

THE EFFECTS OF ALFAPROSTOL (PGF_{2α} analogue) AND eCG ON REPRODUCTIVE PERFORMANCES IN POSTPARTUM RABBITS

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ABSTRACT: The aim of this study was to verify the effectiveness of post partum pharmacological oestrus synchronisation with alfaprostol or eCG in improving rabbit doe reproductive performances and to evaluate the incidence of pseudopregnancy in this species. One hundred and eighty commercial hybrid rabbit does, multiparous and lactating, were randomly divided into three groups (n=60) and treated on day 8 post partum (pp) as follows: Group A, 1 mL s.c. vitaminic solution with 200 µg alfaprostol, a synthetic analogue of PGF_{2α}; Group B, 1 mL sc vitaminic solution with 20 I.U eCG; Group C (Control) 1 mL sc vitaminic solution. On day 11 pp (3 days after treatment), all the rabbits were artificially inseminated (AI), and injected with 0.8 µg of buserelin i.m. to induce ovulation. Concurrently, blood samples were taken for progesterone (P4) analysis by RIA with the following schedule: day 8 pp (before treatment), day 11 pp (before AI and the induction of ovulation) and day 16 pp (5 days after AI). Fertility was not influenced by hormonal treatments (71.7% and 71.2% respectively) compared to control (66.1%). Conversely, both PGF_{2α} and eCG hormonal synchronisation treatments increased ($P<0.05$) litter size. P4 values before treatment on day 8 pp testify to the absence of functional CL ($P4<2$ ng/mL) in the early post partum period in 98.9% of the does in this breeding farm. Before AI and the induction of ovulation on day 11 pp 5.6% of the does had P4 concentrations, indicating non-oestral status ($P4>1$ ng/mL). On day 5 after insemination 95.5% had P4 values, which attest to the presence of functional corpora lutea (CL) ($P4>2$ ng/mL). The results of this study show that as long as rabbitry is properly managed zootechnically and sanitarilly, drugs such as alfaprostol and eCG, while not ameliorating fertility rate when used for post partum oestrus synchronisation, can increase litter size.

Key words: PGF_{2α}, eCG, reproductive performance.

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INTRODUCTION

The widespread use of AI in industrial rabbit production has created the need for a pharmacological aid for inducing ovulation, which is easily obtained by i.m. injecting GnRH synthetic analogues. Moreover, oestrus synchronisation by means of eCG injection two or three days before insemination has become very common in industrial management, as this practice usually improves reproductive performances (ANGELI *et al.*, 1990; BONANNO *et al.*, 1990, 1991a, 1991b; CASTELLINI *et al.*, 1991) especially of young lactating does (REBOLLAR *et al.*, 1989; BOURDILLON *et al.*, 1992). Several authors (ARMERO *et al.*, 1995; BOITI *et al.*, 1995; MAERTENS *et al.*, 1995; STRADAIOLI *et al.*, 1997) have expressed doubts about a generalised use of this gonadotropin for oestrus synchronisation. This is because not only non-lactating or receptive does show no improvement from it, but also its antigenic properties cause a decrease in fertility after repeated treatments. Nevertheless it is still commonly used in rabbit production. In contrast, other authors have suggested using PGF_{2 α} to improve doe receptivity and to prepare the animals better for AI (FACCHIN *et al.*, 1992). Puerperium is a critical rabbit doe reproduction period during which the negative effects of lactation on at least some of the stages of the mating acceptance rate, as well as on ovulating frequency and embryo mortality, can be quite pronounced (THEAU-CLÉMENT and ROUSTAN, 1992). Progesterone blood concentration during post partum has also been linked to receptivity, ovarian response, embryo quality and development (BOITI *et al.*, 1996).

Post partum high progesterone levels could be a sign of pseudopregnancy. In this condition, which affects fertility and prolificacy, doe reproductive efficiency is low (THEAU-CLÉMENT *et al.* 2000). The aim of this study was to verify the effectiveness of post partum pharmacological oestrus synchronisation with alfaprostol or eCG in improving rabbit doe reproductive performances and to evaluate the incidence of pseudopregnancy.

MATERIALS AND METHODS

Animals

This research was carried out in a North Italian breeding farm in which controlled environmental conditions of temperature (19-25°C) and light (16 h L:D) were assured. One hundred and eighty commercial hybrid rabbit does were used. They had a parity of 2-4, were clinically healthy and did not differ significantly in production from the breeding unit average. The does were housed in flat-deck wired cages with external nest boxes. The access to the nest boxes was limited to 30 minutes a day for the first 9 days.

Two days before insemination, matutinal nursing was postponed till the afternoon, while one day before AI nursing was prevented; nests were then reopened immediately before insemination, thus applying a mother-litter separation for 36 hours. Does were fed *ad libitum* with a commercial pelleted diet (17.2% crude protein and 14.6% crude fibre). Access to food on the day preceding insemination was prevented for 6 hours. Litters were averaged at birth to 8-9 young within group. A 42 day reproduction rhythm was followed using 2 batches of does inseminated at a 21day interval. Free suckling and weaning at 35 days of age were adopted.

Treatments

All the does were nursing and randomly divided into three groups (n=60 per group). On day 8 post partum (pp) they were treated in the following manner:

Group A: 1 mL s.c. vitaminic solution (Vitatox, Fatro SpA, Ozzano Emilia, Italy) with 200 µg alfaprostol, a synthetic analogue of PGF_{2α}, (Gabbrostim, Centralvet, Vetem, Agrate Brianza, Italy);

Group B: 1 mL s.c. vitaminic solution with 20 IU eCG (Ciclogonina, Fort Dodge Animal Health, Parma, Italy);

Group C (Control): 1 mL s.c. vitaminic solution.

Three days after treatment, on day 11 pp, all the rabbits were inseminated. AI was performed by depositing 0.5 mL of fresh semen deeply in the vagina by means of a sterile catheter. A pool of sperm, collected from bucks of proven fertility by means of an artificial vagina, was used. After removing the mucous plug, if present, sperm was assessed for mass motility and diluted 1:2 with a rabbit diluting medium (IMV Technologies Italia S.r.l., San Bonico, Italy) at 37°C, stored at 20°C and used within 4 hours after collection (BRIVIO *et al.*, 1989).

The insemination was immediately followed by the administration of 0.8 µg of buserelin i.m. (Receptal – Hoechst Roussel Vet SRL, Milano, Italy) in order to induce ovulation.

Concurrently, blood samples were taken from the central artery of the ear for progesterone (P4) analysis in accordance with the following schedule: on day 8 pp (before treatment), on day 11 (before AI) in order to detect eventual pseudopregnant does ($P4 < 2$ ng/mL), and on day 16 pp (5 days after AI) to assess the presence of functional corpora lutea (CL) after ovulation ($P4 > 2$ ng/mL). All blood samples were collected in heparinized tubes, and immediately centrifuged. Plasma was stored at –20°C until assayed.

Hormonal Analysis

Plasma progesterone was measured by radioimmunoassay in accordance with the procedure described by Boiti *et al.* (1974). Progesterone was extracted with ethyl ether and the efficiency of the extraction procedure monitored by the addition of a controlled amount of [3H] progesterone. The efficiency of the ether extraction ranged from 82 to 95%.

For extraction, 0.2 mL plasma was used and each sample was assayed in duplicate. The assay sensitivity was 0.08 ng/mL for a sample volume of 200 µL of plasma. Intra- and inter-assay coefficients of variations were 5.3% and 10.2%, respectively, for n=8 replications of a pool of normal rabbit plasma containing 4.3 ± 0.23 ng/mL.

Measurements

At parturition, the fertility rate (number of parturitions/number of inseminations x 100) was recorded, as were also the total number of young born and the number of born alive.

Statistical Analysis

A Chi-square test was used to analyse the fertility data, while those relating to the prolificacy were evaluated by ANOVA, followed by a Tukey test for multiple comparisons.

RESULTS

Fertility was not significantly influenced by hormonal treatments (Table 1) although it was higher in A and B groups (71.7% and 71.2% respectively) compared to the control group (66.1%). Conversely, both PGF_{2α} and eCG hormonal synchronisation treatments increased ($P < 0.05$) litter size as reported in Table 1.

Table 1: Number and percentage of does delivering and related litter size (total born) in the A, B and control groups (mean \pm SD).

	A	B	Control
Does (n)	60	59	56
Fertility (%)	71.7	71.2	66.1
Litter size (n)	7.9 \pm 2.3 ^a	7.9 \pm 2.3 ^a	6.6 \pm 2.9 ^b

Means in the same row with different superscripts differ significantly. $P < 0.05$.

Progesterone values before treatment on day 8 pp were found to be higher than 2 ng/mL in only two does (2.5 and 8.9 ng/mL, respectively), both of which belonged to group B (Figure 1). These data document the absence of functional CL in the

early post partum period in 98.9% (178/180) of the does in this breeding farm.

Before AI and the induction of ovulation on day 11 pp, 94.4% (169/179) of the does had progesterone concentrations lower than 1 ng/mL. In contrast, 10 does (5.6%), equally distributed among the groups (3, 3 and 4 for groups A, B and Control, respectively), had progesterone concentrations higher than 1 ng/mL and ranging from 1.1 to 7.5 ng/mL, confirming the presence of luteal activity.

On day 5 after insemination, 95.5% (170/178) had progesterone values greater than 2 ng/mL, which attests the presence of functional CL, while 8 animals (4.5%), 2 belonging to group A and 6 to Control group, had progesterone values ranging from 0.1 to 1.9 ng/mL (Figure 1).

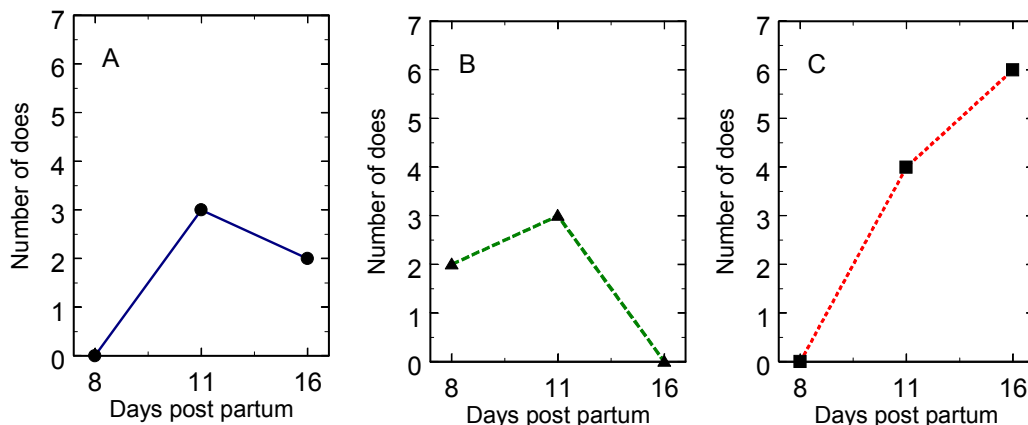


Figure 1: Dynamic pattern of does having abnormal progesterone basal values on day 8 post partum (>2 ng/mL), on day 11 (>1 ng/mL) prior to insemination, and five days later (<2 ng/mL) in groups A and B, synchronised with alfaprostol and eCG respectively, compared to control group (C). Abnormal progesterone levels on both day 8 and 11 post partum point to the presence of functional CL, while on day 16 indicate an ovulation failure.

DISCUSSION

The end of the 8th day pp to synchronise oestrus was chosen mainly for management reasons, as the 11th day pp is considered the ideal time for insemination without interfering with the standard six weeks reproductive cycle length and also for the presence of a follicles growth wave between days 5–9 pp (DIAZ *et al.*, 1987 as in UBILLA and REBOLLAR, 1995). Moreover BATTAGLINI *et al.* (1991) reported that eCG administered 64 hours before AI gave better results in terms of receptivity compared with the administration 48 hours before AI.

From an endocrine point of view, during the post partum period, both oestrogen and progesterone production patterns are significant as they greatly affect the receptivity of the does (UBILLA and REBOLLAR, 1995). With regard to estrogens, one should consider that while decreasing after delivery, they begin to increase again in the early post partum. The increased plasma E₂ levels on day 1 after parturition, and on days 5-7 of the pp are probably related to the follicular growth and the increase in follicle steroidogenic activity that occurred between the last days of pregnancy and the first day after parturition, and between days 5-9 of the pp (UBILLA and REBOLLAR, 1995). Prolactin, which is typical during the puerperium phase, can also concur in negatively influencing receptivity and fertility when secreted at high concentrations (THEAU-CLÉMENT and ROUSTAN, 1992; UBILLA *et al.*, 2000). This finding could explain why the days in which the estrogen peaks are known to occur during puerperium do not necessarily correspond to those in which there is behavioural sexual receptivity. The rationale of eCG for rabbit oestrus synchronisation is justified by its FSH-like mechanism. It has been shown, in fact, that eCG increases the number of preovulatory follicles (PINGEL *et al.*, 1981; BONANNO *et al.*, 1990). Repeated eCG treatment, however, has been demonstrated to induce an immune response (CANALI *et al.*, 1991; BOURDILLON *et al.*, 1992, BOITI *et al.* 1995), which not only decreases the efficacy of further treatments, but also seems to have a negative effect on fertility (CASTELLINI *et al.*, 1991). Nevertheless other authors (LEBAS *et al.* 1996) after more than seven injections did not observe a relation between immunogenicity and productivity.

Moreover, considering that eCG is a biological product whose dosage is based on evoked effects rather than on molar basis, its longer half-life and, being a hormone, its more complex legal prescription regime in several European countries, all these remarks induced a search for possible alternatives.

Some authors proposed the use of PGF_{2 α} , which, together with the well-known effects on partum induction, luteolysis and sperm motility, seem to have an impact on ovulation and oestrus synchronisation (CASTELLINI, 1996, FACCHIN *et al.*, 1998). According to ALVARIÑO *et al.* (1995) PGF_{2 α} has a positive effect on fertility when does are treated before insemination. Similarly, DRAGAN *et al.* (1996) reported that prostaglandins have a stimulating effect on inactive ovaries, preparing them better for the subsequent AI, even if the physiological basis of their actions are still unclear.

A vitaminic solution was injected into the animals of the control group in order to create a similar stress condition in all the does due to capture, restraint, injection and blood sample collection when scheduled.

Our results show that there was not really any improvement in fertility as a result of hormonal treatments, although an increase in litter size was obtained in the treated groups (7.7 and 7.9 vs 6.6), thereby confirming the positive effect already described.

Our current results, compared with those of an analogous study reported by FACCHIN *et al.* (1998), show only slight differences in fertility and average litter size.

Spontaneous ovulation (BOITI *et al.*, 1999), investigated in this case by means of progesterone concentration on the 8th day pp, was found to be a rare occurrence in this farm. In fact, out of 180 samples, only 1.1% had progesterone levels greater than 2 ng/mL, a value indicative of the presence of functional CL. Although numerically irrelevant in this specific trial, it remains to be seen what the possible underlying causes are because a greater incidence was reported (THEAU-CLEMENT *et*

al., 2000) in other instances.

Three days later, on the 11th day pp, we checked to see whether the artificially inseminated does had a progesterone concentration that was coherent with oestrus or whether handling, treatments, or other unknown stimulus, had induced ovulation and a consequent luteal phase. Results show that more than 94% of the animals had normal progesterone values below the threshold of 1.0 ng/mL when AI was performed. Interestingly, none of those 10 does, which equally distributed among groups had a progesterone concentration greater than 1 ng/mL on the day of AI, became pregnant. This confirming previous results (BOITI *et al.*, 1996, THEAUCLÉMENT *et al.*, 2000). Among these animals, two had already showed luteal activity (P4>2ng/mL) even before synchronising treatment.

Progesterone concentration was determined by a third blood sample on the 5th day following AI to check whether ovulation and CL development, which are needed for pregnancy, had taken place. On the basis of our data, we considered the threshold of normal luteal activity for 5-day old CL to be 2.0 ng/mL, because none of the does which had delivered had lower values. This value was used also by BOITI *et al.* (1996) to determine arbitrarily which does manifested luteal activity. Out of a total of 178 determinations only 4.5%, whose P4 values were < 2 ng/mL, indicated luteal activity. This was certainly not compatible with pregnancy and none of these does gave birth.

In this study we considered a rabbitry that was well-managed, zootechnically and sanitarly. Mother-litter separation and a 6 hour fasting the day before AI were used for biostimulation. Our results show that, in such a situation, the drugs used for post partum oestrus synchronisation, such as alfaprostol and eCG, while do not ameliorate fertility rate, can improve litter size. This effect, though understandable for eCG, is hard to explain in the case of prostaglandins because it is difficult to undertake a mechanism of action on the basis of current knowledge. Looking at the progesterone values we can conclude that spontaneous ovulation in the early post partum period does not appear as a major problem in our study. Moreover, we can

assume that progesterone levels >1 ng/mL at AI are not compatible with a successful fecundation and, finally, that the P4 level of 2 ng/mL is a suitable threshold for luteal activity, compatible with pregnancy at Day 5 of gestation.

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