Influence of an antiprogestin (onapristone) on in vivo and in vitro fertilization

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Abstract

The effects of a progesterone antagonist (onapristone) on heat synchronization, luteinizing hormone (LH) surge, ovulation, oocyte maturation and fertilization of superovulated ewes were studied. Its effects on in vitro bovine oocyte maturation and fertilization were also studied. Estrus synchronization and superovulation treatments were applied to 39 adult ewes using an intravaginal sponge with flugestrogen acetate for 9 days with injections of prostaglandin F2α and pregnant mare’s serum gonadotrophin given 24 h before sponge withdrawal. The animals were randomly assigned to four different groups; T1 receiving only the synchrony treatment (n = 11); T2 ewes received two injections of onapristone (1 mg kg⁻¹, i.v.) 12 h apart from 3 h after sponge withdrawal (n = 10); T3 ewes received two injections of progesterone 12 h apart from sponge withdrawal (n = 10); and, T4 ewes received both onapristone and progesterone as described (n = 8). Ewes were mated by a fertile male during estrus. Progesterone and LH were measured during the superovulation period in plasma samples taken every 4 h. Uterine flushings for ova recovery were performed at 5 days (n = 25), 48 h (n = 5) and 24 h (n = 5). Non-fertilized oocytes collected at 24 and 48 h were checked for meiosis resumption. The effects of two doses of onapristone (D1 and D2) on in vitro bovine oocyte maturation (control = 100, D1 = 100 and D2 = 100) and fertilization (control = 107, D1 = 40 and D2 = 75) were also studied.

The percentage of animals showing heat signs was significantly lower in group T3 (50% vs. 100%). The onset of oestrus (27.6, 24.8, 68.8 and 25.5 h, respectively for T1, T2, T3 and T4) and an LH surge (32.3, 28.8, 76.5 and 30.5 h, respectively for T1, T2, T3 and T4) after sponge withdrawal were significantly delayed in group T3. There were no significant differences in the

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intervals between estrus and LH surge among groups (4.61 ± 0.75 h). The response and ovulation rates until 40 h after sponge withdrawal (group T3 excluded) were similar among groups, but the fertilization rates were significantly lower in groups T2 and T4 when compared with T1 (2% and 3% vs. 41%, respectively; P < 0.001) due to sperm arrest in the cervix. Ova recovery rate decreased significantly from 24–48 h to 5 days and was not affected by treatments (76.9% vs. 37.1% respectively). Onapristone did not affect the resumption of meiosis. Fertilization of bovine oocytes in vitro decreased significantly only in group D2 when compared to control (48% vs. 62.6%, respectively). In conclusion, onapristone treatment during the preovulatory period did not interfere with normal synchronization of estrus, ovulation and oocyte maturation but severely compromised fertilization by arresting spermatozoa in the cervix.

Keywords: Ovine; Superovulation; Fertilization; Oocyte maturation; Gonadotropin; Progesterone antagonists; Onapristone; ZK98299

1. Introduction

In cows and ewes superovulated with pregnant mare's serum gonadotrophin (PMSG), it was found that during the follicular phase plasma levels of progesterone could abnormally increase up to levels found during the luteal phase (Callesen et al., 1988; Cavaco Goncagalves et al., 1992). In cattle, these deviating patterns of progesterone which interfered with luteinizing hormone (LH) surge, were associated with inferior superovulatory response and embryo quality (Callesen et al., 1988). It was reported that these premature high levels of progesterone, in superovulated ewes, delayed signs of heat until they dropped to basal concentrations (Cavaco Gonçalves et al., 1992). This asynchronous synthesis of progesterone after administration of exogenous gonadotrophins is likely to be an effect of their LH activity (Boland et al., 1991) which stimulates progesterone synthesis by granulosa cells (Kuran et al., 1995).

To date no other antiprogestins have been studied so extensively as mifepristone (RU486) and onapristone (ZK98299) (Neef et al., 1984). Onapristone, a potent steroidal (1β-aryl substituted) antiprogestin being unnaturally configured at C-13 and bearing a terminal hydroxylated 17β-propyl side chain, is chemically different from mifepristone, the first described progesterone antagonist. Onapristone binds to the progesterone, glucocorticoid and androgen receptors. Depending on the model used, onapristone exhibits antiprogestagenic activity equal or superior to that of mifepristone (Elger et al., 1988; Chwalisz et al., 1995). However, its antiglucocorticoid activity is lower than that of mifepristone (Elger et al., 1988). Studies using mifepristone during the luteal phase of the estrous cycle and gestation confirmed an antiprogestin action of the drug in ewes (Morgan et al., 1993; Gazal et al., 1993). To our knowledge, the only report dealing with the administration of antiprogestins during the follicular phase in the ewe is that of Cavaco Gonçalves et al. (1992). In this work, mifepristone could reverse the inhibitory action of endogenous progesterone on estrous behaviour of superovulated ewes.

The purpose of this work was to study the effects of the antiprogestin onapristone on estrus behaviour, ovulation, oocyte maturation, fertilization and embryo production in superovulated ewes after synchronization treatments. The drug effects on in vitro bovine oocyte nuclear maturation and fertilization were also studied.
2. Materials and methods

2.1. In vivo experiments

A total of 39 adult Merino ewes weighing 45.5 ± 6.3 kg (mean ± standard deviation) were used to study the effect of onapristone (ZK98299, Schering) during the superovulation process.

All groups were synchronized with intravaginal sponges containing fluorgestone acetate (FGA, 40 mg/animal, Chronogest, Intervet) for nine days. PMSG for superovulation purpose (1500 IU intramuscularly/animal, Intergonian 500, Intervet) and prostaglandin F2α for luteolysis purpose (PGF2α, 7.5 mg intramuscularly/animal, Dinolytic, UpJohn), were both administered 24 h prior to sponge withdrawal. Animals of group T1 (n = 11) received only the above treatment and served as controls.

The progesterone antagonist onapristone (11β-(4-dimethylaminophenyl)-17α-hydroxy-17-(3-hydroperoxypropyl)-13α-estra-4,9-dien-3-one, Schering) was administered twice, 12 h apart, beginning at 3 h after sponge withdrawal to animals of groups T2 (n = 10) and T4 (n = 8) (1 mg kg⁻¹ body weight, intravenously). Prior to injection, onapristone was dissolved in NaCl with 1 N HCl with a pH of 3.0 reaching a final solution of 5 mg ml⁻¹.

Progesterone (P4, Faciligest, SYVA Laboratorios) was administered twice, 12 h apart from sponge withdrawal (12.5 mg/animal, intramuscularly), to animals of groups T3 (n = 10) and T4. This treatment, as tested before in an adult ram, would maintain plasma progesterone levels higher than 0.5 ng ml⁻¹ for 24 h.

The onset of estrus was checked in all groups using adult fertile males every 4 h for a 72 h period (or until mating had occurred), beginning 24 h after sponge withdrawal. Four males were used and each ewe was allowed to be mated three times.

Blood samples from all animals were collected by jugular venipuncture at sponge insertion, superovulation treatment, sponge withdrawal and every 4 h for a 72 h period starting at 24 h after sponge withdrawal. Plasma samples were used to measure progesterone, and LH. Progesterone concentrations were measured by direct solid-phase radioimmunoassay using 50 μl of each plasma sample running in duplicate, according to the 125I-Progesterone Coatria RIA Kit protocol from BioMérieux, as previously described (Vasques, 1990). Intra- and inter-assay progesterone coefficients of variation (calculated by a method described by Solari, 1983) were 1.8 and 5.1%, respectively (10 assays for a 0.9 ng ml⁻¹ pool). LH measurements were performed by an “enzyme-linked immunosorbent assay sandwich” type assay, using two polyclonal antibodies produced from the same antigen, ovine LH, from two different animal species (produced by INRA and commercialized by Sanofi under the name REPROKIT). Plasma samples of 10 μl were used in duplicate and the absorbance was measured by a spectrophotometer using a 405 nm wave length filter, after a 1 h reaction time at room temperature. Results were expressed as optical density units (ODU) and the LH surge for each animal was identified with the highest ODU reading in between two basal series of readings. Intra- and inter assay coefficients of variation for validation of LH measurements were 4.6% (from a total of 70 samples running in duplicate in 6 assays) and 8.76% (from the same sample running in 6 assays), respectively.
Ovulations were detected by laparoscopies performed under local anesthesia from 12 to 48 h after matings and further confirmed by the presence of early corpora lutea at the time of uterine flushings. The presence of oocytes, fertilized embryos and spermatozoa was checked in uterine flushings after hysterectomy under a stereo microscope. Hysterectomies were performed only in animals in which ovulations were detected by laparoscopy and were done at 24 h \((n = 5: \text{T2} = 3, \text{T4} = 2)\), 48 h \((n = 5: \text{control} = 4, \text{T2} = 1)\) and 5 days \((n = 25)\) after matings. Genitalia flushings were performed with a phosphate buffered solution with 5% of fetal calf serum and the oviductal, uterine and cervical fractions were observed separately for ova detection. Sperm presence, without quantification, was checked by microscopy in all those fractions after centrifugation. Oocytes obtained from animals flushed at 24 and 48 h were used to check nuclear maturation and fertilization. These oocytes were fixed in a solution of acetic acid and ethanol (in a proportion of 1:3) during 24 h starting immediately after collection, and nuclei were stained thereafter with lacmoid solution (1 g of lacmoid, 45 ml of acetic acid and distilled water giving a final volume of 100 ml). The resumption of meiosis was checked using a stereo microscope, by identifying any stage between diakinesis and metaphase II. Fertilization of oocytes collected at 48 h was assessed by cleavage detection. From flushings performed at 24 h we could only assume that fertilization did not take place by the observation of free-sperm zonae and a lack of sperm in the flushing media.

From all groups, animals with a detected heat and ovulation prior to 40 h after sponge withdrawal were selected to compare the following rates:
1. Response rate: Number of ovulated animals/number of synchronized animals;
2. Ovulation rate: Number of ovulations/number of ovulated animals;
3. Recovery rate: Number of collected ova (oocytes + embryos)/number of ovulations;
4. Percentage of fertilized embryos/ovulation;
5. Fertilization rate: Number of collected embryos/total number of collected ova;

2.2. In vitro experiments

An in vitro model using bovine oocytes was used to study the effects of onapristone on either oocyte maturation or fertilization. The method used for producing in vitro bovine embryos of oocytes collected from ovaries of slaughtered cows was described elsewhere (Marques et al., 1995). An oocyte culture medium (TCM 199 supplemented with 10% of oestrous cow serum and antibiotics) was used to mature oocytes for a 24 h period incubated at 39°C with a humidity saturated atmosphere with 5% CO₂. Only oocytes surrounded by granulosa cells and without visual signs of pycnosis were selected for incubation. After culture, oocytes were washed in a proper medium to remove cumulus cells. Only expanded oocytes surrounded of approximately four granulosa cell layers, were selected for in vitro fertilization. Thawed sperm of previously tested bulls for in vitro fertilization was incubated in a Ca²⁺ free Tyrodes medium for one hour. After swim-up, the upper layer containing sperm was collected and centrifuged (500 g). The concentrated sperm was added to the fertilization medium containing matured oocytes and they were incubated together for 24 h. After this period, fertilized oocytes were transferred to an embryo culture medium where granulosa cells
monolayers were previously developed. Forty eight hours after in vitro fertilization, embryos were checked for cleavage.

In experiment 1, where a total of 300 bovine oocytes were used, the effect of onapristone on oocyte nuclear maturation was assessed by adding two different concentrations of the drug to the oocyte maturation medium (D1 = 5.56 × 10^{-6} \text{ mol}^{-1}, n = 100; D2 = 2.22 × 10^{-5} \text{ mol}^{-1}, n = 100). From the control group (n = 100) and from the groups receiving onapristone, a pool of 10, 13 and 12 oocytes of each, respectively, were used to check nuclear maturation. Nuclear maturation was assessed after culturing oocytes for 24 h as described before. In experiment 2, a total of 222 bovine oocytes matured in vitro were used as previously described. The effect of onapristone on in vitro fertilization was assessed by adding the same drug concentrations as before (D1 and D2) to the fertilization medium. D1, D2 and control groups used 40, 75 and 107 mature oocytes respectively. Fertilization was checked at 48 h after insemination by counting cleaved embryos.

Differences between groups on time intervals (h) between sponge withdrawal and estrus and LH surge, and between estrus and LH surge, were determined by analysis of variance (ANOVA) and least significant differences (LSD) (Steel and Torrie, 1982). All the other qualitative variables were statistically compared by Chi-square tests with Yate's correction (Bailey, 1969). Significance was tested at the $P < 0.05$ level and results are expressed as average ± standard error when applicable.

3. Results

Table 1 indicates the numbers of animals showing estrus, demonstrating an LH surge and actually ovulating after the various treatments. All ewes from group T1 showed estrus, LH surge and ovulation. Concerning the number of animals showing LH surge and ovulations, there were no significant differences between groups. One animal in each of the groups treated with onapristone (T2 and T4) presented an LH surge without ovulation. In group T3, one of the ewes showed ovulation without a detected LH surge. Considering animals from groups T1, T2 and T4 the time limit for estrous behaviour

<table>
<thead>
<tr>
<th>Group</th>
<th>Treated (n)</th>
<th>Onset of heat (n)</th>
<th>LH surge (n)</th>
<th>Animals ovulating</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1 (Control)</td>
<td>11</td>
<td>11</td>
<td>27.64 ± 1.74</td>
<td>11</td>
</tr>
<tr>
<td>T2 (ZK)</td>
<td>10</td>
<td>10</td>
<td>24.80 ± 0.53</td>
<td>10</td>
</tr>
<tr>
<td>T3 (P4)</td>
<td>10</td>
<td>5</td>
<td>68.80 ± 6.50</td>
<td>7</td>
</tr>
<tr>
<td>T4 (P4 + ZK)</td>
<td>8</td>
<td>8</td>
<td>25.50 ± 1.05</td>
<td>8</td>
</tr>
</tbody>
</table>

Within columns, groups with different superscripts are significantly different.
For heat (ANOVA $F_{[3,30]} = 61.824$); $P < 0.0001$.
For LH surge (ANOVA $F_{[3,32]} = 63.733$); $P < 0.0001$. 

Table 1
Effect of treatments on intervals between sponge withdrawal to the onset of heat and LH surge, and animals ovulating
ranged from 24 to 40 h after sponge withdrawal. In the 5 ewes receiving progesterone (T3) and responding to synchronization, estrous behaviour ranged from 52 to 84 h. As expected, progesterone treatment after sponge withdrawal (T3) when compared with group T1, significantly delayed the time interval from sponge withdrawal to estrus and to LH surge (68.80 and 76.57 h vs. 27.64 and 32.36 h, respectively; \( P < 0.0001 \)). Estrus and LH surge were observed significantly sooner in animals of group T4 than in group T3, in spite of showing high peripheral progesterone levels (25.50 vs. 68.80 h for estrus, and 30.50 vs. 76.57 h for LH surge, respectively; \( P < 0.0001 \)). None of these patterns were affected by onapristone given alone (T2) or in association with progesterone (T4). None of the treatments affected the time interval between estrus and LH surge which occurred on average 4.6 ± 0.75 h after the onset of heat for all groups.

Patterns of peripheral progesterone concentrations in different groups are shown in Fig. 1. The drop of progesterone levels in group T3 (P4; \( n = 10 \)) occurred at 52.8 ± 11.4 h (mean ± standard deviation) after injection of the hormone, which was not significantly different (ANOVA) from that of the group T4 (P4 + ZK; 45.1 ± 5.0 h; \( n = 7 \)). Animals in group T4 (P4 + ZK) resumed the luteinization process sooner after LH surge than the other groups. In groups not receiving exogenous progesterone [T1 (control) and T2 (ZK)], plasma levels of this hormone were found to be lower than 0.5 ng ml\(^{-1}\) in all animals from sponge withdrawal to ovulation.

LH surge was synchronous in groups T1 (control), T2 (ZK) and T4 (P4 + ZK), and was delayed and asynchronous in animals of group T3 (P4), as shown in Fig. 2.

Heat response, ovulation and fertilization rates are shown in Table 2. Heat response rate was significantly lower in animals of group T3. Ovulation rate was not affected by
Fig. 2. Peripheral plasma LH levels (mean optical density units ± sem).

Table 2
Response (heat/synchronised), ovulation (ovulations/animals ovulating) and fertilization (fertilized/ovulations and fertilized/total recovered ova) rates

<table>
<thead>
<tr>
<th>Group</th>
<th>Response rate</th>
<th>Ovulation rate</th>
<th>Fertilized embryos/ovulation (%)</th>
<th>Fertilization rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1 (Control)</td>
<td>1 (11/11) a</td>
<td>10.46 (115/11) a</td>
<td>23/115 (20.0) a</td>
<td>0.41 (23/56) a</td>
</tr>
<tr>
<td>T2 (ZK)</td>
<td>1 (10/10) a</td>
<td>9.11 (82/9)  a</td>
<td>1/82 (1.2) b</td>
<td>0.02 (1/43) b</td>
</tr>
<tr>
<td>* T3 (P4)</td>
<td>0.5 (5/10) b</td>
<td>7.13 (57/8)  a</td>
<td>13/57 (22.8) a</td>
<td>0.77 (13/17) c</td>
</tr>
<tr>
<td>T4 (P4 + ZK)</td>
<td>1 (8/8) a</td>
<td>8.14 (57/7)  a</td>
<td>1/57 (1.8) b</td>
<td>0.03 (1/30) b</td>
</tr>
</tbody>
</table>

Within columns ($\chi^2$): for different superscripts $P \leq 0.02$.

* Heat and ovulations observed beyond the normal period.

Table 3
Ova recovery rate (%) at different flushing times (in brackets: recovered ova/number of ovulations)

<table>
<thead>
<tr>
<th>Groups</th>
<th>Ova recovery rate at different flushing times</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>24–48 h</td>
</tr>
<tr>
<td>T1 (Control)</td>
<td>75.6 (34/45) a</td>
</tr>
<tr>
<td>T2 + T4 (ZK groups)</td>
<td>78.3 (36/46) a</td>
</tr>
<tr>
<td>Total</td>
<td>76.9 (70/91) a</td>
</tr>
</tbody>
</table>

$\chi^2$: within rows $P < 0.0001$; within columns not significant.
onapristone treatment (T2 and T4), the number of ovulations ranged between 8.1 and 9.1 per animal. The same rate was also not affected by progesterone treatment (T3) but ovulations occurred significantly later in this group. Onapristone, either alone or in association with progesterone (T2 and T4 groups), significantly reduced the number of fertilized embryos/ovulation (1.4% vs. 20%; \( P < 0.001 \)) and the number of fertilized embryos/total number of ova collected (fertilization rate: 2.7% vs. 41.1%; \( P < 0.001 \)), when compared with control group. The two embryos collected after onapristone administration (from flushings performed at 5 days) presented signs of cell division asynchrony and degeneration. These embryos when co-cultured in vitro did not succeed in developing and were dead 24 h after culture. In spite of delayed ovulations, animals of T3 group presented a significantly higher fertilization rate than controls (77% vs. 41%; \( P < 0.01 \)).

The ova recovery rate compared between T1 group and those receiving ZK (T2 + T4) was not different either in flushings performed at 24–48 h (\( n = 4 \) and \( n = 6 \), respectively) or at 5 days (\( n = 6 \) and \( n = 10 \), respectively) after matings. There was a significant decrease in ova recovery rate between 24–48 h and 5 days after matings in both T1 and T2 + T4 groups (\( P < 0.0001 \), Table 3).

In the animals of groups T2 (\( n = 4 \)) and T4 (\( n = 2 \)), receiving onapristone, which were hysterectomized at 24 (\( n = 5 \)) and 48 h (\( n = 1 \)) after mating and checked for sperm presence, not a single sperm was found in the flushing medium either from the uterus, the oviduct or attached to the zonae. In these animals, sperm presence could only be detected in flushings obtained from the cervix and fertilized oocytes were not observed. In four ewes from the control group flushed at 48 h, sperm presence was detected in all uterine portions. Oocytes sampled for nuclear maturation and collected at 24 and 48 h after mating (groups T1: \( n = 10 \); T2: \( n = 13 \); T4: \( n = 7 \)), showed unequivocal signs of meiotic resumption through the identification of a polar body characterizing metaphase II. In the four ewes of the control group flushed at 48 h after mating, 39.4% of the recovered oocytes were fertilized. In one ewe of group T2 flushed at the same time, thirteen unfertilized matured oocytes were detected.

Neither of the doses of onapristone added to the oocyte maturation medium interfered with the in vitro resumption of meiosis (Table 4). The higher onapristone dose added to the fertilization medium significantly reduced the number of cleaved embryos when compared to the control group (48% vs. 62.6%, respectively; \( P < 0.05 \)).

### 4. Discussion

In the ewe, progesterone is not synthesized by follicular granulosa cells during proestrus, as confirmed by little cytological evidence of luteinization before ovulation.
the hormone peripheral levels during the follicular phase being below 0.5 ng ml$^{-1}$ (Scaramuzzi et al., 1993). After LH surge, there is a shift in follicular steroid production from oestradiol towards progesterone (Hansel and Convey, 1983). Studies in sheep confirm a progesterone rise in follicular fluid only after LH surge and 12 h prior to ovulation (Baird and McNeilly, 1980), and its levels are only hardly detected in ovarian venous blood (Weeler et al., 1975). In contrast, in primates and rats, a rise in progesterone produced by granulosa cells is detected peripherally as early as 12 h before LH surge (Batista et al., 1992). In these species, progesterone seems to be involved either in follicle growth and ovulation (Puri, 1995; Sanchez-Criado et al., 1990) by its participation in gonadotrophin release (Batista et al., 1992) or in the mechanism of follicle rupture (Lipner and Greep, 1971). In the ewe, progesterone produced by granulosa cells after LH surge seems to participate in follicular rupture through a local effect (Murdoch et al., 1986).

In this work, the objective of administering onapristone was to block progesterone activity from sponge withdrawal to predicted LH surge. The results clearly show that the antiprogestagenic activity of the drug was maintained during this period, since estrous behaviour and LH surge were not compromised in animals under the influence of exogenous progesterone. On a previous study, the administration of mifepristone to an ewe presenting deviating patterns of progesterone after PMSG stimulation produced the same effect (Cavaco Gonçalves et al., 1992). Whether onapristone action was extended beyond LH surge could not be confirmed in this study but we suspect that antagonizing activity did not exist at the time of follicular rupture. This assumption arises from the work of Murdoch et al. (1986) where follicular rupture in the ewe was blocked by the administration of a progesterone synthesis inhibitor (isoxazol) during the ovulation period. In superovulated cows, the administration of progesterone alone or associated with mifepristone after estrus and LH surge, did not affect ovulation rate (McBride et al., 1986), suggesting differences concerning the effect of the hormone during follicular rupture among species (Tsafri and Dekel, 1994).

In the present study, none of the superovulated ewes not receiving exogenous progesterone showed abnormal patterns in circulating levels of the hormone, meaning that the incidence of this occurrence is low. This does not exclude that an abnormal increase in intrafollicular synthesis of the hormone before LH surge might have occurred since PMSG stimulates progesterone synthesis in in vitro cultured granulosa cells (Kuran et al., 1995), this effect being explained by the LH activity of the gonadotrophin (Boland et al., 1991). Luteinization after ovulation was resumed sooner in animals receiving onapristone and progesterone, suggesting an interaction of both drugs in stimulating corpus luteum formation.

Onapristone administration did not interfere with LH patterns but exogenous progesterone inhibited estrus and endogenous LH release. Gonadotrophin surge occurred in a less synchronized way than in controls and only after peripheral levels of progesterone reached basal values. This confirms that at this stage in the ewe, differently from other known species, instead of stimulating, progesterone inhibits LH release.

The average number of ovulations per ewe after PMSG stimulation was within the normal range for this breed (Bindon et al., 1986).

The ova recovery rate was not affected by the administration of onapristone.
However, the number of recovered ova significantly decreased from 24-48 h to 5 days after matings independently of whether the animals received onapristone or not. In a previous study (S. Cavaco Gonçalves, 1994, unpublished results,) it was found that mifepristone significantly reduced the ova recovery rate in superovulated ewes. Mifepristone is known to stimulate uterine contractions in pregnant rats (Arkavichien and Kendle, 1992). Previous work in mice clearly demonstrated that mifepristone provokes a retention of ova in the oviduct for a longer period and a faster clearance of embryos when in the uterus (Vinijsanum and Martin, 1990). The differences on the ova recovery rate between both drugs suggest that they may have a distinct effect on the type of contractions at this stage.

Normal oocyte maturation in ewes during the ovulatory period was not affected by onapristone treatment. Bovine oocytes were also allowed to mature normally under in vitro culture after onapristone addition. So, the impairment on fertilization provoked by onapristone was due to causes other than dysfunction of oocyte maturation.

As shown in the present results, progesterone administration during proestrus disturbed estrus synchronization but fertilization was higher in these animals than in controls. This suggests that the delay observed in this group between PMSG treatment and estrus (around 92 h) allowed the reduction of the known negative effects of PMSG on fertilization. These negative effects are known to be caused by excess circulating oestrogens (McIntosh et al., 1975) and abnormal steroidogenesis from the granulosa cells (Moor et al., 1985). Onapristone significantly affected fertilization of matured sheep oocytes in vivo. This was probably due to a direct effect of the drug in inhibiting sperm transport or progression to the oviduct since not a single sperm could be found after hysterectomy in the examined animals. According to Quinlivan and Robinson (1969), sperm is expected to be found in all uterine portions (cervix, uterus, isthmus and ampulla) from 1-48 h after insemination. According to Hunter (1980), sperm transport depends on the physical properties of the cervical mucus, myometrial activity and ciliate oviductal cells activity, all under endocrine control. Previous studies demonstrated that PMSG superovulating treatments in the ewe reduced the number of sperm reaching the oviduct but did not affect fertilization rates (Hawk et al., 1987).

The mechanism by which onapristone blocked sperm progression from the cervix to the uterus remains to be clarified. Onapristone might have induced significant changes in cervix structure similar to those found after mifepristone administration, causing cervical softening and dilatation in pregnant guinea pigs (Elger et al., 1987), pregnant women (Rådestad et al., 1988; WHO, 1990) and non-pregnant women (Gupta and Johnson, 1990). It was found that ewes exposed to plant oestrogen presented significant alterations in volume, viscosity and arborization of cervical mucus (Cantero et al., 1993) and that a lower viscosity of this mucus may alter sperm migration through the cervix (Hunter, 1980; Adams, 1990). Cervical softening induced by antiprogestins might be associated with significant changes in cervical mucus physical properties which may disturb sperm passage to the uterus. Mifepristone was shown to induce uterine contractions in pregnant rats and a subsequent rise in PGE2 and PGF2α (Arkavichien and Kendle, 1992). These contractions were predominantly towards the cervix, before implantation (Vinijsanum and Martin, 1990). We must not exclude a possible role of these changes at uterine level as contributors to the observed retention of sperm in the
cervix. Since PGE2 is implicated in cervical softening, through its action on collagen metabolism (Ellwood, 1980), an involvement of PGs on cervix antiprogestin induced alterations may be admitted. In addition, a lack of fertilization associated with antiprogestin treatment was also observed in monkeys using RU486 (Hodgen, 1994). In this study, where low doses of RU486 were administered, the ovulatory/menstrual cycles were maintained but fertilization did not occur. Although in that experiment the presence of sperm in the uterine flushings was not checked, it is noteworthy that the collected oocytes had not got sperm attached to them, suggesting that they may have been arrested as in our study. However, a direct effect of onapristone in the fertilization process must not be excluded since the in vitro studies have shown that bovine oocyte fertilization was also inhibited when high concentrations were used. A toxic effect of the drug upon oocytes, when added in higher concentrations to the fertilization medium, may be a possible explanation of the antifertility effect in vitro as previously suggested by Chen et al. (1995), using mifepristone.

In conclusion, onapristone administration during the follicular phase does not interfere with folliculogenesis, estrous behaviour, LH surge, ovulation and oocyte maturation in superovulated ewes. However, the drug significantly affected fertilization in vivo by arresting sperm in the cervix. A negative effect on in vitro fertilization of bovine oocytes was only evident when high doses of the drug were used. Further studies should confirm this antifertility effect of onapristone in other species as well as the mechanism involved.

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