been demonstrated that progesterone could play an immunosuppressive role, allowing the implantation of allotrasplantation. However, the individual plasmatic levels of the substance needed to produce that effect still unknown. With the aim of determining the certain immunosuppressive progesterone levels in women with normal ovulatory function, and in order to determine if the other principal steroids might also have immunosuppressive effects, in 47 women affected by sine causa infertility, treated with 100 mg. die of Clomiphene Citrate (from day 3 to day 7) we evaluated the plasma levels of progesterone, 17-OH-Progesterone and 17β-Estradiol. The assays were made on the 7th, 11th and 14th post-ovulation days both in women who conceived (immunosuppressive effect present) and in women who did not achieve pregnancy (immunosuppressive effect absent). The results achieved showed a significant difference only in the progesterone values, while those of the other steroids were not significantly different, indicating thus that progesterone is the main element responsible for the immunosuppressive phenomenon and that the seriated evaluation in the luteal phase of this steroid could be used as a marker of achieved implantation.

Key words: Progesterone; Implantation; 17-OH-Progesterone; Estradiol; Clomiphene Citrate; Immunosuppression.

INTRODUCTION

In the field of the therapy of female infertility it has been demonstrated that the treatment with Clomiphene Citrate is highly effective in the induction of ovulation.

In literature we find ovulation percentages between 73% and 95% ([3, 9, 23]). The ovulation rate grows remarkably in women with endocrine based infertility only, in whom a certain endogenous estrogenic activity coexists with a hypothalamic-pituitary dysfunction and consequent anovulation. These patients are endocrinologically identified in the pre-ovulatory phase by an estrogenuria >10 mcg/24h, by plasmatic values of 17-beta-estradiol >80

University of Rome "La Sapienza",
II Institute of Obstetrics and Gynaecological
(Dr. Prof. E. V. Cosmi)
(*) Teaching of Prenatal Puericulture
(Prof. L. Scarpellini)
(**) Teaching of Gynaecological Urology
(Prof. N. Dino)

pg/ml and by a gonadotropinemia >10 mIU/ml, with scarce or no increase of the plasmatic levels of progesterone, even in the luteal phase, or with a late and inadequate increase of the same levels.

These women are included in group II of the classification of infertile women made by the scientific group of the WHO; the treatment with Clomiphene Citrate is positive for them ([13]). This compound, Clorotrianisene is analogous (TACE), it has a trifenyl-ethylenic structure, like one of the Diethyl-Stilbestrol (DES), a powerful synthetic estrogen, and acts in a similar way to 17-beta-estradiol from which it differs only in its kinetics ([4, 9]). The latter is the basis of its mechanism of action which is not yet completely defined, even after almost 30 years in clinical use. Nevertheless all the authors agree that it acts mainly by receptorial competition wherever target organs and tissues for the estrogens are present, particularly at hypothalamic and hypophysis level ([4, 12, 14, 15]). In these structures the Clomiphene Citrate could bind and behave in the same way as the estrogenic endogenous compounds because of the similarity of its stereoisomeric endogenous compounds because of the similarity of its stereoisomeric configuration.

However while for the latter this process happens more rapidly and in relation to the specific half life in each steroid, for Clomiphene it takes place slowly, determining sequestration of the estrogenic receptors. In fact, after oral administration, the elimination of the marked compound occurs in 94% of cases within 5 days ([23]).

In this case the endogenous estrogenic compounds cannot bind with the receptorial proteins also because of their more rapid turnover, thus they lose the possibility of acting. Consequently the hypothalamic receptors occupied by the Clomiphene molecules, give a false message of estrogenic deprivation which determines a fast release of GnRH and then of FSH and LH ([1, 15]).

Though the evolution of the follicular phase induced by the administration of the Clomiphene is relatively well known, in literature little attention has been given to the correspondent postovulatory phase.

In this context it is necessary to remember that some authors have invoked a certain direct steroidogenic effect of the Clomiphene on the luteal cells ([10, 17, 25]) with relative progesteronic hyperincretion.

This hypothesis would be supported by the increase of the progesterone levels to Clomiphene administration during the initial luteal phase (II - VI dys after ovulation) ([17]). This effect is denied by those who think that the increase of the steroid levels is simply the result of an improvement of the follicular phase by way of a gonadotropic effect and consequent estrogenic hyperincretion and recruitment of a greater number of progesterone receptors ([7, 8, 22]).

Whatever the cause, a marked increase in the progesteronemia is nevertheless demonstrated in the luteal phase, after Clomiphene Citrate administration in the initial follicular phase ([11, 20]).

This observation may explain the increase in the percentage of pregnancies in the patients with normal ovulatory and menstrual functions. This datum may be explained by the immunosuppressive capacity of the progesterone which could, directly or undirectly, through substances induced by it, allow the implantation of the pregnancy allograft ([18, 24]). It will be very useful to determine the progesteronemic and 17-OH-progesterone levels, as expressions of the functionality of the luteal body, in cycles with and without conception induced by Clomiphene Citrate, considering the latter as those where the immunosuppressive effects are fulfilled.

By estimating the differences of these two steroids in both types of cycles we

can learn the minimal steroid dose at which the immunosuppression occurs. The aim of our study is, thus, to compare the endocrine gradient between fertile and infertile patients after pharmacological induction of ovulation with Clomiphene Citrate, in order to obtain a luteal hormonal model to which to refer in researching clinically assisted pregnancy.

MATERIALS AND METHODS

47 patients with primary infertility of an endocrine and/or ovulatory origin underwent induction of ovulation during 137 cycles. None of them was affected by infertility due to male causes, malformative genesis, endometriosis, hyperprolactinemia, thyroidal pathologies, systemic disendocrinies or other pathologies contraindicating the pharmacological induction of ovulation.

Besides, none of them had received previous treatment, hormonal or otherwise which could have influenced the cycle. Each of these patients, in the preceding cycle, had been subjected to an endocrine evaluation of her own menstrual cycle with a periovulatory estrogenemia > 80 pg/ml and a gonadotraphin level > 10 mIU/ml for both the FSH and the LH. Therefore these patients were included in group II of infertile women according to the WHO classification, thus potentially able to respond to the therapy with Clomiphene Citrate.

Each patient underwent pharmacological induction of ovulation with Clomiphene Citrate at a dose of 100 mg/day for 5 days from the 3rd to the 7th day, after echographical exclusion of local pathologies contraindicating such pharmacological therapy.

The response of each patient was monitored by ultrasonography, basal body temperature, evaluation of the cervical smear and titration of plasmatic values of 17-beta-estradiol and progesterone. The echographical monitoring was realized with an ultrasonograph Aloka SSD-500, starting day 9 till the day of the follicular dehiscence. Transbdominal probe with transverse and longitudinal scanning of both ovaries and endometrium and the transvaginal one with transversal and parasagittal scanning of the same structure were used. The survey of the thermical curve or basal temperature was done by the rectum every morning at the same hour from day 1 of the cycle and transferred to a graphic.

The evaluation of the cervical smear was realized by means of the semi-quantitative criteria of the Insler score every day from day 9 of the cycle.

The plasmatic titration of the 17-beta-estradiol and progesterone was carried out with a RIA dosage according to the standard technique (17-beta-estradiol and progesterone - I 125 Kit Radim). The samples were collected between 8.00 and 8.30 to minimize the possible oscillations due to the various personal circadian biorhythms.

When the dominant follicle was over 19 mm, reaching preovulatory dimensions, 5000 IU of HCG were administered if the verification of the cervical smear had reached a maximal or submaximal score (11 or 12) for more than 2 days, the 17-beta-estradiol was not inferior to 250 pg/ml per follicle and the progesterone was not superior to 2 ng/ml.

After the dehiscence had occurred, during the luteal phase, no therapy was administered to the patients. On the 7th, 11th and 14th days after ovulation, they underwent the plasmatic RIA dosage with standard technique of the 17-OH-progesterone, progesterone and 17-beta-estradiol (by I 125 Kit Radim).

On day 17 of the postovulatory phase a titration of the plasmatic beta HCG was carried out to check an eventual pregnancy.

The mean age of the patients was 27.54 years (range 24-32), the mean body mass index (BMI) was 23.71 kg/m (range 21.7 - 25.8) and the mean duration of infertility was 3.6 years (range 2-6), 2.91 stimulation cycles per woman were carried out.

RESULTS

Of the 137 induced cycles with Clomiphene Citrate, 31 (22.62%) resulted in pregnancies and 106 (77.37%) did not. From this last group, 39 (36.79%) showed a clinical evidence of anovulation. Considering the total of the 47 patients, 27 were pregnant (57.4%), 4 of whom (8.5%), after a first abortion, had a second pregnancy with physiological course, and 11 (23.4%) ended in abortion. The remaining 16 patients had a pregnancy with physiological course, with the exception of 6 cases (12.7%) of threatened abortion in the first trimester, which receded after pharmacologic therapy.

In the fertile cycles the RIA dosage showed on the 7th day, after ovulation, a mean estrogenemia of 552 ± 187 pg/ml, a mean progesteronemia of 22.6 ± 4.2

ng/ml and mean plasmatic levels of 17-OH-progesterone of 274±87 ng/ml, while on day 11 the mean plasmatic concentration of 17-beta-estradiol were of 647± 185 pg/ml, those of progesterone and of 17-OH-progesterone were respectively 39.65±9.8 ng/ml and 494±9.8 ng/ml and 494±212 ng/ml.

On day 14 these cycles showed mean values of estrogenemia of 854±227 pg/ml, of progesteronemia of 62.15±26.3 ng/ml and of 17-OH-progesterone of 1152±537 pg/ml.

On day 7 the infertile cycles showed mean plasmatic estrogen rates in the postovulatory phase of 526±165 pg/ml, on day 11 of 498±163 pg/ml and on day 14 finally of 183±83 pg/ml. In the same cycles, the mean progesteronemia resulted on day 7 in the postovulatory phase of 18.2±3.8 ng/ml, on day 11 of 14.3±7.1 ng/ml and on day 14 of 6.5±2.3 ng/ml, while the mean serous levels of 17-OH-progesterone were, in the same cycles and on the same days in the postovulatory phase, respectively, 222±73 ng/ml, 183±61 ng/ml and 75±32 ng/ml.

Out of 31 pregnancies obtained, 15 resulted in abortion (48.3%); of these, 3 in the 6th week (9.6%), 5 in the 7th week (16.1%), 2 in the 9th week (6.4%), 3 in the 11th week (9.6%) and 2 in the 2nd trimester (6.4%), respectively in the 17th (3.2%) and 19th (3.2%) week.

DISCUSSION

Luteal steroidogenesis appears very different from the follicular one, and, unlike it, characterized by the production of estrogens and particularly of 17-beta-estradiol, it is characterized by progesterone metabolism, whose main product is progesterone. This substance, whose production is enhanced by estrogens, is bound to its own cytoplasmatic receptors; it is transferred into the cytoplasm, determines, like all steroids, a genic activation that produces a corresponding proteic synthesis ([2, 4]). Under the morphological profile, progesterone acts principally by blocking cellular mitotic activity induced by the estrogens, in the target tissues, thus determining the cellular maturation of these structures.

In particular, at the endometrial level, apart from the above-mentioned effects, the decidualization of the stroma, cytogenic or otherwise, is very important for embryo implantation. Though the effect is not yet clear, progesterone may also represent an immunosuppressive effect at a local level, markedly evident in the T lymphocytes of cellular immunity ([6, 18, 24]).

Among the progestative substances (C - 21 structure) this property seems to be possessed of progesterone only, while other components of the same class, for example 17 - H - progesterone lack it ([18]).

Progesterone dependent immunosuppression will be necessary, as the embryo is biologically like an allograph, having half of the genetic heritage in common with the father, and the other half with the mother; therefore half of its genetic heritage thus results "non-self" to the other, and so able to provoke an antibody response which may be the immunity biological basis of rejection.

It is known that in fertile cycles ovulation has surely taken place.

It is also known that in infertile cycles the mean concentration of progesterone is generally lower than the one present in the cycles that evolve in pregnancies.

On the bases of such considerations, assuming the progesteronemic levels of the fertile cycles to be the correspondent hematic concentration at the local dose effective for performing the immunosuppressive effect, and those not followed by conception to be correspondent to an ineffectual dose, we have compared the differing levels of the steroid mentioned in both types of cycle.

Our purpose has been to individuate the certain blood level of progesterone necessary for producing an immunosuppressive effect.

The survey of the plasmatic values of 17-OH-progesterone gives us useful data on the functionality of the luteal body, since such a steroid, unlike progesterone, which is partly produced by the adrenal gland, derives exclusively from the ovaries ([26]).

Finally, the 17-beta-estradiolemia also has an extremely important role in the process of implantation, though this it is not yet very clear ([16, 27]).

In the cycles we studied, the plasmatic concentration of the three steroids was substantially similar on day 7 after ovulation, minimal differences between the blood levels of the fertile and infertile cycles were coexisting. A difference became evident in the hormonal results of day 11 after the ovulation in all three steroids reported, though less evident in the values of estradiolemia.

This could be explained by the fact that in the period between the 7th and the 11th postovulatory days the modifications of the implantation are established. Particularly, where pregnancy is present, the biochemical-endocrinological differences of the cycles begin to be obvious on the 11th day. Therefore, it is now that the serum concentration correspondent to the local dose of immunologically active of progesterone must be looked for, not differences on the 14th day after ovulation (the day of the absence of menstruation) when the hormonal differences between the pregnant and non-pregnant cycles are very clear.

On the 11th post-ovulatory day the levels of progesterone in the two groups were different; in fact on that day, in the fertile cycle they were never less than 35 ng/ml, while in the infertile cycle the highest progesterone levels were 24.7 ng/ml.

This observation has double value, diagnostic and therapeutic. As a matter of fact, it is possible to anticipate the final level of hematic progesterone in the cycles treated with Clomiphene Citrate and to individuate the concentration of progesteromenia necessary to obtain the local immunosuppression of the implantation. Therefore, it is useful to integrate the progesterone to keep constant the steroid blood levels, thus preparing the most favourables conditions for the implantation of the probable embryo; it is important that the pharmacological intervention takes place on the 7th day after ovulation, when the plasmatic values of progesterone in both types of cycles present slight variations, sustantially negligable.

A possible objection to this point of view comes from the evident existance of a negative steroidopoietic feedback during the exogenous administration of progesterone ([21]). In this case it is possible to proceed with a progressively increased administration of the steroid.

Besides, the isolated pharmacological preservation of high progesteronemic levels will have scarce significance if it is not matched by good functioning of the luteal body, fundamental structure for the maintenance of the initial human gestation ([5]), secured by the growth "in toto" of all steroids and, in particular way, of the 17-OH-progesterone. From this also emerges the importance of the seriate evaluation of the estrogens, which have a paracrine role in the stimulus of progesterone secretion ([27]), and especially of the 17-OH-progesterone, direct and exclusive product of the luteal body ([26]).

The comparison between an increase of estradiol and 17-OH-progesterone on the 11th postovulatory day justifies continuing to give progesterone, as it indicates the persistance of a stimulating action on the luteal body and, also a tendency to lack of involution. In fact, on this day, the clear endocrine divergence of both types

of cycles (fertile or not) emerge, thus permitting the identification of the cycles that result in pregnancy.

The survey of high progesteronemia (>35 ng/ml), even if in coincidence with a progesteronic therapy, in concomitance with high levels of the other two steroids (estrogenemia >700 pg/ml; 17-OH-progesteronemia >350 ng/ml) permits the hypothesis of the existence of a pregnancy not yet clinically evident. The reason for this increase of the steroid production probably lies in the stimulating action that the blastocyst exercises, through the HCG, in the ovarian luteal body. Therefore, where there are signs of increased luteal activity, substantially persistent, there also good probabilities of pregnancy. However, until that period (11th day) the supplementary progesteronemia of the infertile cycle must be carried out, to guarantee, at the moment of the implantation, an adequate local immunosuppressive dose suitable for permitting it, as otherwise there is the possibility of interruption in an extremely precocious phase of the gestational event.

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Address resprints requests to:
N. DINO
Piazza Monteleone di Spoleto, 36
00191 Roma (Italy)