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ORIGINAL ARTICLE ***Fertility control***

In vitro and *in vivo* effects of ulipristal acetate on fertilization and early embryo development in mice

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STUDY QUESTION: Does ulipristal acetate (UPA), a selective progesterone receptor modulator used for emergency contraception (EC), interfere with fertilization or early embryo development *in vitro* and *in vivo*?

SUMMARY ANSWER: At doses similar to those used for EC, UPA does not affect mouse gamete transport, fertilization or embryo development.

WHAT IS KNOWN ALREADY: UPA acts as an emergency contraceptive mainly by inhibiting or delaying ovulation. However, there is little information regarding its effects on post-ovulatory events preceding implantation.

STUDY DESIGN, SIZE, DURATION: This was an *in vitro* and *in vivo* experimental study involving the use of mouse gametes and embryos from at least three animals in each set of experiments.

PARTICIPANTS/MATERIALS, SETTING, METHODS: For *in vitro* fertilization experiments, mouse epididymal spermatozoa capacitated in the presence of different concentrations of UPA (0–1000 ng/ml) were used to inseminate cumulus-intact or cumulus-free eggs in the presence or absence of UPA during gamete co-incubation, and the percentage of fertilized eggs was determined. For *in vivo* fertilization experiments, superovulated females caged with proven fertile males were injected with UPA (40 mg/kg) or vehicle just before or just after mating and the percentage of fertilized eggs recovered from the ampulla was determined. To investigate the effect of UPA on embryo development, zygotes were recovered from mated females, cultured in the presence of UPA (1000 ng/ml) for 4 days and the progression of embryo development was monitored daily.

MAIN RESULTS AND THE ROLE OF CHANCE: *In vitro* studies revealed that the presence of UPA during capacitation and/or gamete co-incubation does not affect fertilization. Whereas the *in vivo* administration of UPA at the same time as hCG injection produced a decrease in the number of eggs ovulated compared with controls (vehicle injected animals, $P < 0.05$), no effects on fertilization were observed when UPA was administered shortly before or after mating. No differences were observed in either the percentage of cleaved embryos or the cleavage speed when UPA was present during *in vitro* embryo culture.

LIMITATIONS, REASONS FOR CAUTION: Considering the ethical and technical limitations inherent to the use of human gametes for fertilization studies, the mouse model was used as an approach for exploring the potential effects of UPA on *in vivo* sperm transport and fertilization. Nevertheless, the extrapolation of these results to humans requires further investigation.

WIDER IMPLICATIONS OF THE FINDINGS: This study presents new evidence on the lack of effect of UPA on gamete interaction and embryo development, providing new insights into the mechanism of action of UPA as an emergency contraceptive method with potential clinical implications. These new findings could contribute to increase the acceptability and proper use of UPA as an emergency contraceptive method.

STUDY FUNDING/COMPETING INTEREST(S): This study was partially supported by a National Agency of Scientific and Technological Promotion (ANPCyT), Argentina grants PICT 2011-061 to D.J.C. and PICT 2011-2023 to P.S.C. None of the authors has any competing interests to declare.

Key words: emergency contraception / ulipristal acetate / sperm / fertilization / embryo development

Introduction

Emergency contraception (EC) is an important back-up method in cases of unprotected intercourse or failure of a regular contraceptive method. The efficacy of emergency contraceptive pills depends on several factors including how and when they are used and their mechanism of action. An ideal emergency contraceptive method should be safe and acceptable, readily available and act through mechanisms that make it effective during the entire fertile window (Gemzell-Danielsson and Trussell, 2013).

Progesterone (P) is a key modulator of normal female reproductive functions and its actions are required for ovulation as well as for providing an appropriate uterine environment for maintenance of pregnancy (Niswender *et al.*, 2000; Wira *et al.*, 2015). These biological effects of P are mediated by modulation of the transcriptional activity of two specific intracellular P receptors (PRs) termed PR-A and PR-B (Mulac-Jericevic and Conneely, 2004). In addition, it has been reported that P also facilitates sperm-egg interaction within the female genital tract in several species (Libersky and Boatman, 1995; Holt and Fazeli, 2010) through the regulation of different sperm capacitation-associated processes. In this regard, it has been shown that in spermatozoa, P is able to induce calcium influx (Blackmore *et al.*, 1990, 1991; Lishko *et al.*, 2011; Strunker *et al.*, 2011), protein tyrosine phosphorylation (Chung *et al.*, 2014) and other signalling cascades that end in the occurrence acrosome reaction, hyperactivation and chemotaxis (Uhler *et al.*, 1992; Teves *et al.*, 2006; Baldi *et al.*, 2009; Sagare-Patil *et al.*, 2012). As spermatozoa are transcriptionally silent cells, all these effects are most likely mediated through non-genomic membrane P receptor/s whose identity and localization have not yet been completely elucidated.

Considering the importance of P in the fertilization process, synthetic compounds ranging from pure antagonists to a mixture of agonistic/antagonistic actions on nuclear PR have been developed with applications in contraception. Ulipristal acetate (UPA; 17-alpha-acetoxy-11-beta-[4-*N*,*N*-dimethylaminophenyl]-19-norpregna-4,9-diene-3,20-dione; derivative of 19-norprogesterone) is a selective PR modulator, which has been introduced into the market as an emergency contraceptive pill in different countries. UPA binds with high affinity to both PR-A and PR-B isoforms exhibiting mixed agonistic/antagonistic properties (Blithe *et al.*, 2003; Chabbert-Buffet *et al.*, 2005; Gemzell-Danielsson and Meng, 2010). Although UPA is highly effective for EC (Glasier *et al.*, 2010; Cheng *et al.*, 2012), the mechanisms underlying its action are not completely understood. Its biological effects depend on the stage of the menstrual cycle in which it is administered. Before the LH peak, its primary mode of action is to inhibit or delay follicular rupture, improving the effectiveness in pregnancy prevention compared with pre-existing contraceptive methods (Brache *et al.*, 2013). However, when administered at or after the LH peak, UPA cannot prevent ovulation (Brache *et al.*, 2010). Considering that unprotected sexual intercourse may occur at any time of the female menstrual cycle, it is important to establish whether UPA can work for EC even when administered at or after the LH peak. After ovulation, the oviduct constitutes an excellent site for UPA action as it can interfere with gamete transport and storage, fertilization or early embryo development. It was previously reported that human spermatozoa exposed *in vitro* to UPA concentrations similar to those found in the serum of emergency contraceptive pill users (100–200 ng/ml) (Blithe *et al.*, 2003) present no differences in viability, protein tyrosine phosphorylation or spontaneous or follicular fluid-induced acrosome reaction (Munuce *et al.*, 2012). However, a significant decrease in sperm DNA fragmentation was observed in the presence of UPA, probably due to the capacity of this compound to capture oxygen-free radicals (Munuce *et al.*, 2013). Whereas these results do not indicate a direct effect of UPA on sperm function, recent reports have revealed that UPA inhibits ciliary beat and muscular contraction of the human Fallopian tube *in vitro* (Li *et al.*, 2014), opening the possibility of post-ovulatory effects of UPA on gamete transport, fertilization and/or embryo development.

Considering the unfeasibility of sperm transport and *in vivo* fertilization studies in humans owing to ethical and technical limitations, the mouse model represents a tool for exploring the potential effects of UPA on these processes. In this regard, it has been shown that the administration of UPA up to 8-h post-hCG (ovulation occurs 12-h post-hCG) inhibits mouse ovulation (Nallasamy *et al.*, 2013) indicating that female mice respond to UPA in a similar way than humans. In view of this, the aim of this work was to evaluate the potential of UPA to regulate *in vitro* and *in vivo* mouse gamete interaction or embryo development.

Materials and Methods

Ethical approval

Approval for the study protocol was obtained from the Bioethics Committee of IBYME-CONICET (no. CE010-Abril/2014) and the Institutional Review Board of the School of Biochemical and Pharmaceutical Sciences, National University of Rosario. Experiments involving animals were performed at IBYME-CONICET in strict accordance with the Guide for Care and Use of Laboratory Animals approved by the National Institutes of Health (NIH).

Animals and reagents

Adult (60–120 days old) male, immature (21–35 days old) and young adult (60–120 days old) female hybrid (C57BL/6xBALB/c)F1 mice were used. Animals were maintained with food and water *ad libitum* in a temperature-controlled room (23°C) with light:dark (12:12 h, lights on: 7:00 AM) cycle. All reagents and chemicals were of molecular biology grade and were purchased from Sigma-Aldrich Chemicals (St Louis, MO, USA), unless otherwise specified. For *in vitro* experiments, a stock solution of UPA (1 mg/ml, HRA Pharma, Paris, France) was prepared in ethanol and stored at −20°C until use. Working solutions were further diluted on the day of the experiment to obtain the range of UPA concentrations used.

Sperm capacitation

Mouse spermatozoa were recovered by incising the cauda epididymis in 300 µl capacitation medium (Fraser and Drury, 1975) supplemented with 0.3% (w/v) bovine serum albumin under paraffin oil (Ewe, Sanitas SA, Buenos Aires, Argentina). Aliquots of the suspension were added to 300 µl fresh medium previously placed in tissue culture dishes containing UPA (10–1000 ng/ml) or an equivalent volume of ethanol (control) to give a final concentration of $1–10 \times 10^6$ cells/ml. These concentrations of UPA were chosen from the pharmacokinetic data of the 30 mg oral tablet rendering a peak plasma concentration of UPA (bound and unbound) 1 h after ingestion of 176 ± 89 ng/ml (Blithe *et al.*, 2003; Snow *et al.*, 2011). Sperm suspensions were then incubated for 90 min under paraffin oil at 37°C in an atmosphere of 5% (v/v) CO_2 in air.

Acrosome reaction assays

Mouse spermatozoa were incubated for 90 min in the presence of different concentrations of UPA (10–1000 ng/ml) and then evaluated for both spontaneous and P-induced acrosome reaction. For induction of the acrosome

reaction, P (15 μM final concentration) in dimethylsulfoxide was added to spermatozoa for the last 15 min of incubation under capacitating conditions. The acrosomal status was evaluated by Coomassie Brilliant Blue staining as previously described (Busso *et al.*, 2007). At least 400 spermatozoa were evaluated in each treatment slide in a light microscope (×400) and the percentage of spermatozoa without an intact acrosome was calculated.

Sperm viability determination

Sperm viability was assessed by dye exclusion using 0.5% (v/v) Eosin Y and the percentage of viable spermatozoa was calculated as the number of spermatozoa that did not incorporate the dye divided by the total number of spermatozoa counted in the light microscope (×400).

Egg collection and *in vitro* fertilization assays

In vitro fertilization was performed as previously described (Curia *et al.*, 2013). Briefly, 3–4 female mice per experiment were superovulated by an injection (i.p.) of equine chorionic gonadotrophin (eCG; 5UI; Syntex, Argentina) 1 h before the lights turned out, followed by the administration (i.p.) of human chorionic gonadotrophin (hCG; 5UI) 48 h later. Egg–cumulus complexes were collected from the oviducts 13–14 h after hCG administration and pooled. When required, cumulus cells were removed by incubating the complexes for 3 min in 0.3 mg/ml hyaluronidase (Type IV). Cumulus-intact eggs or cumulus-free zona pellucida-intact eggs were inseminated with capacitated spermatozoa (final concentration: $5–6 \times 10^5$ spermatozoa/ml) and gametes were co-incubated for 5 h in the presence or absence of UPA (10–1000 ng/ml) or ethanol at 37°C in an atmosphere of 5% (v/v) CO_2 in air. Eggs were then transferred to fresh medium and 15 h later the number of 2-cell embryos was recorded.

Effect of UPA on ovulation

UPA was dissolved in sesame oil and administered i.p. at 40 mg/kg body weight at the same time as the hCG injection. Females injected with the same volume of vehicle (100 μl) were used as controls. In both cases, eggs were collected as described above 14 h after administration of hCG and counted.

In vivo fertilization assays

Estrous cycle stage of mature females was determined every morning by the analysis of vaginal smears. Briefly, vaginal epithelial cell samples were collected in 30 μl of PBS with a pipette, placed on glass slides and visualized. The different stages are characterized by the proportion of the three cell types, i.e. leukocytes, cornified and nucleated epithelial cells present in the sample. In proestrous stage, there is a predominance of nucleated epithelial cells (Caligioni, 2009). A pair of females in the proestrous stage were superovulated and caged with proven male breeders 12 h after hCG administration. Forty minutes later, successful mating was confirmed by the presence of a copulatory plug. One female mouse received an i.p. injection of 40 mg/kg UPA and the other one, sesame oil (vehicle) before or after mating according to the experiment. This dose was chosen because it was previously shown to be the most effective in preventing ovulation in mice (Palanisamy *et al.*, 2006; Nallasamy *et al.*, 2013). Seven hours after confirmation of mating, eggs were recovered from the oviducts, stained with 10 μg/ml Hoechst 33342, mounted on slides and analysed in a Nikon Optiphot microscope (Nikon, Tokyo, Japan) equipped with epifluorescence optics (×250). Eggs presenting either decondensing sperm heads or pronuclei in the cytoplasm were considered as fertilized.

Effect of UPA on *in vitro* mouse preimplantation embryo development

Immature female mice (two per experiment) were superovulated (eCG: 4 h before lights turned out, hCG: 6 h before lights turned out) and mated with male mice of proven fertility. Effectiveness of mating was confirmed by identification of a copulatory plug. Twenty hours after hCG injection, zygotes were recovered from the oviducts in KSOM medium (Erbach *et al.*, 1994). Embryos were transferred to 50 μl drops of fresh KSOM medium supplemented with 1000 ng/ml UPA or control at 37°C in a 5% (v/v) CO_2 atmosphere in air and incubated for 4 days. Embryo development was evaluated daily in an inverted microscope (Nikon Eclipse TS100, Nikon) and the developmental stage of each embryo was determined.

Statistical analysis

Values are presented as mean ± standard error of the mean (SEM) from at least three independent experiments. Statistical analyses were performed by using the GraphPad Prism software (San Diego, CA, USA). Statistical significance of the data was analysed with two-way analysis of variance with the Bonferroni post-test for acrosome reaction and *in vitro* fertilization assays or Student's *t*-test for viability, ovulation, *in vivo* fertilization and embryo development assays. In all cases, data were considered significantly different when the *P*-value was <0.05.

Results

To evaluate the effect of UPA on gamete interaction *in vitro*, mouse spermatozoa were capacitated in the presence of different concentrations of UPA (10–1000 ng/ml) and used to inseminate cumulus-intact eggs in the presence or absence of UPA during gamete co-incubation. Evaluation of sperm viability at the end of the capacitation period with the maximum concentration of UPA (1000 ng/ml) showed no differences in this parameter compared with the controls (data not shown), confirming the lack of toxicity of the drug on spermatozoa during capacitation. No significant differences were observed in the percentage of eggs fertilized at any of the concentrations and conditions tested (Fig. 1a). Considering the beneficial effects that cumulus cells exert on spermatozoa (Yanagimachi, 1994), the ability of UPA-treated spermatozoa to penetrate cumulus-free eggs was also analysed. No differences in the percentage of fertilized eggs were observed when the maximum concentration of UPA was present during sperm capacitation and/or gamete co-incubation (Fig. 1b). Consistent with fertilization results, no differences in the percentage of spontaneous or P-induced acrosome reaction were detected at any of the concentrations of UPA assessed (Fig. 1c).

Considering that the *in vivo* capacitation process is associated with sperm transport within the female tract and sperm interaction with the oviductal cells (Yanagimachi, 1994), we also evaluated whether UPA could affect these events and, thus, *in vivo* fertilization. For this purpose, a single dose of UPA (40 mg/kg body weight) or vehicle (control) was administered to superovulated females shortly before or after mating, and the percentage of penetrated eggs was evaluated. In parallel, to confirm the inhibitory effect of UPA on ovulation, a group of females received UPA together with the hCG injection, and the number of ovulated eggs was counted. Results revealed that whereas the administration of UPA together with hCG caused a significant decrease in the number of eggs recovered from the ampulla compared with controls (Fig. 2a), its administration before or after mating produced no differences in either the total number of ovulated eggs (Fig. 2b) or the percentage of *in vivo* fertilized eggs (Fig. 2c).

Additionally, to determine whether UPA impairs mouse preimplantation embryo development, zygotes were cultured in the presence of 1000 ng/ml UPA and the progression of development was monitored daily. No significant differences in either the percentage of developed

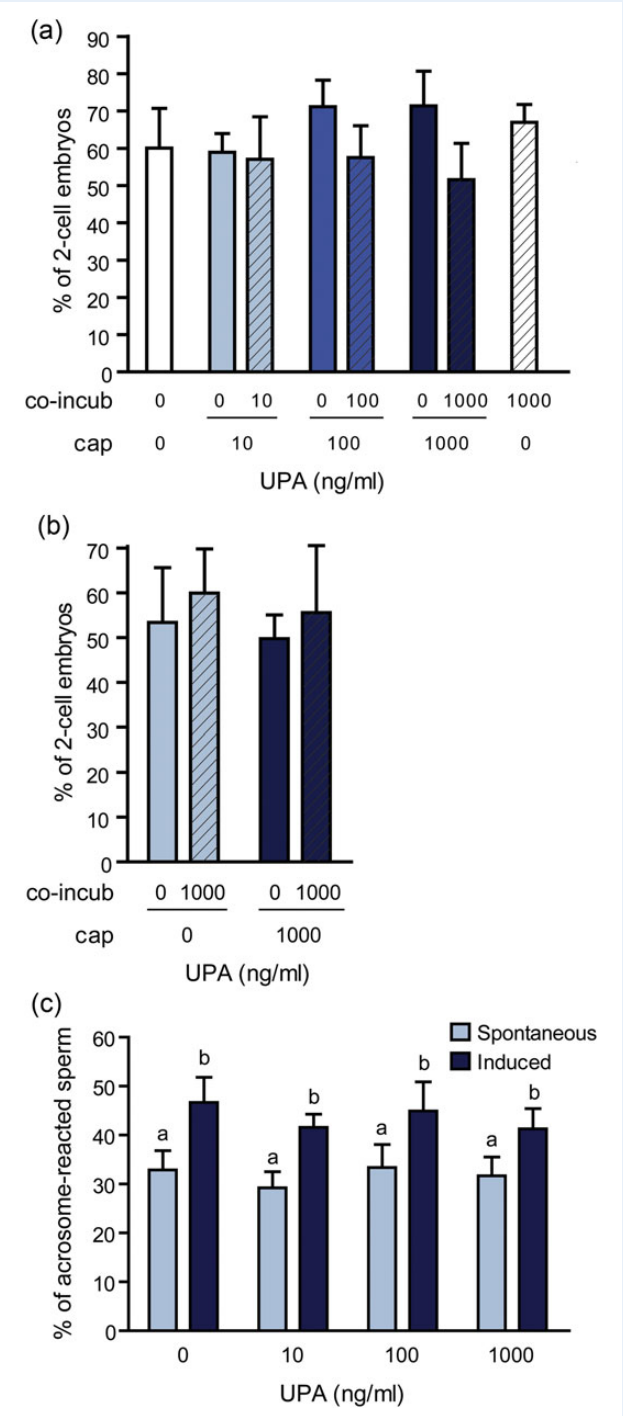


Figure 1 Effect of UPA on sperm fertilizing ability *in vitro*. Mouse spermatozoa were capacitated for 90 min in the presence of different concentrations of UPA (cap, 0–1000 ng/ml) and used to inseminate cumulus-intact (**a**) or cumulus-free eggs (**b**) in the presence or absence of UPA during gamete co-incubation (co-incub). The number of 2-cell embryos was scored 20 h later. The values are presented as mean ± SEM of 5 (a) or 3 (b) independent experiments. (**c**) Spontaneous and progesterone (15 μM)-induced acrosome reaction were evaluated by staining spermatozoa with Coomassie Brilliant Blue. The values are presented as mean ± SEM of 8 independent experiments. Bars with different letters are significantly different, $P < 0.05$.

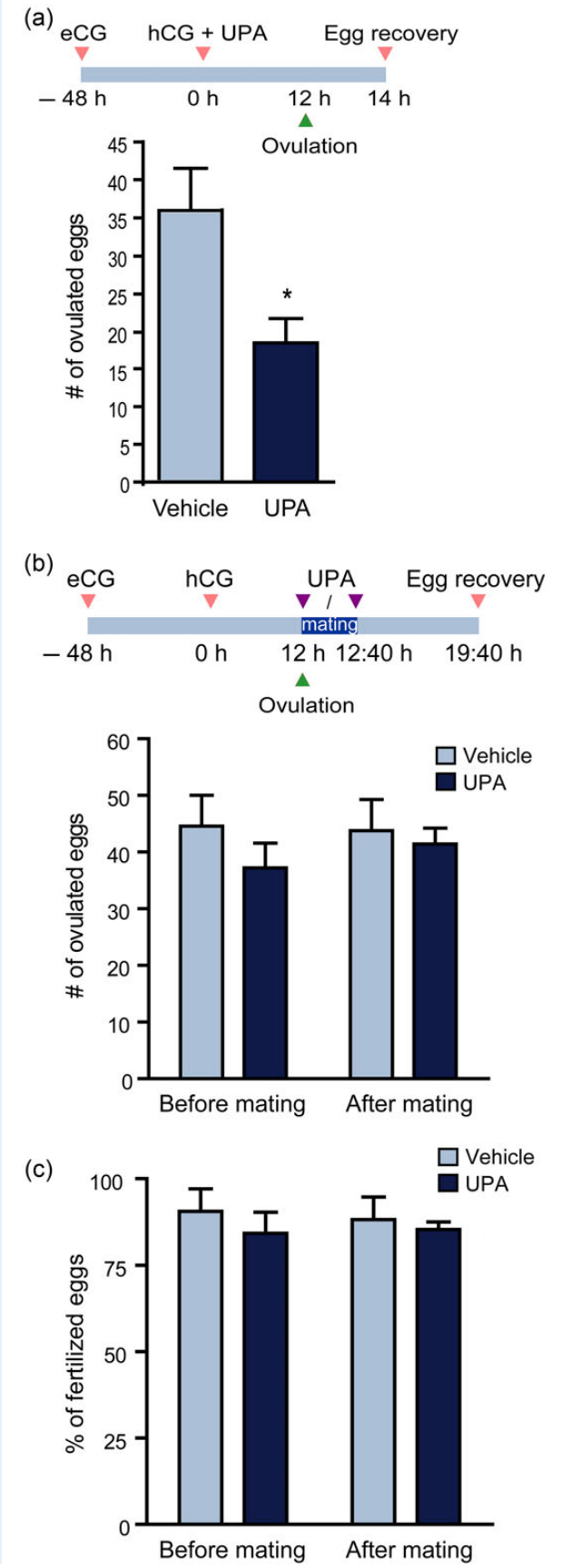
embryos (percentage of blastocysts, control: 99 ± 1 versus UPA: 98 ± 3) or the kinetics of development were observed between embryos cultured in the presence or absence of UPA (Fig. 3).

Discussion

The emergency contraceptive pill is becoming more commonly used worldwide. In the recent years, UPA, a selective PR modulator, has been introduced in the market as a more efficient emergency contraceptive option than the existing methods (Glasier *et al.*, 2010) by extending the window of action to up to 5 days after intercourse compared with the 3 days obtained with the widely used levonorgestrel pill. Although it is well established that the main action of UPA is to delay or to inhibit follicular rupture (Brache *et al.*, 2010), it is crucial to elucidate its mechanisms of action for correct clinical recommendations and use. This study shows that UPA, at concentrations similar to those reached in the female serum after oral intake of the emergency contraceptive pill, does not interfere with sperm transport through the female tract, *in vivo* and *in vitro* gamete interaction or embryo development.

It has been clearly stated that P modulates different events in spermatozoa that are associated with the capacitation process they undergo while migrating through the female tract (Yanagimachi, 1994; Baldi *et al.*, 2009). Therefore, after unprotected intercourse and UPA intake, spermatozoa may be exposed to the drug affecting their functions. Furthermore, it has been proposed that muscular contractions of the human oviduct at the time of ovulation could activate spermatozoa stored in the isthmic region, increasing their chances of encountering the egg, and that the tubal microenvironment plays an important role in ensuring normal embryo development (Holt and Fazeli, 2010). In this way, factors that modify tubal ciliary beating may interfere with conception by affecting gametes or embryos, or by altering the timing of embryo arrival at the uterine cavity. As it has been reported that UPA *in vitro* modulates muscle contractions and ciliary beating in Fallopian tube samples (Li *et al.*, 2014), this might be one of the potential mechanisms of action of this drug. However, scarce information was available on the effects of UPA on events occurring after ovulation and before implantation. In this regard, our *in vitro* assays allowed the evaluation of the effects of UPA on sperm capacitation, sperm-egg interaction and embryo development whereas the *in vivo* approach also allowed the analysis of its effects on sperm transport.

The cumulus cells that accompany the egg after ovulation continuously synthesize and secrete P that modulates sperm function (Baldi *et al.*, 2009). In this regard, the presence of UPA did not affect *in vitro* fertilization when cumulus–egg complexes were used supporting the view that both hyperactivation and acrosome reaction occurred normally in UPA-incubated spermatozoa. Furthermore, no effect of UPA was observed on the P-induced acrosome reaction. Altogether, these results suggest that the interaction of P with its receptor/s in spermatozoa is not antagonized by UPA at the tested concentrations. Although it has been shown that UPA blocks the two isoforms of the genomic PR in other cellular systems (Attardi *et al.*, 2004), this inhibitory mechanism is unlikely to occur in spermatozoa which are transcriptionally inert cells. A non-genomic plasma membrane PR, probably involved in the induction of the acrosome reaction, has been found in the acrosomal region of sperm from several species (Contreras and Llanos, 2001; Wu *et al.*, 2005, 2006). The lack of effect of UPA on the P-induced acrosome reaction in mouse is consistent with previous observations



showing that UPA does not prevent the occurrence of the follicular fluid-induced acrosome reaction in human sperm (Munuce *et al.*, 2012). A recent study shows an inhibitory effect of UPA on P-induced acrosome reaction in human sperm (Ko *et al.*, 2014); however, this inhibition was only observed at drug concentrations higher than those present in the sera of UPA users (Blithe *et al.*, 2003; Snow *et al.*, 2011). Recent reports have shown that P modulates in human spermatozoa the activity of CatSper (Lishko *et al.*, 2011; Strunker *et al.*, 2011), a key Ca^{2+} channel localized in the sperm flagellum essential for hyperactivation and male fertility (Ren *et al.*, 2001; Smith *et al.*, 2013). However, as mouse CatSper is not regulated by P (Lishko *et al.*, 2011), further experiments with human spermatozoa will be required to evaluate the potential effects of UPA on CatSper-mediated events.

In vitro fertilization experiments were also performed with cumulus-free eggs, a more restricted condition in which deficiencies in sperm fertilizing ability can be more clearly evidenced (Nishimura *et al.*, 2004; Da Ros *et al.*, 2008). In addition, under this condition P is not present during sperm capacitation or gamete interaction. In spite of this, no differences in the percentage of fertilized eggs were observed between groups, supporting the non-interference of UPA with sperm-egg interaction. From these observations, it can be postulated that sperm plasma membrane PRs would not be modulated by UPA.

Considering the unfeasibility of *in vivo* studies in humans, we designed an *in vivo* fertilization protocol using the mouse as an experimental model. The decrease in the number of ovulated eggs recovered from the ampulla of females injected with UPA at the moment of the hCG injection confirmed that the administration route and dose of UPA chosen in our studies were effective. The inhibition of ovulation observed however was lower than that previously reported (Nallasamy *et al.*, 2013). This difference could be due to the fact that in the previous study the authors used immature females and longer times to confirm ovulation, while our studies involved the use of adult females and checking times closer to ovulation, resembling the situation faced during EC. When UPA was injected just before or after mating, no effect on the number of eggs present in the oviduct was observed, confirming that the efficacy of UPA as an antiovulatory agent declines markedly when administered after the LH/hCG peak (Brache *et al.*, 2013). Our results constitute the first *in vivo* evidence showing that the administration of UPA to females after mating does not affect sperm transport and fertilization. The finding that injection of UPA just before mating did not produce a decrease in fertilization indicates that the rapid transport of spermatozoa described both in mouse and humans (Suarez, 2006) is also unaffected by UPA. Although the ciliary beating and muscle contraction inhibitions produced *in vitro*

Figure 2 Effect of UPA on sperm fertilizing ability *in vivo*. (**a**) Female mice were superovulated by injection of eCG followed by the administration of hCG 48 h later. Vehicle (sesame oil) or UPA (40 mg/kg) was administered at the same time as hCG, and the number of ovulated eggs was counted 14 h later. $^*P < 0.05$. (**b** and **c**) Superovulated females were caged 12 h post-hCG with males of proven fertility for 40 min. Vehicle or UPA was injected before or after mating. Seven hours after mating was confirmed, the number of total eggs (b) and the percentage of fertilized eggs (c) were determined. The values are presented as mean ± SEM of at least 5 independent experiments.

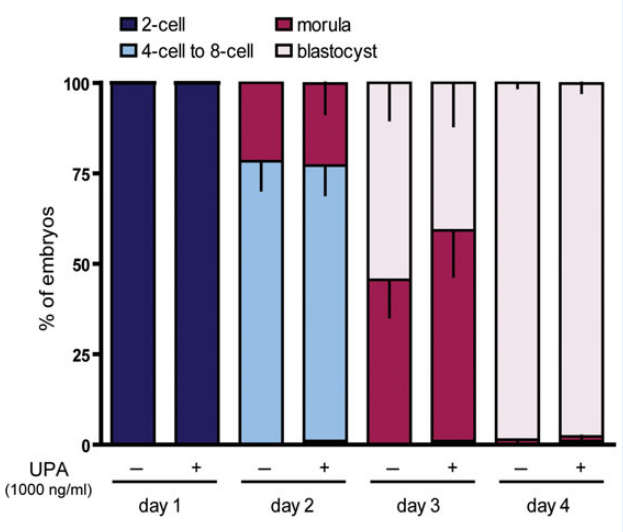


Figure 3 Embryo development in the presence of UPA. Zygotes were incubated in medium alone (control) or containing 1000 ng/ml UPA. The percentage of embryos in each developmental stage, i.e. 2-, 4- and 8-cell embryos, morula and blastocyst was determined during the following 4 days. The values are presented as mean ± SEM of 5 independent experiments using 7–17 embryos/drop.

by UPA have been proposed as an additional mechanism for its contraceptive action (Li *et al.*, 2014), our *in vivo* results showing high levels of fertilized eggs in females treated with UPA do not support this possibility.

Our *in vitro* experiments showed the lack of effect of UPA on embryo development as judged by the similar cleavage rate and percentage of blastocysts obtained in the UPA-treated and control groups. These results are similar to those obtained with levonorgestrel (Munuce *et al.*, 2005) but different from those showing that mifepristone, another EC method used in several countries (Benagiano *et al.*, 2014), retards *in vivo* and *in vitro* development of mouse embryos (Roblero *et al.*, 1987; Yang and Wu, 1990), supporting that the mechanism of action of mifepristone might be different from that of UPA and levonorgestrel. It has been shown that UPA has post-coital antifertility effect when given orally to rats during the implantation day (Reel *et al.*, 1998). In this regard, our observations, together with a recent report showing that UPA does not affect human embryo attachment to endometrial constructs *in vitro* (Berger *et al.*, 2015), support the idea that UPA would not affect embryo development or its interaction with the endometrium in the dosage used for EC. Nevertheless, the possibility that UPA affects embryo transport to the implantation site cannot be excluded. Taking into account that the proportion of time the fertilized eggs remain in the ampulla and the ithsmus differs between human and mouse (Croxatto, 2002), another experimental model should be used to explore this possibility.

Altogether, our results provide additional information on the mechanism of action of UPA with potential clinical implications as they show that a direct action of UPA on sperm function or transport would be of low significance. These new findings improve our knowledge on the mechanism of action of UPA and could contribute to increase its acceptability and proper use.

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Authors' roles

M.D.G.E. conducted all the experimental procedures and analysed the data. M.J.M. designed the experimental protocols, analysed the data and participated in drafting the manuscript. L.B. provided reagents and participated in designing the study. P.S.C. conceived the study, analysed the data and participated in drafting the manuscript. D.J.C. was responsible for conducting the study, analysing the data and writing the manuscript which was critically discussed, edited and approved by all co-authors.

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Conflict of interest

None declared.

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