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INDUCTION OF LUTEOLYSIS BY ADMINISTRATION OF A SYNTHETIC PROSTAGLANDIN $F_{2\alpha}$ ANALOGUE IN PSEUDOPREGNANT RABBITS.

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INTRODUCTION

Several studies indicate that in pseudopregnant rabbits the corpus luteum life span reaches a maximum level from day 7 to 10 after ovulation, and declines from day 14-15 gradually to basal levels on day 17 of pseudopregnancy (Scott,R.S. & Rennie,P.J.C.,1970; Keyes,P.L.,et al.,1983). Decreasing progesterone concentrations suggest that luteolysis starts earlier, between days 12 to 14 after HCG injection (Caillol,M., et al.,1983; Browning,J.Y., et al.,1980; Carlson,J.C. & Gole,J.W.D.,1978).
Higher rates of fertilization and implantation in pseudopregnant rabbits are obtained on days 17 and 18 after HCG administration, that means 1-2 days after clear signs of epithelial regeneration are observed in the endometrium, and when many large follicles are present. These events are well correlated with the decline in the progesterone concentrations observed (Fischer,B., et al.,1986; Villemant-Dauphin,CH.,1980).
A 33% to 88% conception rate is observed in rabbits after $PGF_{2\alpha}$ administration on days 9,10 or 11 of pseudopregnancy, followed by artificial insemination (A.I.) 72 hours later (Carlson,J.C.,et al.,1990; Rodriguez,J.M.,et al.,1989; Lammers,H.J. & Petersen,J.1987; Dubiel,A.,et al.,1982). PMSG treatments administered in rabbits 48-64 hours before A.I. sincronizes the oestrus phase, allowing that 41% to 65% of the animals present a high sexual receptivity after treatment (Costantini,F.,1989). An administration of 25 IU PMSG/does improves sexual receptivity and fertility in rabbits with a low reproductive performance (Khalifa,K.M., et al.,1989).

This study was designed to find an effective dose of a prostaglandin $F_{2\alpha}$ analogue to reduce pseudopregnancy, and when administered together with PMSG improves the conception rates obtained when using $PGF_{2\alpha}$ alone.

MATERIAL AND METHODS

Pseudopregnancy was induced by administration of 25 (IU) PMSG (Serigan, Ovejero Lab.) on day 4 or 11 postpartum followed by 20μg GnRH (Fertagyl, Intervet Lab.) 60 hours later, in 37 multiparous, lactating, does of the California x Newzealand breed, with 4-10 pups/litter. Pseudopregnancy was checked 4 day later observing the presence of corpora lutea by means of a laparotomy performed under general anesthesia (Acepromazyne acetate 1-2 mg/kg, i.m., Beecham Lab.; + Penthotal sodium, 33 mg/kg, intravenously, Abbot Lab.). 32 rabbits became pseudopregnant after this treatment. They were randomly allocated to 4 groups of 8 animals and i.m. injected on day 11 of pseudopregnancy as follows: a Control group with 1.0 ml saline solution, and three groups with 50μg, 100μg, or 200μg of the synthetic $PGF_{2\alpha}$ analogue-Etiproston (Prostavet, Virbac Lab.),

respectively (Treated groups). Similar doses of the same $PGF_{2\alpha}$ analogue were previously used in the induction of parturition with good results (Ubilla,E., et al.,1988; Ubilla,E. & Rodriguez,J.M.1989).
Blood samples were obtained from the margin ear vein using sterile heparinized tubes, on days 11 to 15 of pseudopregnancy. The first samples were taken before saline or $PGF_{2\alpha}$ analogue injections (day 11 of pseudopregnancy at 08.00 hr), and the following samples every 12 hours until day 13. From this day until day 15 every 24 hours. Plasma obtained after centrifugation was stored at -20º Celsius degrees until analyzed. Plasma progesterone concentrations were measured by immunoenzimatic assay (ELISA) (Munro,C. & Stabenfeld,C., 1984). The sensivity of the analysis was 0.1pg/well, and the intra and inter-assay variation coefficient was less then 5% .

69 multiparous rabbits were used in the second part of our study. All animals were diagnosed as non pregnant by abdominal palpation on day 11 after a previous A.I. performed with fresh diluted semen and 20 μg GnRH. . 66% - 100% of these animals were considered pseudopregnant according to our previous results on ovulation rates when using 20μg GnRH (Rodriguez,J.M.,et al.,1988). These rabbits were randomly allocated to 3 groups of 23 animals receiving i.m. on the same day (day 11 after A.I.) the following treatment: 25 IU PMSG + 6.25 IU Hialuronydase (Ciclogonina, Prochena Lab.) the Control group, or 25 IU PMSG + 6.25 IU Hialoronydase + 100μg synthetic $PGF_{2\alpha}$-(Etiproston), or 25 IU PMSG + 6.25 IU Hialuronydase + 200μg synthetic $PGF_{2\alpha}$ the treated groups, respectively. Hialuronydase was used as a difusion factor. All groups were artificially inseminated 60 hours later, using fresh diluted semen and 20μg GnRH. Conception rate, live born/litter and born dead/litter were measured after parturition in all groups of animals.

Plasma progesterone concentrations data were statistically analysed using ANOVA. The fixed considered factors were: treatment, day and interaction (treatment) x (day). Means obtained were compared using the Duncan test (SAS/STAT.,1987), or the Mann-Withney U test (Siegel,S.1956). Percentages were compared using the Chi-square test (Yates,F.1949).

RESULTS

The results of the two parts of our study are seen in Figure 1 and Table 1. No differences among groups in plasma progesterone levels on day 11 of pseudopregnancy before injections were detected. 50μg, 100μg, or 200μg synthetic $PGF_{2\alpha}$ decrease plasma progesterone concentrations in a similar manner from days 11,5 to 12,5 of pseudopregnancy when compared to the Control ($p< 0.001$). A new decline on days 13, 14 and 15 in rabbits injected with 200μg synthetic $PGF_{2\alpha}$ compared to other groups was observed ($p< 0.01$). The other two treated groups exhibited similar progesterone levels on days 13, 14 and 15 of pseudopregnancy. Variations in progesterone levels detected from day 11 to 13 in Control group were no significative. Low levels were observed on day 14 and 15 of pseudopregnancy in Control rabbits when compared to days 11 to 13 of the same group ($p< 0.01$).
Higher conception rates were observed in rabbits previously diagnosed as non pregnant on day 11 after A.I., and treated on the same day with PMSG + Hialuronydase togheter with 200μg synthetic $PGF_{2\alpha}$, and inseminated again 60 hours later, compared to the other groups ($p< 0.05$). No differences in the number of live born/litter, or neonatal mortality among groups were detected.

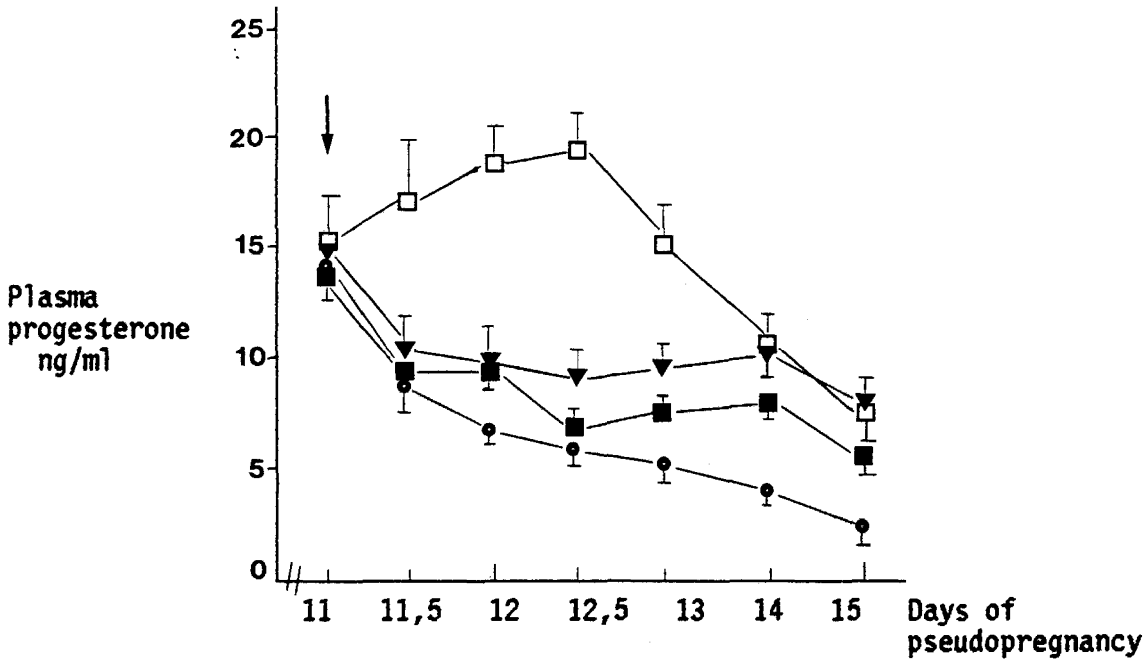

Fig.1. Plasma progesterone mean levels ($\bar{x}$ ± S.E.M.) during days 11 to 15 of pseudopregnancy after injections of 1 ml saline solution (□–□), 50µg (▼–▼), 100µg (■–■), or 200µg (●–●) of a synthetic $PGF_{2\alpha}$ on day 11. Arrow indicate the day of injections.

Treatments:	Conception rate (%)	Litter size at birth ($\bar{x}$ ± S.E.M.)	Born dead per litter (%)
25 IU PMSG + 6.25 IU (HL)	15.0	7.1 ± 0.5	3.9
25 IU PMSG + 6.25 IU (HL) + 100µg $PGF_{2\alpha}$ analogue	34.8	7.5 ± 0.9	3.3
25 UI PMSG + 6.25 IU (HL) + 200µg $PGF_{2\alpha}$ analogue	* 69.5	7.3 ± 0.7	3.6

Table 1. Reproductive parameters in rabbits previously determined as non pregnant on day 11 after a.i. and treated on the same day with PMSG + HL (Hialuronydase) and a synthetic $PGF_{2\alpha}$, or without $PGF_{2\alpha}$. All rabbits were artificial inseminated 60 hours later. * = ($p < 0.01$).

DISCUSSION

Plasma progesterone concentrations measured in all groups of animals from days 11 to 15 of pspg. are in accordance with those observed during this period by other authors (Kwack,D.O.,et al.,1988; Gadsby,J.E.,1989). The evolution of plasma levels of this hormone during these days in Control animals was comparable to other results (Hilliard,J.,1973; Browning,J.Y.,et al.,1980). The similar fall in progesterone concentrations from days 11 to 12,5 in rabbits treated with the synthetic $PGF_{2\alpha}$ suggest that the three prostaglandin doses injected induce luteal regression with the same efficacy. Only the 200μg dose shows a new reduction in progesterone levels from day 13 to 15 of pspg. In our opinion it is important to emphasize that rabbits injected with 200μg synthetic $PGF_{2\alpha}$ reach mean progesterone levels of 4,5 ± 0,8 ng/ml on day 14. Normally in pseudopregnant rabbits a similar level is only reached on day 15 to 16 togheter with an epithelial endometrium regeneration that allows fertilization and implantation on days 17-18 of pspg. (Fischer,B., etal.,1986). From this point of view our results obtained after injecting 200μg of the $PGF_{2\alpha}$ analogue on day 11 of pspg. suggest an advancement by about 1-2 days of all these changes, offering more chances to obtain a new pregnancy before the end of natural pspg.

On the other hand, conception rates obtained after administration of PMSG + (Hialuronydase) together with 200μg synthetic $PGF_{2\alpha}$ in non-pregnant rabbits on day 11 after a previous A.I., suggest a clear response to this treatment compared to rabbits injected with PMSG + (Hialuronydase) alone, or togheter with 100μg synthetic $PGF_{2\alpha}$. That is to say, among all non-pregnant animals treated there would be a high proportion of pseudopregnant rabbits on day 11 after the A.I. was performed, confirming the ovulatory response results obtained post-administration of 20μg GnRH (Rodriguez,J.M.,et al.,1988). These rabbits could not respond to the administration of PMSG + (Hialuronydase) without $PGF_{2\alpha}$, due to the presence of active corpora on day 11 post-A.I. The 15% of rabbits that became pregnant probably were not pseudopregnant on the day of injections.
Only animals treated with PMSG + (Hialuronydase) + 200μg synthetic $PGF_{2\alpha}$ showed a normal conception rate. 100μg $PGF_{2\alpha}$ probably failed in advancing luteolysis in most of the rabbits of this group. The higher levels of plasma progesterone found on days 13, 14 and 15 of pspg. when using this dose compared to those found when using the 200μg dose would explain these results.

We feel that the luteolytic effect of the 200μg synthetic $PGF_{2\alpha}$ dose, would also better compliment the PMSG stimulus on follicle growth. In this case the largest follicles observed only on days 17-18 of pspg. (Villemant-Dauphin,CH.1980), could appear earlier, on day 14-15 of pspg.

Our results suggest an advance in a technique which would permit the sinchronization of the oestrus phase in pseudopregnant rabbits resulting from a previous A.I. We consider it necessary to continue studying these effects, using a larger dosage of this synthetic $PGF_{2\alpha}$ analogue together with PMSG, to improve the conception rate obtained.

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