Reduction of the developmental competence of sheep oocytes by inhibition of LH pulses during the follicular phase with a GnRH antagonist

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A GnRH antagonist (Antarelix) treatment was used during the breeding season of Romanov ewes, to investigate whether LH pulses are required the day before the preovulatory surge for normal early embryo development *in vivo* (Expt 1) and *in vitro* (Expt 2). In Expt 1, at the onset of oestrus after removal of a fluorogestone acetate sponge, group A0.5 ($n = 22$) received a subcutaneous injection of 0.5 mg Antarelix, and ovulation was induced with an intravenous injection of 3 mg pig LH 24 h later. The control group (group C, $n = 20$) were untreated. All ewes were mated naturally at 36 and 48 h after oestrus and embryos were recovered 8 days after sponge removal. There were significant differences in the decrease in LH and in the increase in FSH concentration after Antarelix treatment between treated and control groups. The ovulation rate and embryo recovery rate were not significantly different between the two groups but the blastocyst rate was lower ($P < 0.0001$) in group A0.5 than in group C, with more unfertilized or degenerated oocytes in group A0.5 (69.2%). In Expt 2, 24 h after sponge removal, group A ($n = 10$) and group B ($n = 10$) received one subcutaneous injection of 0.5 mg Antarelix. The control group (group C, $n = 10$) was left untreated. LH pulsatility was re-established in group B with hourly intravenous injections of 5 µg ovine LH for 24 h. Oocytes were collected by flushing the oviducts 28 h after the LH surge, and were fertilized and cultured *in vitro* for 7 days. Ovulation and cleavage rates were not significantly different among the three groups but a higher rate of blastocysts ($P < 0.01$) was obtained after Antarelix treatment when LH pulsatility was re-established (group B). Oestradiol concentration was strongly depressed ($P < 0.0003$) after Antarelix treatment in group A, but was maintained after injection of LH pulses in group B, although at a lower value than before the preovulatory surge in the control group. In conclusion, inhibition of endogenous LH pulses 1 day before the preovulatory surge was not essential for ovulation and *in vitro* fertilization but was associated with a decrease in plasma oestradiol concentrations and inferior embryo development both *in vivo* and *in vitro*. When LH pulsatility was re-established, oestradiol concentrations increased and embryo development was restored.

Introduction

GnRH antagonists can be used to delay and synchronize the LH surge and ovulation in superovulated heifers (Madill *et al.*, 1994), goats (Baril *et al.*, 1996) and in ovarian stimulation cycles for IVF in humans (Frydman *et al.*, 1992). However, it is not known whether there are deleterious effects of inhibition of LH on final follicular and oocyte maturation.

It has been generally accepted that FSH is the key gonadotrophin responsible for the recruitment, growth and

differentiation of follicles, whereas LH has a role in the acute control of follicular steroidogenesis (Baird *et al.*, 1976). Baird *et al.* (1981) suggested that the pattern of LH secretion during the follicular phase of the ovine oestrous cycle controls the final stages of follicular development. The increase in LH pulse frequency, combined with a decrease in plasma concentrations of FSH, may play an important role in the selection of the preovulatory follicle and its final development to ovulation (McNeilly *et al.*, 1992). However, the specific gonadotrophin requirements for normal oocyte maturation in the preovulatory period are unclear (for review, see Staigmiller, 1988). When LH pulsatile release is inhibited by chronic treatment with a GnRH agonist,

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infusion of FSH alone allows the growth of large oestrogenic follicles and normal ovulation after hCG injection in ewes (Picton and McNeilly, 1991). Furthermore, fertilized oocytes and viable embryos are obtained after intrauterine insemination (Evans *et al.*, 1994). Evans *et al.* (1994) concluded that pulsatile LH during the follicular phase is not required for normal oocyte maturation and early embryonic development. Further confirmation of this hypothesis comes from primates, in which viable oocytes have been recovered from females stimulated with recombinant hFSH that contains no LH activity (Hornnes *et al.*, 1993; Zelinski-Wooten *et al.*, 1995). However, against this hypothesis is the fact that embryo yield is improved by LH supplementation after FSH treatment in primates (Weston *et al.*, 1996) and sheep (Cognié *et al.*, 1986).

The inhibition of endogenous pulsatile LH secretion results in a marked decrease in the ovarian secretion of androstenedione and oestradiol (Campbell *et al.*, 1990), but the consequence of such changes on the quality of the oocyte has not been evaluated. In cattle, follicles exhibiting high aromatase activity are more likely to contain oocytes capable of developing to the blastocyst stage (Driancourt *et al.*, 1998). In addition, the hatching rate of blastocysts is positively correlated with oestradiol concentrations in the follicular fluid of the follicles from which they originate (Van de Leemput *et al.*, 1998). Similarly, oocytes recovered for *in vitro* production (IVP) from large oestrogenic follicles may give rise to a higher blastocyst yield compared with those originating from small follicles (cattle: Pavlok *et al.*, 1992; Lonergan *et al.*, 1994; goat: Crozet *et al.*, 1995; sheep: Cognié *et al.*, 1998).

Before ovulation, the oocyte undergoes several modifications in the dominant follicle (Hyttel *et al.*, 1997). These changes are concomitant with increased follicular steroid production, and it is thought that they are a prerequisite for the oocyte to acquire full developmental competence (Assey *et al.*, 1994). It is not known whether LH pulses or subsequent oestradiol secretion induced by these pulses in the preovulatory period of the ovine natural oestrous cycle are required for normal oocyte maturation and development.

The aims of this experiment, using ewes during the breeding season, were (i) to determine whether oocyte competence is altered after LH pulse suppression by a GnRH antagonist administered 1 day before the preovulatory surge; and (ii) to examine whether changes in oestradiol production by the preovulatory follicle are associated with oocyte developmental competence.

Materials and Methods

Animals and products

Adult Romanov ewes ($n = 79$) were used during the breeding season. The average ovulation rate of this race is 3.9 in middle of the breeding season (Ricordeau *et al.*, 1990), with a mean interval from the onset of oestrus to the preovulatory LH surge of 18 h (Land *et al.*, 1973). All animals received a vaginal sponge (Chronogest; Intervet, Angers) for 14 days to synchronize oestrus. Oestrus detection was carried out every

4 h between 20 and 32 h after sponge removal with the aid of harnessed rams. The GnRH antagonist, Antarelix (EP 24332, AcDNal-DCpa-DPal-Ser-Tyr-DHci-Leu-Lys(iPr)-Pro-DAla-NH₂; Europeptides, Argenteuil) (Deghenghi *et al.*, 1993) was diluted in pyrogen-free distilled water (2 ml mg⁻¹) and administered subcutaneously at a dose of 0.5 and 1.0 mg in the preliminary experiment ($n = 7$ ewes per group) and 0.5 mg in the remaining experiments. Heparinized saline to 50 iu ml⁻¹ was used for ovine LH (oLH) preparation. Pulses of 5 µg LH h⁻¹ were delivered for 24 h by a pump connected to the jugular vein by a Silastic catheter. Ovulations were induced by intravenous injection of 3 mg pig LH (pLH; JF Beckers) for the Antarelix-treated animals.

Experiment 1: embryos produced in vivo

At the onset of oestrus, 24 h after sponge removal (day of sponge removal = day 0), 22 ewes received 0.5 mg Antarelix (group A0.5); 20 untreated ewes served as controls (group C). Ovulation was induced by an injection of 3 mg pLH (group A0.5) 24 h after Antarelix treatment. The ewes were exposed to different rams at 36 and 48 h after detection of oestrus (group C) or 12 to 24 h after pLH injection (group A0.5). Blood samples were taken from 12 ewes (group A0.5) before Antarelix injection and from ten control ewes (group C) and then every 4 h for 24 h (group A0.5) or for 32 h (control) for measurement of the LH and FSH concentrations in plasma. Five ewes from groups A0.5 and C were also sampled at days 5, 6, 7 and 8 after sponge removal for measurement of plasma concentrations of progesterone.

Embryos were recovered at day 8 after general anaesthesia and laparotomy by flushing the uterine horns with 10 ml PBS per horn and classified by morphology (Wintenberger-Torres and Sevellec, 1987). The number of cells per embryo was determined after fixation and staining with Hoechst 33342 to determine the number of non-fertilized oocytes, retarded embryos (2–16 cells), morulae (16–60 cells) and blastocysts (> 60 cells).

Experiment 2: embryos produced in vitro

Ten ewes received 0.5 mg Antarelix (group A) 24 h after sponge removal and ten others received 0.5 mg Antarelix plus a pulse of 5 µg oLH h⁻¹ for 24 h (group B). The control consisted of ten untreated ewes (group C). Ovulation was induced 24 h after Antarelix injection by administration of 3 mg pLH (groups A and B) or 50 µg GnRH given i.m. 40 h after sponge removal (group C) to synchronize LH surges at the time when the first preovulatory LH releases occurred (Expt 1).

Blood samples were collected from the jugular veins of the ewes every 30 min, 2 h before and after Antarelix injection, then 3 h before pLH injection (groups A and B) for measurement of plasma concentrations of LH and to determine plasmatic concentrations of oestradiol in samples pooled before or 24 h after Antarelix treatment and 3 h before GnRH injection (group C). Blood sampling was also carried out every 10 min for 2 h between 12 and 14 h after Antarelix injection for measurement of LH pulsatility in five ewes from each group.

In vitro fertilization and embryo culture

Oocytes were recovered 28 h after pLH (groups A and B) or GnRH (group C) by flushing the oviducts with 5 ml PBS per oviduct. A motile sample of frozen-thawed ram spermatozoa was obtained by centrifugation at 500 g for 20 min on a Percoll density gradient (45% on 90%).

After centrifugation, the motile spermatozoa were diluted in capacitation medium (Brackett's medium supplemented with 10 mmol Hepes l^{-1} and 20% oestrus ewe serum, DMH-S). Fertilization took place in DMH-S (pH 7.6) supplemented with 8 mmol calcium lactate l^{-1} (Hunneau and Crozet, 1989). The oocytes were placed into 900 μl fertilization medium to which was added 100 μl of the sperm suspension (1×10^6 spermatozoa ml^{-1}) in a closed tube at 38.5°C. After 18 h of co-incubation, oocytes were washed and placed in culture in 25 μl drops of synthetic oviduct fluid medium under oil (Carolan *et al.*, 1996), at 38.5°C in an atmosphere of 5% CO_2 , 5% O_2 and 90% N_2 for 7 days. The droplets were supplemented with 10% FCS 24 h later and the cleavage rate was evaluated. Eight days after fertilization the numbers of morulae and blastocysts were counted and the embryos were fixed and stained with Hoechst 33342 and the number of cells per embryo was counted.

Blood sampling and radioimmunoassay

Blood samples were stored at -20°C until assayed. For LH, the potency of the standards used relative to NIH was $1.8 \times \text{NIH-LHS1}$; the sensitivity of the assay was 0.3 ng ml^{-1} ; the intra-assay coefficient of variation was 8% and the volume of plasma assayed in duplicate was 50 μl (Pelletier *et al.*, 1982). For FSH, the potency of the standards used relative to NIH standards was $0.1 \times \text{NIH-FSHS6}$; the sensitivity of the assay was 0.2 ng ml^{-1} . The intra- and interassay coefficients of variation were 10 and 9.2%, respectively, and the volume of plasma assayed in each duplicate was 100 μl (Blanc and Poirier, 1979). For progesterone, the volume of plasma assayed was 0.2 ml after extraction with hexane. The sensitivity of the assay was 0.1 ng ml^{-1} ; the intra-assay coefficient of variation was 11% (Saumande *et al.*, 1985). For oestradiol, the volume of plasma extracted by dichloromethane was 5 ml; the sensitivity of the assay was 0.6 pg ml^{-1} ; and the intra-assay coefficient of variation was 8% (Saumande, 1981).

Statistical analysis

Comparisons between proportions were carried out by chi-squared analysis. Comparisons of hormonal concentrations were carried out by ANOVA and a P value < 0.05 was considered significant.

Results

In a preliminary experiment, there was no difference in the decrease in LH and in the increase in FSH concentrations between groups treated with 0.5 ($n = 7$) or 1 mg ($n = 7$) of Antarelix. Injection of 1 mg Antarelix resulted in a number of corpora lutea (2.9 ± 1.5) that was not significantly different from the other groups (A0.5: 4.6 ± 2.1 ; C: 4.2 ± 1.3), but also

Fig. 1. (a) Plasma concentrations of LH (mean $\pm$ SEM) in samples taken every 4 h for 24 h after injection with 0.5 mg Antarelix (●) at the onset of oestrus ($n = 12$) in ewes. ○, Controls ($n = 7$). (b) Plasma concentrations of FSH (mean $\pm$ SEM) in samples taken every 4 h for 24 h after injection with 0.5 mg Antarelix (●) at the onset of oestrus ($n = 12$) in ewes. ○, Controls ($n = 7$).

resulted in a number of cystic follicles (1.3 ± 0.8). Therefore, administration of 0.5 mg Antarelix, which gave rise to an ovulation rate similar to that of the control group, without the appearance of cystic follicles, was used for the subsequent experiments.

Experiment 1

After injection of Antarelix, the plasma concentration of LH dropped rapidly from $0.84 \pm 0.02 \text{ ng ml}^{-1}$ to a basal concentration of 0.4 ng ml^{-1} and remained significantly lower than the control until ovulation was induced at 24 h (Fig. 1a). A

Table 1. Oocyte development *in vivo* at day 8 in ewes treated with 0.5 mg Antarelix at the onset of oestrus and in the control group

Treatment	<i>n</i>	Number of corpora lutea*	Number of embryos recovered (%)	Non-fertilized/retarded (%)	Morulae (%)	Blastocysts (%)
Antarelix (0.5 mg)	22	97	65 (67)	45 (69.2)	11 (17)	9 ^a (13.8)
Control	20	77	52 (67.5)	0	4 (7.6)	48 ^b (92.3)

*Mean $\pm$ SEM: 0.5 mg Antarelix = 4.3 ± 2.0 ; control = 3.8 ± 1.5 ; values with different superscripts within columns: $P < 0.0001$.

Table 2. Oocyte development *in vitro* at day 7 after IVF in ewes treated with 0.5 mg Antarelix, 0.5 mg Antarelix plus pulses of 5 μ g LH every hour and in the control group

Group	<i>n</i>	Number of corpora lutea*	Number of oocytes recovered* (%)	Number of oocytes to IVF	Cleaved (%)	2–16 cells (%)	Morulae (%)	Blastocysts (%)
Antarelix	10	3.2 ± 0.8	2.6 ± 1.3 (84)	27	21 (78)	13 ^a (48)	1 (4)	7 ^b (26)
Antarelix + LH pulses	10	3.9 ± 0.8	2.8 ± 1.5 (71.7)	28	26 (93)	2 ^b (7)	7 (25)	17 ^a (61)
Control	10	3.7 ± 0.8	2.8 ± 1.4 (75.6)	28	26 (93)	11 ^a (39)	2 (7)	13 ^{ab} (46)

*Mean $\pm$ SEM; values with different superscripts within columns: $P < 0.01$.

significant increase in plasma FSH concentration was observed 8 h after Antarelix injection compared with the control group (1.70 ± 0.1 versus 0.88 ± 0.1 ng ml⁻¹) and concentrations remained high for 24 h until ovulation was induced (Fig. 1b).

Three control ewes from group C exhibited an LH and FSH peak 16 h after the onset of oestrus. One ewe had a delayed LH peak at 32 h and six other ewes had not exhibited a peak by this time. Mean LH and FSH concentration in the control group (group C) are represented by these seven ewes.

Plasma concentrations of progesterone in control ewes were not different from those treated with Antarelix at day 5 (4.2 ± 0.7 versus 4.2 ± 0.6 ng ml⁻¹), day 6 (6.7 ± 0.89 versus 6.5 ± 0.9 ng ml⁻¹), day 7 (7.8 ± 1.1 versus 7.6 ± 1.3 ng ml⁻¹) or day 8 (10.8 ± 0.94 versus 8.2 ± 1.9 ng ml⁻¹).

The overall ovulation and oocyte development data *in vivo* are presented (Table 1). There was no difference in the number of corpora lutea (4.3 ± 2 versus 3.8 ± 1.5) or the number of embryos recovered at day 8 (67 versus 67.2%) between the group treated with Antarelix (A0.5) and the control group (C), respectively. Of the oocytes recovered at day 8, 69.2% were not fertilized (39/65) or retarded (6/65) in the group treated with Antarelix compared with 0% in the control group.

The number of morulae was not different between the groups, but development to the blastocyst stage was significantly ($P < 0.0001$) higher in the control group than in the Antarelix-treated group (92.3 versus 13.8%).

Experiment 2

The LH fluctuation (Fig. 2a) observed 2 h before Antarelix injection was not observed 1 h after treatment and the

concentration remained low at 0.2–0.3 ng ml⁻¹. In the 2 h during which blood samples were taken every 10 min, no LH pulsatility was observed in the five ewes from group A exposed to this sampling regimen. In group B (Fig. 2b), after each injection of 5 μ g oLH there was an increase in LH of 3–4 ng ml⁻¹.

Plasma oestradiol concentrations before the Antarelix injection did not differ among the three groups (9.95 ± 0.8 , 7.98 ± 1.05 and 8.52 ± 0.6 pg ml⁻¹ for groups A, B and C, respectively). Oestradiol concentrations decreased significantly ($P < 0.0002$) to 1.37 ± 0.27 pg ml⁻¹ 24 h after Antarelix treatment (group A). The exogenous LH pulses (group B) re-established the plasma concentrations of oestradiol (7.47 ± 0.54 pg ml⁻¹; $P < 0.0003$), but this concentration remained significantly lower ($P < 0.0003$) than that of the control 24 h after Antarelix injection. For the control (group C), plasma oestradiol concentrations were significantly higher (13.18 pg ml⁻¹; $P < 0.001$) at the moment of ovulation induction than 16–18 h before GnRH injection.

The overall ovulation and oocyte development data from *in vitro* studies are presented (Table 2). No differences were observed among the three groups (A, B, C) in the number of corpora lutea on the ovaries (3.2 ± 0.8 , 3.9 ± 0.8 and 3.7 ± 0.8 , respectively), in the number of oocytes recovered (84, 71.7 and 75.6%, respectively) or in the cleavage rate (78, 93 and 93%, respectively).

There were more embryos at the 2–16-cell stage ($P < 0.01$) in groups A (48%) and C (39%) than in group B (7%). There was no difference in the number of morulae among the three groups. However, the number of blastocysts was significantly higher ($P < 0.01$) in group B (61%) than in group A (26%). The proportion of blastocysts in the control group was intermediate (46%).

Fig. 2. (a) Plasma concentrations of LH in ewes in samples taken for 2 h before and after injection with Antarelix and at the end of treatment (22, 23 and 24 h) in five ewes (Expt 2, group A), and LH pulsatility in periods of 2 h with blood sampling every 10 min between 12 h and 14 h after Antarelix injection. (b) Plasma concentrations of LH (mean $\pm$ SEM) in ewes treated with Antarelix plus LH pulses every hour (Expt 2, group B; $n = 5$) measured 2 h before Antarelix treatment and after each injection of 5 μ g LH (indicated by arrows) in periods of 2 h, with blood sampling every 10 min between 12 h and 14 h after Antarelix treatment.

Discussion

These results indicate for the first time that, in cyclic ewes, inhibition of endogenous LH pulses for 1 day before the ovulatory surge was not essential for ovulation and *in vitro* fertilization but was associated with decreased plasma concentrations of oestradiol and a lower rate of embryo

development. When LH pulsatility was re-established, oestradiol concentrations and embryo development were restored.

Administration of a GnRH antagonist during the follicular phase of the sheep oestrous cycle immediately inhibits endogenous LH secretion and reduces the ovarian secretion of androstenedione and oestradiol (Campbell *et al.*, 1990). In the present study, injection of 0.5 mg Antarelix induced a similar inhibition of LH and oestradiol to that described by Campbell *et al.* (1990) using another GnRH antagonist. The increase in FSH observed 8 h after Antarelix administration was probably the consequence of the decrease in oestradiol secretion, since Campbell *et al.* (1990) reported that inhibin concentrations were not affected by GnRH antagonist treatment. The FSH increase was not significantly different after injection of 0.5 or 1 mg Antarelix. Cystic follicles were induced only with the dose of 1 mg Antarelix and there was a consequent decreased ovulation rate. Similarly, significantly fewer transferable embryos were obtained in superovulated goats when the preovulatory surge was blocked with 1 mg as opposed to 0.5 mg Antarelix (Baril *et al.*, 1996). The presence of cystic follicles may indicate a direct effect of the high dose of Antarelix on the ovary, although GnRH receptors have not been identified in sheep follicular cells as has been reported in rats (Hsueh and Jones, 1981).

A similar number of ovulations and the same plasma progesterone concentrations were observed in the group treated with 0.5 mg Antarelix compared with the control group. The temporary suppression of LH pulses by a GnRH antagonist in the follicular phase does not affect ovulation rate and luteal activity, as shown after LH inhibition with a GnRH agonist (Picton and McNeilly, 1991). In addition, the use of a GnRH antagonist during the follicular phase to delay the LH surge and ovulation in superovulated heifers had no effect on ovulation rate, but there was a non-significant tendency for the number of transferable embryos to be decreased (Madill *et al.*, 1994). In Expt 1, the majority of recovered oocytes (69.2%) were not fertilized or fertilized and blocked before the 16-cell stage. Only 13.8% of embryos reached the blastocyst stage. This finding is in contrast to the controls, in which all recovered oocytes were fertilized and developed to the morula-blastocyst stage. The weak oocyte developmental rate *in vivo* after Antarelix treatment indicates either an alteration of oocyte quality or an alteration of uterine function caused by unbalanced steroid concentrations, or both. It has been shown that steroids secreted during the follicular phase play an important role in the establishment of a uterine environment suitable for gamete transport (Allison and Robinson, 1972) and normal embryo development (Miller and Moore, 1976).

Bypassing the effect of uterine environment using *in vitro* fertilization and embryo culture, in Expt 2, the hypothesis that temporary suppression of LH pulses in the late follicular phase affect oocyte developmental competence in cyclic ewes was confirmed. The cleavage rate was not significantly affected, but more embryos were blocked before the 16-cell stage ($P < 0.01$) compared with the group given exogenous LH pulses. Consequently, the blastocyst rate was significantly lower (26 versus 61%; $P < 0.01$) when LH pulses were inhibited. In the control group of Expt 2, the lower than

expected blastocyst yield may be explained by the fact that the ovulatory surge, induced with GnRH 40 h after sponge removal, may have been too soon before the preovulatory increase of oestradiol in some animals. It has been shown that the premature induction of the preovulatory surge with GnRH in the follicular phase of synchronized ewes is associated with a decrease in the fertility rate *in vivo* (Walker *et al.*, 1989).

The discrepancy between the present results and the hypothesis that pulsatile LH secretion is not required for oocyte developmental competence (Evans *et al.*, 1994) may be explained by the fact that these authors used superovulated ewes. The infusion of FSH alone after GnRH agonist treatment in ewes is known to enhance the growth of large oestrogenic follicles (Picton *et al.*, 1990). If oocyte competence is correlated with oestradiol secretion, then inhibition of endogenous LH pulses may be of less importance in superovulated animals, in which follicular activity is supported by the infused FSH, than in cyclic ewes. In primates treated with a GnRH antagonist, a high fertilization rate was detected after stimulation with recombinant hFSH alone (Zelinski-Wooten *et al.*, 1995), but the developmental rate of the fertilized oocytes was lower than in the treatment associating recombinant hLH and recombinant hFSH (Weston *et al.*, 1996). The present results demonstrate that the blastocyst rate was lower in Expt 2 when plasma oestradiol concentrations were significantly depressed by the inhibition of LH pulses with Antarelix. Re-establishment of LH pulsatility restored plasma oestradiol concentrations and was associated with a high rate of blastocyst development.

Is the full establishment of oocyte developmental competence largely dependent on the presence of LH pulses in the follicular phase? Sheep follicles > 3 mm in diameter express LH receptors in their granulosa cells (Carson *et al.*, 1979) and are able to respond not only to the LH surge but also to the pulsatile LH secretion that precedes it. The positive effect of LH pulses on the oocyte in the preovulatory follicle may be exerted through the increased steroid secretion by follicular cells or by stimulation of other locally acting growth factors, such as epidermal growth factor (Harper and Brackett, 1993; Lonergan *et al.*, 1996) and insulin-like growth factor 1 (Lorenzo *et al.*, 1995) involved in cytoplasmic oocyte maturation *in vitro*. The involvement of oestradiol in oocyte maturation is supported by the reduced rate of embryo viability observed when sheep oocytes were matured in follicles in the presence of a steroid inhibitor (Osborn and Moor, 1983). The similar cleavage rates of oocytes in the control and Antarelix-treated groups in Expt 2 indicate that a suppression of LH pulses and oestrogen production do not affect the resumption of meiosis. The inhibition of follicular steroidogenesis during oocyte maturation has been associated with abnormal patterns of proteins synthesized by the oocyte and low developmental ability (Moor *et al.*, 1981). The suppression of LH pulses and steroid production by granulosa cells may affect protein synthesis and cytoplasmic maturation in oocytes. A positive correlation has been observed between oestradiol concentrations in follicular fluid and the blastocyst rate in cattle (Van de Leemput *et al.*, 1998) and IVF efficiency in women (Carson *et al.*, 1982).

The relatively low efficiency of techniques for producing viable mammalian embryos *in vitro* after years of efforts by numerous teams, indicates that the follicular environment must provide crucial signals to the oocyte to acquire developmental competence in the few days preceding ovulation (Bevers *et al.*, 1997) during which the oocyte undergoes ultrastructural modifications (Hyttel *et al.*, 1997).

In conclusion, the present study demonstrates that, in cyclic ewes, inhibition of endogenous LH pulses for 1 day before the ovulatory surge does not affect ovulation and *in vitro* fertilization but is associated with decreased plasma concentrations of oestradiol and a lower rate of embryo development.

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