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REVIEW ARTICLE

Ulipristal Acetate (UPA) in Emergency Contraception: The Mechanism of Action, Toxicity and Perspectives

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ABSTRACT

After recalling that Levonorgestrel Emergency Contraceptive Pills never delay or suppress ovulation, but impair the luteal body functions, and, consequently, the embryo-implantation, we focus on the mechanism of action (MOA) of ellaOne®: micronized UPA (Ulipristal Acetate) 30mg and on UPA- toxicity.

EMA and, after it, the National Drug Regulatory Institutions and the most renowned International Gynecological Societies present ellaOne® as an anti-ovulatory drug which is safe even in repeated assumption, also during the same menstrual cycle. We'll try to understand whether this dogma is supported by experimental data in literature.

As to the MOA, EMA reports (EMEA-261787-2009) that *Ulipristal blocks the synthesis of the proteins necessary to begin and maintain pregnancy*, and that *Ulipristal and mifepristone were approximately equipotent* as to their *ability to terminate pregnancy*. Besides, EMA further evidences (EMA/73099/2015) that *it is unknown whether it is possible to use ellaOne® for abortion.*

Data in Literature evidence that ellaOne® can delay ovulation only when is taken in the very first fertile days of the cycle. In the pre-ovulatory, most fertile, days – when most intercourses do occur and over 70% fertilizations ensue – it never prevents ovulation, like placebo. On the contrary, whenever it is taken in the cycle, it consistently impairs the endometrium that becomes an inhospitable ground for the embryo: endometrial gene expression is completely subverted compared with that of the normal luteal phase.

Recently, an UPA-based drug used for uterine fibroids-treatment (Esmya®) was withdrawn from the market because it caused fulminant hepatitis requiring transplantation (EMA/455818/2020). It was prescribed by the hospital for 3-6 months and carefully followed-up. The strict post-marketing surveillance allowed to link UPA-administration and tissue-accumulation to liver-failure. Surprisingly EMA, while revoking Esmya®, warranted for the safety of ellaOne®, though it is taken by millions of women unaware of the risk, repeatedly without prescription, without medical supervision and any possibility of post-marketing surveillance, in UPA-cumulating doses even greater than with Esmya®.

Finally, we criticize the attempt to propose UPA in daily contraceptive pills, at doses even double than in Esmya® and for much longer periods, to fertile women aiming at preserving their fertility and health.

Keywords: ellaOne, ella, UPA, Ulipristal, Emergency Contraception, Mechanism of Action, Ovulation, Endometrium, Embryo-implantation, Toxicity, Esmya

INTRODUCTION

Emergency contraception (EC) is defined as the use of any drug, or the intrauterine insertion of devices, after unprotected sex intercourse (UPSI) with the aim of preventing an unwanted pregnancy. UPSI can lead to pregnancy only if it occurs in the fertile period of the cycle: the four-five days preceding ovulation and the ovulation day itself. Only in these days, in fact, does the cervical mucus allow the sperms to enter female internal genitalia. Among them, the pre-ovulatory is the day on which the probability of conception is highest, followed by the ovulation day and by the second day preceding ovulation [1-5]. On these three days the frequency of both protected and unprotected intercourse peaks [2,6].

The use of Emergency Contraceptive Pills (ECPs) must face at least two facts: (1) the sperms have already entered and no day-after drug can reverse their ascent that already happened; (2) ovulation is imminent.

Within this setting, the clinical appearance of pregnancy can only be avoided in two ways: by preventing ovulation *in extremis,* thereby preventing fertilization, or by making sure that the embryo will not find the fertile ground he needs to implant within the uterus.

Correct information on the mechanism of action (MOA) of these drugs is dutiful: it is the essential requirement for the woman to express a fully free and informed consensus to their use. The MOA is one of the main criteria that determine the choice among the different contraceptives [7-10]. Moreover, a complete information about possible risks of the drug should be warranted.

In previous papers (they will be quoted subsequently) we dealt with the mechanism of action of the two drugs used for EC and evidenced that both Levonorgestrel (LNG), a potent synthetic progestogen, and Ulipristal Acetate (UPA), a potent anti-progestogen, mainly work as anti-implantation drugs.

Though the WHO [11], the producer (HRA-Pharma) [12], the Food and Drugs Administration (US-FDA) [13], the European Medicines Agency (EMA) [14], the most highly reputed international and national gynecological Scientific Societies [15] affirm that Emergency Contraceptive Pills (ECPs) work by either inhibiting or delaying ovulation (therefore preventing fertilization), without affecting implantation in any way, our evaluation – strictly based on scientific and experimental evidence – leads to an opposite conclusion: these drugs, in fact, consistently prevent fertilization only when they are taken at the very beginning of the fertile period. In the subsequent fertile days, on the contrary, and mainly in those closest to follicular rupture, both ECPs have no effects on either ovulation or fertilization. In those days, in which most fertilizations do occur [1-3,12], ECPs transform the endometrium into an inhospitable environment for the embryo.

As to Levonorgestrel (LNG;Norlevo®,Levonelle®,Escapelle®), we showed that it does never affect ovulation [16-18]. Besides, we highlighted that the FIGO (International Federation of Gynecology & Obstetrics) and ICEC (International Consortium for Emergency Contraception) share a false information in their 2008, 2011 and 2012 joint Statements [15]: in fact, they state as a dogma that LNG-ECPs delay or inhibit ovulation and consequently prevent fertilization, without ever affecting embryo-implantation, while – on the contrary – in the studies quoted in support [15,19-23] ovulation is never inhibited when LNG is taken in the most fertile days of the cycle.

Moreover, the Statements' authors Brache and Faundes [24], in their own studies [24-27] report that when LNG is taken in the advanced pre-ovulatory phase it "*resulted in follicle rupture inhibition in 7/48 women (14.6%) of the LNG-studied cycles*", evidencing a very poor anti-ovulatory effect that was reaffirmed in a further paper [28]. In spite of this, in all the FIGO Statements they state exactly the opposite, verbatim: "*that inhibition or delay of ovulation is LNG ECPs' principal and possibly only mechanism of action*".

We also evidenced that EMA itself, in all the European Public Assessment Reports (EPAR) on ellaOne® - since 2014 to the last one, updated 16/11/2022 [14] - reports that in the fertile days LNG is never able to inhibit ovulation (Table 1, page 9).

In our previous papers we also pointed out that LNG-ECPs, though unable to prevent ovulation, are highly effective in avoiding the clinical appearance of pregnancy: when UPSI occurs in the fertile period of the cycle and LNG is taken before ovulation, it prevents the appearance of 70% of expected pregnancies [29], likely through the impairment of the formation of an adequate corpus luteum [20-23].

In this paper our attention will be focused on Ulipristal Acetate (UPA; ellaOne®) and, in particular, on its mechanism of action (MOA), on the possible risks linked to its assumption and on the perspectives of its use.

Each tablet of ellaOne® contains 30mg of micronized Ulipristal Acetate, to be taken in a single oral dose. It is acknowledged that 30mg of micronized UPA are equivalent to 50mg of

unmicronized UPA (the drug used in previous clinical trials, administered in gelatin capsules) [12,30].

Presentation by the EMA

UPA binds to Progesterone Receptors and inhibits the effects of Progesterone, the pro-gestational hormone. In the *CHMP Assessment Report (AR) for Ellaone®* (EMEA-261787-2009) [31] leading to Marketing Authorization, at page 8, the molecule is presented – verbatim – with the following words: "*Ulipristal acetate prevents progesterone from occupying its receptor, thus the gene transcription normally turned on by progesterone is blocked, and the proteins necessary to begin and maintain pregnancy are not synthesized.*". It means that UPA can prevent implantation and also terminate pregnancies.

It works in the same way as does Mifepristone (RU486) and their molecules are quite equivalent. In the just mentioned EMA AR, at page 10, it is reported that "*The ability of Ulipristal Acetate to terminate pregnancy was investigated. Ulipristal and mifepristone were approximately equipotent*" and that "*When using intramuscular administration of 0.5mg/kg, 4/5 foetuses were lost in ulipristal acetate treated animals* (macaques)" [32]. It means that 50mg unmicronized UPA, (ellaOne®), can terminate pregnancy in a 100kg primate, and we know well that the parenteral administration, unavoidable in the animals, is similar to the sublingual one that cannot be asked to an animal.

Indeed, the possibility that UPA is used *off-label* for pregnancy termination is known and is presented as a "*safety concern*" in the Table "Summary of the risk management plan for Ellaone®" at page 41 of the same AR [31], but the strategic choice for the "*proposed risk minimization*" has been "*Omit any sentence in the SPC and the PL suggesting that the product could be used as an abortifacient.*"

The EMA and HRA-Pharma agree that all of the approaches to avoid this abuse suffer from inevitable limitations; the only way seemed to be prescription registries (at pages 45-46). However, the prescriptions were abolished by EMA in 2015 [33].

At last, in 2015 at the page 35 of the Assessment Report "EMA/73099/2015" [33], EMA recalls verbatim that "*During the evaluation process of the ellaOne® registration dossier the MAH (HRA-Pharma) was requested to study any potential off-label use of ellaOne®, in particular during pregnancy, possibly as an abortifacient. No clinical studies have been performed with Ulipristal-Acetate as an abortifacient, and it is therefore also unknown whether it is possible to use it for abortion*". (page 35).

However, to minimize any possible off-label use, in the absence of reassuring scientific evidences, in the same Assessment Report "EMA/73099/2015", at page 31, EMA presented the results of a simple interview to 75 prescribers from Poland and Sweden (HRA2914-544a), *evidently* considered as representative of all the European Doctors' Community: requested whether they ever used UPA for abortion they answered no. This interview is considered a reliable "*demonstration that off-label prescription of ellaOne for abortion does not happen in the real world*" [33].

After presenting the drug in the above reported way the CHMP of the EMA holds that "*Emergency contraceptives work by stopping or delaying ovulation*" [34].

On November 16th 2022 the EMA updated the EPAR on ellaOne®[14]; there appear unchanged the same sentences we can read since 2009: the HRA-Pharma, the producer, affirms that ellaOne®, administered in the fertile period of the menstrual cycle, is able to delay ovulation and hence prevent fertilization (pages 8 and 39). EllaOne® would be able to postpone follicular rupture up to five days even when taken immediately before ovulation is scheduled to occur and its efficacy would be consistently high, over 80%, even when it is taken up to five days since UPSI [12]. This statement, basing on Brache's paper [25], is fully endorsed and shared by the EMA [14].

EllaOne® and ovulation

Curiously enough, the EMA itself publishes data that fully contradict the above statement and might close definitely any discussion on the MOA: they are summarized in the Table 2 at the page 7 of the EMA-CHMP Assessment Report on ellaOne® "EMA/73099/2015" [33]. The EMA reports the HRA2914-554 study, subsequently published by Jesam et al [35], that examined the effect of single repeated doses of ellaOne® on ovulation. The drug was taken weekly (Q7D) or every 5 days (Q5D) for 8 consecutive weeks. Ovulation was observed in 91.7% of the women in the group who took ellaOne® weekly and in 72.7% of those who assumed it every 5 days. In both groups, the pre-ovulatory cervical mucus proved to be normal and suitable to sperm penetration. It means that ovulation takes place normally in women taking ellaOne®, even when they take it weekly for eight consecutive weeks.

As already said, the belief that ellaOne® is an anti-ovulatory drug is based on Brache's paper [25].

She evaluates the effects of ellaOne® on ovulation when it is taken in the different days of the fertile period. The authors conclude that ellaOne® can inhibit or significantly delay follicular rupture for over 5 days, even when it is administered immediately before ovulation and emphasize this point in the title, in the abstract and in the paper conclusions.

Brache enrolled thirty-four women for her study. At first, they are evaluated as a whole and then separately, subdivided into three groups according to whether they took ellaOne® before luteinizing hormone (LH) levels start to increase, or during LH surge, or when LH peak levels are reached.

Overall, ellaOne® taken in the fertile period inhibits or delays ovulation in 58.8% of the women, while 41.2% ovulate regularly and fertilization can ensue. Besides, the effects of UPA are reported to be highly dependent on the levels of LH at the time of administration, in the three subsequent phases of the fertile period. Ovulation is consistently delayed (100%) only in eight women who took ellaOne® before LH levels start to increase. After the onset of LH surge but prior to its peak, ovulation is delayed in eleven women out of fourteen (78.6%). In the patients treated at the LH peak ovulation is delayed in only one woman out of twelve, thus 92% of women ovulate.

Moreover, in the *results* section of the same paper, the authors state that when UPA is taken at the LH peak, that is one-two days before follicular rupture, the drug has no ability to either avoid or delay ovulation and behaves exactly like a placebo: "*when UPA was given at the time of the LH peak, the time elapsed to rupture was similar to placebo (1.54±0.52 days versus 1.31±0.48 days)*.". These days are the most fertile in the cycle, those in which most fertilizations do occur. These are the days in which UPA, which is demonstrated to have a steadily high contraceptive efficacy (over 80%), should prevent ovulation with the highest efficacy if its MOA were truly anti-ovulatory.

On the contrary, when ellaOne® is taken in these most fertile days, it does not exhibit any anti-ovulatory effect.

Unfortunately, at this point, I must remark an unpleasant episode of *incorrect information*: though Brache's clearly explains that "*when UPA was given at the time of the LH peak, the time elapsed to rupture was similar to placebo (1.54±0.52 days versus 1.31±0.48 days)*.", some Swedish authors, quoting her paper, pretend to sentence that "*Even on the day of the LH peak, UPA could delay ovulation for 24 to 48 hs after administration*". This sentence appears in two distinct papers, respectively at pages 302 [36] and 93 [37], and is evidently the opposite of what Brache wrote: it clearly appears as an intentionally deceiving information.

We understand that ellaOne®'s ability to delay ovulation decreases sharply from the first to the third fertile day and becomes almost null (8%) 36 hours before ovulation. In spite of this, its effectiveness in preventing pregnancies remains very high (≥80%) and does not decrease depending on which of the five days it is taken after UPSI [38,40-42]. It appears, consequently, unlikely that its anti-ovulatory effect, that is decreasing, can explain its effectiveness that is steadily high [18,38].

A further confirmation that ellaOne® cannot delay ovulation, when administered in the one to two days preceding its scheduled occurrence, comes from a paper by Lira-Albarràn [39], who evidenced that ellaOne®, intentionally given at a time of the cycle in which the probability of pregnancy is highest, has no effect on ovulation.

At last, the administration of unmicronized UPA (10, 50 or 100mg) to women in their mid-follicular phase led to a delay in ovulation that was greatest at the highest doses. On the contrary, even the lowest dose of 10mg inhibited luteal phase endometrial maturation in the same way as the higher doses, evidencing – as Stratton remarks – that UPA threshold for altering endometrial morphology was lower than that for altering folliculogenesis [40].

Unmicronized UPA 50mg is equivalent to the micronized UPA 30mg of ellaOne®. It can delay ovulation when is taken before the start of the fertile period (mid-follicular) and also in the first fertile day [25], but when it is taken in the most fertile pre-ovulatory days it has no effect on follicular rupture. Stratton's data point out that UPA's negative effects appear consistently in the luteal phase endometrium, both when UPA succeeds in delaying ovulation and when it doesn't. This means that, once ovulation occurs and fertilization ensues, the endometrium will always be unsuitable for embryo-implantation.

EllaOne® and endometrium

One single dose of unmicronized UPA (10,50,100mg) leads to a reduction in endometrial thickness consistently and modifies deeply endometrial receptivity, at whichever time it is given: either in the mid-follicular phase, before the fertile days [40], and at mid-cycle following ovulation and the eventual fertilization [41], and in

the mid-luteal phase in the implantation-window [42]. The pro-gestational effects of Progesterone on the endometrium are lost and, among them, the expression of those proteins that make the uterus hospitable for the embryo, as was anticipated clearly in EMA 2009 Assessment Report. In particular, taken in the early luteal phase [41], after ovulation, the doses of 50mg (equivalent to ellaOne®) and 100mg increase endometrial Progesterone receptors and reduce significantly the markers of endometrial receptivity (Node-Addressin). Embryo-implantation becomes impaired.

Again, for the second time, a still more evident episode of *incorrect information* shall be pointed out, always by the same authors from the renowned Karolinska Institute [43]. Quoting the above Stratton's data [41], they fully acknowledge that unmicronized UPA, at the doses of 50mg (ellaOne®) and 100mg, makes the endometrium unsuitable for embryo-implantation, but affirm that "*in the doses relevant for EC use (30mg) UPA has no significant effect on the endometrium*" (page 5). Their conclusion is maintained at the end of the paper – even if some pages later (page 9) they acknowledge, within brackets, that "*50mg UPA equals 30mg micronized UPA*" – and is repeated one year later[44] and even subsequently [45].

Going on in evaluating the data in literature, UPA effects appear identical to those observed after the administration of 200mg of Mifepristone, the dose used for pregnancy termination, but UPA is effective at a much lower doses: 10mg (one fifth of ellaOne®) [41].

As anticipated in the presentation of UPA by the EMA (2009 AR), endometrial inhibition is direct and is due to the inhibition of Progesterone receptors (the same as RU486) [46-51]. EllaOne® occupies Progesterone receptors. The hormone is present but cannot act and the endometrium will not become a hospitable ground.

However, the definitive demonstration of this anti-implantation MOA comes from the above mentioned study by Lira-Albarràn [39]. He evidences that ovulation always occurs, consistently, when ellaOne® is taken in the most fertile days; at the same time, however, ellaOne® induces in the luteal endometrium changes associated with a non-receptive phenotype, an endometrium unsuitable for embryo-implantation.

He enrolled 14 healthy fertile women and followed them longitudinally in two consecutive menstrual cycles, in which each woman served as control of herself. In the first cycle, untreated, the mayor characteristics of the cycle were determined. In the following cycle a single dose of ellaOne® was administered when the follicle reached 20mm diameter, intentionally in the most fertile days of the cycle. In both the control and the treated cycle ovulation took place regularly. At the day LH+7 of both cycles an endometrial biopsy was taken from every woman and the expression of 1183 genes was determined on each specimen.

EllaOne® did not alter the luteal progesterone plasma levels, but induced a remarkable anti-progestin effect in the endometrium: the genes that were up-regulated in the hospitable pro-gestational endometrium were, on the contrary, down-regulated in the UPA-treated endometrium; vice versa, the genes that were down-regulated in the pro-gestational endometrium were up-regulated in the UPA-treated endometrium.

The gene expression typical of the receptive endometrium is subverted completely after ellaOne®, going in a quite opposite direction. The author concludes that the "*changes observed in gene expression in endometrial samples from women exposed to UPA are associated with a non-receptive endometrial phenotype*". One year later the same authors compared their own data with those in Altmae's meta-analysis on receptive human transcriptomic [52] and verified that 39 of the genes whose endometrial expression was subverted by ellaOne® were included in the list of 57 genes recognized by Altmae as putative receptive markers. Out of these 39 common genes, 37 were down-regulated and two up-regulated by UPA in an opposite fashion to that observed in the meta-analysis [53].

In summary, Lira-Albarràn evidences that the women who take ellaOne® after UPSI in the most fertile days have regular ovulation and fertilization can ensue [39]. UPA cannot interfere with human sperm fertilizing ability and nothing can prevent fertilization [54]. Unfortunately, the endometrium is unhospitable and the embryo has no chance of surviving.

Quite recently, Jimenez Guerrero et al. [55] reported that UPA, administered after ovulation, down-regulates or delays the action of the main genes involved in conditioning the endometrium for implantation and in the dialogue with the embryo.

In previous studies of their own in the mouse, they had evidenced that the post-ovulatory administration of UPA impairs pregnancy reducing the number of conceptus, probably due to an effect of UPA on endometrial receptivity [56]. For the recent *in vivo* study they included four healthy parous women with tubal sterilization, regular

menses, no hormonal therapy, breastfeeding, or pregnancy in the 6 months prior to the enrolment.
Each woman was evaluated for three non-consecutive menstrual cycles: a *pre-treatment*; a *treatment* and a *post-treatment cycle* two months after the treatment.
EllaOne® (micronized UPA 30mg) was administered 2 days after the LH peak (LH + 2). Transvaginal ultrasound (TVU) was also performed at that time to assess the rupture of the dominant follicle (≥50% in follicle size reduction) and endometrial thickness.
Endometrial biopsies were obtained on day LH + 7 (in the implantation window) in the three studied cycles. The expression of 192-carefully-selected genes related to endometrial receptivity and maternal immune response was studied on each tissue specimen.
Transcriptomic analysis of endometrial biopsies showed a significant reduction in total gene expression in both the treatment ($p<0.0001$) and post-treatment ($p<0.0001$) cycle samples compared to the pre-treatment condition, without differences between the treatment and post-treatment group. In particular, the authors observed a tendency to down-regulation of very important functions related to the regulation of metal ions (VCAM, MMP group and KCNG1) such as zinc, relevant in implantation; negative growth regulation, essential for endometrial conditioning and a proper implantation and matrix remodeling (MT group). In addition, as a result of a discriminant analysis the authors identified three populations clearly differentiated. A deep study on which genes exert an effect on the grouping analysis, allowed the identification of 11 genes that are able to explain 98.2% of the variability of the samples. These genes (LIFR, VEGF, EZR, MARK1, or SERPINA1 among others), are closely related to implantation, receptivity and endometrial functionality as previously described.
They conclude that UPA down-regulates or delays the action of the main genes involved in conditioning the endometrium for implantation and in the dialogue with the embryo. Besides, they observed that this effect was maintained two months after the pill intake, adding novel information on the reversibility of UPA impact on endometrial gene expression.
These results are in agreement with the data reported by Stratton in 2010 [41] and confirm that ellaOne® plays its anti-implantation effect even when it is taken after ovulation and the eventual fertilization.
Li et Al. [57] suggest partially different conclusions. The failure rate of ellaOne® in avoiding pregnancy when it is taken after ovulation would be apparently higher than that following a pre-ovulatory assumption, although statistical significance was not reached. Indeed, the authors acknowledge that in their study it is difficult to ascertain the time when a woman actually ovulates, as the date of ovulation is very variable and is difficult to be ascertained on the base of the cycle anamnestic data, with the help of an isolated ultrasound evaluation and a blood drawing for hormones. The discordance mainly applies to subjects in the peri-ovulatory phase, that is in the most fertile days of the cycle.
Besides, the time elapsed since the UPSI is not considered in Li's study. If ellaOne® is taken in the luteal phase after an UPSI in the most fertile days, the number of expected pregnancies would be the highest possible and not lower than after a pre-ovulatory assumption.
Indeed, there is no agreement in literature on the rate of expected pregnancies related to the time of ECPs assumption: while Lì calculates that it is 6.2% in women taking UPA before ovulation and 3.3% if the drug is taken after ovulation, the data reported by Noé [29] in a similar study on LNG-ECPs are quite different: 17% in women taking ECPs before ovulation and 20% if the drug is taken after ovulation. With Noé's reference data, the results of Lì would be the opposite, with a greater efficacy for post-ovulatory assumption which appears even logical: the highest number of UPSI and fertilizations occur in the 36 hours before ovulation and in the ovulation day and, consequently, most of the five days since UPSI – in which ellaOne® is taken - necessary fall in the early luteal phase.
At last, some considerations on the in vitro effects of UPA on human endometrial stromal cells (ESC) isolated from endometrium in the cycle follicular phase and pre-exposed to 17β-estradiol and progesterone. UPA interferes with estradiol and progesterone actions in ESC by decreasing actin cytoskeleton rearrangement, focal adhesion formation, and reducing FAK and Moesin phosphorylation/activation. This leads to an alteration of cell morphology and to the inhibition of cell motility [58].
The above discussion lets us understand that the main MOA of ellaOne® is its anti-implantation effect on the endometrium, whenever it is taken in the menstrual cycle. Scientific evidences are strong, but even a simple reasoning suggests that the main MOA of ellaOne® is post-fertilization (https://www.sipre.eu/does-really-ellaone-delay-ovulation/).

Again, we disagree with the Swedish authors that, after trying to demonstrate that ellaOne® doesn't affect human embryo-*attachment* to a 3D-endometrial construct, pretend to conclude that ellaOne® does not disrupt the *implantation process* [59]. The conclusion is unjustified and constitute, again, a *deceiving information*.

As we read, the endometrium used for their 3D-construct was obtained from healthy women with normal untreated cycles, in the cycle day LH+4. It was a hospitable endometrium, primed by Progesterone, already endowed with the machinery for embryo-attachment. It was not obtained from women previously treated with ellaOne®.

Moreover, the cultures were exposed to UPA 200ng/ml, a concentration similar to that observed in the women's blood one hour after ellaOne® intake (176+89 ng/ml). However, UPA concentration in tissues is higher than in the blood after UPA-assumption, both in the endometrium and elsewhere; the experimental conditions, consequently, might not reproduce what happens in vivo.

Lastly, only the very initial step of embryo-attachment could be imagined as reproducible in vitro, while implantation cannot be tested in this model, as the authors acknowledge. In spite of this, the authors conclude verbatim that "the mechanism of action of UPA when used as an EC does not disrupt the *implantation process*", and always refer to *implantation* in the abstract's Study Question, Summary Answer and Conclusions. This message seems *intentionally deceiving*,

It should be clear, at this point, that the prevalent MOA of ellaOne® is linked to its anti-progestational effect on the endometrium and not to any effect on the process of ovulation [60].

All this information, however, was already evident when ellaOne® was introduced into the market as an anti-ovulatory drug [31]: the papers describing its effects in women are the same discussed above. HRA2914-505: *Stratton* [40]. HRA2914-506: *Stratton* [41]. HRA2914-503. *Passaro* [43]. HRA2914-511: *Brache* [25].

Besides evidencing – as reported at the beginning of this paper – that UPA is able to avoid embryo-implantation and terminate pregnancies (*the proteins necessary to begin and maintain pregnancy are not synthesized*) and that its off-label use as an abortifacient cannot be avoided in any way, EMA acknowledges many other important issues in the *CHMP Assessment Report for Ellaone®* (EMEA-261787-2009) [31] leading to Marketing Authorization:

(1) "*The threshold for altering endometrial morphology appears lower than for inhibition of ovulation*" (page 22). (HRA2914-505 by Stratton) [40].

(2) "*At early-luteal phase significant delay in endometrial maturation occurred in the 50mg (ellaOne®) and 100mg groups compared to the placebo and 10mg groups*" (page 22). (HRA2914-506 by Stratton) [41].

(3) In UPA-use for emergency contraception "*alterations to the endometrium may also contribute to the efficacy of the product*" (page 23). This is never mentioned in ellaOne® package leaflet.

At last, in the *CHMP Assessment Report for Ellaone®* (EMA/73099/2015), in the Table at page 64 [33], the "*Effect on pregnancy maintenance/Off-label use as an abortifacient*" is still presented as a Safety Concern. Nevertheless, the EMA-CHMP recommended that the contraindication "*pregnancy*" be removed from the information.

In spite of all the above information and aware of the fact that women mostly ovulate, even if they take ellaOne® weekly for 8 weeks, the CHMP of the EMA holds that "*Emergency contraceptives work by stopping or delaying ovulation*" [61].

On November 16th 2022 the EMA updated the EPAR on ellaOne® [14] for the nth time, always maintaining the same deceiving sentences: "*When used for emergency contraception the mechanism of action is inhibition or delay of ovulation*" (page 8) and "*ellaOne® works by postponing ovulation*" in the *Package leaflet: Information for the user* (page 39).

TOXICITY

When the Members of EMA-CHMP (Committee-Human-Medicinal-Products) recommended ellaOne® for marketing-authorization in 2009, they acknowledged that UPA accumulates in tissues, with a high tissue-to-plasma ratio (EMEA/261787/2009, page 13) [31]. They acknowledged that repeated UPA-administrations (even scheduled monthly) lead to a progressive accumulation in the liver - eventually resulting in toxicity - but also in the kidney, clitoris, ovary, uterus, adrenals, fat, uveal tract, pigmented skin and the mucosa of the gastro-intestinal tract [31]. Consequently, EMA-CHMP authorized only single-dose administration and warned against repeated assumption.

However, in 2015 this scenario changed: the EMA-CHMP removed the warning against repeated assumption and made ellaOne®-supply "*not subject to medical prescription*" (EMA/73099/2015) [33].

Since then, the repeated assumption of ellaOne® in the same cycle is allowed and suggested as safe, without any medical supervision, with the obvious consequence that millions of women repeatedly take it, whenever an UPSI should occur [33], as is indicated in the package leaflet. Current reports suggest that the percentage of women who take ellaOne® repeatedly is high: it would amount to 42% [62,63].

Given the fact that, among all tissues, only the superficial (functional) layer of the endometrium is shed periodically and renewed, the consequence of repeated assumption is a progressive accumulation of UPA wherever Progesterone receptors are expressed: that is, in the above mentioned tissues and also in the neuronal and glial cells. These cells, in fact, are able to synthesize Progesterone de novo from cholesterol and its metabolites and Progesterone regulates physiological processes, such as neuronal plasticity in normal brain function, besides reducing anxiety and depression [64,65].

Let's examine now the different tissues in which UPA toxicity has been documented or is possible.

Liver toxicity

Ulipristal, the active principle of ellaOne®, is also the active principle of a drug used to reduce the size of uterine fibroids: Esmya® (micronized-UPA, 5mg-tablets in blisters of 28). It was taken daily for cycles of three to six month, after EMA (European Medicines Agency) authorization in 2012. It needed a medical prescription and treatment was supervised by experienced doctors.

Due to the appearance of serious liver injuries in 8 Esmya®-treated patients, the EMA Pharmacovigilance-Risk-Assessment-Committee (PRAC) started an evaluation (EMA/791062/2017) [66] that concluded that UPA had a possible role in those injuries. EMA recommended measures to minimize the risk (EMA/355940/2018) [67]: to avoid prescription in patients with liver problems; to inform correctly the patients about the risk; to evaluate carefully liver-tests before, during and after treatment; to reserve repeated courses only to inoperable women that were not treatable in any other way.

On September 4th 2020, a further review by EMA-PRAC confirmed that UPA 5mg can cause liver injury, including the need for liver transplantation. Since it was not possible to identify either which patients were most at risk, or measures that could reduce the risk, the PRAC concluded that the risks outweighed its benefits and Esmya® should not be marketed in the EU (EMA/455818/2020) [68].

The risk of hepatic failure was pointed out also in Asia: three cases out of 21.000 patients treated with Esmya® were observed in Korea within six months since the treatment start, but without any need of transplantation; besides, over one-sixth of those patients (3675 out of 21.000) developed mild and severe liver diseases [69].

With Esmya® the strict post-marketing surveillance made it possible to link UPA-administration to side-effects and to observe that the time from Esmya® first-intake to hepatic failure was long, ranging from few days to six months [70].

Unexpectedly, the decision of withdrawing UPA from fibroid treatment did not affect its use for emergency contraception. On the contrary, and unnecessarily, both the 2018 and 2020 EMA-PRAC Reports on Esmya®-related risk [67,68] specify that *with ellaOne® there is no concern about liver injury*.

It must be acknowledged that no cases of hepatotoxicity had been reported after single-dose, unrepeated, administration of ellaOne® during the period in which prescription were required; however, it must be pointed out that the patient 2 in Meunier's series [70] evidenced severe liver injury after taking very small amounts of UPA: Esmya® (UPA 5mg) for only 3 days (15mg=half-ellaOne®) to 26 days.

What seems important in terms of toxicity is UPA-accumulation in tissues, whichever drug (Esmya® or ellaOne®) delivers it. The circulating levels of either UPA or its metabolites, on the contrary, seem quite irrelevant [71].

The life-threatening DILI (drug-induced-liver-injury), including autoimmune hepatitis, associated with UPA in post-marketing surveillance of Esmya®-treated patients, may be partially explained by UPA physiochemical (high lipophilicity) and pharmacokinetic (hepatic metabolism, long half-life, inhibition of liver transporters, reactive metabolite formation) features [72].

Other factors potentially contributing to significant side effects with the selective progesterone receptor modulators (SPRMs) include cross-reactivity with other steroid receptors and, in particular, with the endogenous glucocorticoid receptor (GR) [31,73]. Glucocorticoids, in fact, were originally named for their role in the regulation of hepatic gluconeogenesis and, accordingly, the liver is a major target of glucocorticoid action.

Studies based on receptor modeling and reporter assays suggest for UPA a limited GR-modulating activity, but they may not reflect the persistent effect of accumulating UPA on the endogenous receptors in tissues, an effect that might have been underestimated.

Glucocorticoid-mediated responses reflect the ligand-dependent transactivation of GR, which is characterized by receptor phosphorylation, nuclear translocation, and DNA binding. The mechanism by which Ulipristal antagonizes GR is a direct inhibition of all these critical steps of GR transactivation, independently from any action on progesterone receptor. The association between repressed glucocorticoid signaling and liver injury is important and well-supported.

UPA can bind also Androgen Receptor (AR), albeit with much lower affinity than the endogenous ligand, and demonstrates modest anti-androgenic effects. Given the protective role played by AR in regulating liver physiology also this mechanism should be considered in discussing on UPA liver toxicity. On the other hand, acute liver injury has already been reported for patients taking AR antagonists [74-76].

The most challenging form of DILI is the so-called idiosyncratic one: it is unpredictable, usually unrelated to the dose and is characterized by a variable onset-time. DILI is an important public health issue: not only it strengthens the importance of the post-marketing phase, when urgent withdrawal sometimes occurs for rare unanticipated liver toxicity, but also shows the imperfect predictivity of pre-clinical models and the lack of validated biomarkers beyond traditional, non-specific liver-function tests [77].

The burden of DILI is likely underestimated: clinical trials are usually underpowered to identify rare idiosyncratic events and most data come from post-marketing retrospective studies. DILI occurs only in a small fraction of exposed-subjects [77,78]: with UPA the percentage was 1/10.000: 8 out of 80.0000 Esmya®-patients.

Though high, the number of Esmya®-patients is limited, even if the implementation of its use, as well as the registration for its general use, were likely based upon inadequate outcomes and a limited patient representativeness in the registration trial [78].

EllaOne®, on the contrary, is taken by millions and millions of women every year. Its repeated-assumptions cannot be quantified exactly, but is reported to be around 42% [62,63]. As ellaOne® is not subject to medical prescription, no data are available for post-marketing evaluation.

The removal of both the prescription and the warning against repeated use was requested by HRA-Pharma, basing on HRA2914-554 Study (EMA Assessment Report-pages 6-9) [33,35] described above: ellaOne® was given weekly (Q7D, twelve women) or every 5 days (Q5D, eleven women) for 8 consecutive weeks. Ovulation was never inhibited, but no safety-issues emerged for *those* 23 women. HRA-Pharma and the EMA-CHMP agreed that, *should ellaOne® be used more than once in the same cycle, the safety profile is similar to that for a single administration.*

At the time when prescription was removed – in January 2015 – liver-toxicity due to Esmya®-assumption was still unreported, but the EMA-PRAC Experts can't be justified nowadays, when they insist on stating that ellaOne®-assumption is safe, while they themselves assess that UPA is directly responsible of liver-injuries [67,68].

They know very well that the total UPA-dosing for those 23 women was 270mg in Q7D and 360mg in Q5D and that these doses, presented as *safe*, are equal to or greater than Esmya®-dosing in the same 8 weeks (UPA 280mg), that is the UPA-dosing leading at least two patients to liver transplantation [70]; besides, they know well that the single UPA-bolus given to the liver by ellaOne® is six time-higher than with Esmya®.

EMA-PRAC Experts are aware that ellaOne® is taken repeatedly by millions of women, whenever an UPSI occurs, and that repeated assumption can lead to an overall UPA-intake that exceeds the UPA-amounts responsible of the severe DILI they themselves certified after Esmya®-administration [67,68]. Moreover, they know that, differently from Esmya®-treated patients, women who take ellaOne® are unaware of the liver-risks and cannot be followed-up, as prescription-registries were abolished [33].

Other possible adverse effects

Apart from the liver, in which toxicity has been well documented, UPA accumulates and binds Progesterone receptors elsewhere. Studies have been carried out even on the brain [64,65,79-83], where Progesterone is synthesized and is active in the hypothalamic neurons that take part in modulating GnRH regulation, and UPA can impair its physiological effects.

Progesterone seems to exert a protective action on the brain [83]; in rats, after injuries, it exerts a therapeutic action, but this protection has not been confirmed unequivocally in humans.

Besides, UPA may affect brain function even through other metabolic processes: in rats it induces the basal expression of classic glucocorticoid responsive genes in the hypothalamus and pituitary, suggesting GR agonist activity. Ghrelin and neuropeptide Y expression were increased, while the release of prolactin, thyroid secreting hormone, corticotropin-releasing factor, and gonadotropin-releasing factor were

decreased, resulting in an overall suppression of hypothalamic and pituitary activity [79-82].

Current perspectives

Given the proven toxicity of UPA on the liver, particularly evident in the post-marketing survey of the patients treated with Esmya® [84], and the possible interferences with the neuro-regulation of the menstrual cycle, it is surprising to find studies, like the multi-center phase 1 and/or 2 trial by Westhoff et al., that enroll randomized participants to use oral Ulipristal 10 mg or 5 mg daily or a 3 cycle regimen of 5 mg for 24 days followed by four placebo days, with the purpose of evaluating whether Ulipristal might have potential as a daily oral contraceptive [85].

Absence of any progesterone elevations, suggesting consistent ovulation inhibition throughout treatment, was reported only in 52 of 137 (37%) participants; 53%, 45%, and 15% % among those randomized to the 10 mg, 5 mg, and cyclic treatments. Conversely, progesterone elevations occurred among many participants (85 of 137, 62%) at least once during treatment, particularly in those receiving the 5 mg cyclic treatment. Ovulation prevalently occurred during UPA administration, even with these schemes.

UPA (5 or 10 mg daily) had already been experimented as a continuous oral contraceptive in fertile women before the emergence of UPA liver toxicity: one study evidenced a lack of progesterone elevation during the third month of use, but without checking its level during the administration [86]. Others administered UPA as a contraceptive vaginal ring and suggested infrequent ovulation with higher doses or higher circulating levels of Ulipristal [87,88].

Westhoff's study – published in the last Spring - cannot ignore the damages and risks linked to the use of Esmya®, but insists in proposing the same (5mg) and even the double (10mg) daily dose of UPA to fertile women, uninformed of the possible hepatic risk.

Besides, these fertile women are likely young and have a current and future interest in maintaining fertility and nobody knows what can be the long term effects of Progesterone Receptors blocking either in the SNC and in the other non-shading tissues. Furthermore, it is unknown whether Ulipristal possess tissue-specific GR-agonist activity also in the human brain, as shown in the rats, in which UPA has been documented to have the potential to disrupt tissue-selective antagonist and agonist effects on GR in the hypothalamic-pituitary-adrenal axis.

CONCLUSIONS

In spite of what is shared by Scientific Societies and EMA, that present ellaOne® as an anti-ovulatory drug, its main mechanism of action in emergency contraception is an anti-implantation one, and a direct ability to terminate pregnancies cannot be excluded. In the most fertile days of the menstrual cycle ellaOne® never prevents or delays ovulation, and consequently fertilization can ensue, but it consistently transforms the endometrium into a quite unhospitable ground.

The post-marketing evaluation of the administration of Esmya®, which is based on UPA as is ellaOne®, led to its withdrawal from commerce, due to the highly severe liver injuries caused by UPA progressive accumulation in tissues.

Surprisingly, no caution is suggested to women taking ellaOne®: on the contrary, they are reassured that repeated assumption even in the same cycle is safe, without any advice of medical surveillance and without any information on UPA accumulation and toxicity.

Women, mostly teen-agers, are evidently deceived about both the mechanism of action and safety.

Lastly, it appears unbelievable and unjustified that someone can imagine to introduce UPA in continuous contraceptive pills to be offered to healthy fertile unaware women at the daily dose of 5 or 10 mg. Apart the fact that ovulation mostly occurs, so that fertilization cannot be avoided, the administered dosing would be the same as in Esmya®, or even double. These pills would be offered for long-lasting contraception, for periods much longer than with Esmya®; UPA accumulation in the liver and tissues would be far higher than that induced by Esmya®, which led to its removal from the market. Besides, the possible risks for future fertility cannot be excluded.

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