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OVULATION AND EMBRYO IMPLANTATION RATE IN SYNCHRONIZED ARTIFICIAL INSEMINATED MULTIPAROUS LACTATING DOES

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ABSTRACT

The aim of this experiment was to study the response to different methods to synchronize the oestrus in multiparous lactating does on day 9 post-partum. All animals were inseminated on day 11 post-partum with a pool of fresh semen. The methods to synchronize the oestrus were: doe-litter separation during 48 hours prior to artificial insemination (BIOS group, n=14) or administration of 25 UI PMSG (i.m.) (PMSG group, n=14). One control group (CONTROL group, n=14) also received a saline solution injection on day 9 post-partum. Fifteen days later the presence and quantity of corpora lutea and embryos in ovaries and uterus respectively were determined by means of laparotomies. On the other hand, with the aim to study morphological ovary parameters and oocytes quality, two does of each group were killed and their ovaries removed.

No differences among groups in ovulation rate (85.7 , 92.8 and 85.7 %), number of corpora lutea (9.5 ± 0.5 , 11.7 ± 0.7 and 10 ± 0.8), fertility (64.3, 78.6 and 71.4 %), conception rate (75, 84.6 and 83.3%) and number of implanted embryos (9.1 ± 0.6 , 11.3 ± 0.7 and 8.8 ± 1.4) were observed in BIOS, PMSG and CONTROL groups respectively. However, differences in follicular size and quality of oocytes in the ovaries of eight does killed were observed. The results indicate that hormonal and biostimulation methods are not necessary on day 11 post-partum to improve fertility results. Initiate results on ovary morphology and oocytes quality presented here could explain the good results obtained in a long term utilization of these methods.

INTRODUCTION

The application of artificial insemination in commercial rabbit farms is very extensive, for this reason it's necessary to consider the synchronization and induction of oestrus methods to obtain good and competitive results in artificially inseminated lactating does. Normally, the artificial insemination (AI) in rabbits is conducted according to 42 days reproduction rhythm (THEAU-CLÉMENT and BOITI 1998).

Many studies have been made on hormone treatments especially when groups of females in the same reproductive and physiological state are required to apply the artificial insemination (McNITT et al. 1997; BOITI et al., 1995). The use of PMSG before AI to induce and synchronize oestrus is a routine practice in large farms with good fertility and prolificacy results when is applied in lactating does (THEAU-CLÉMENT et LEBAS, 1996). On the other hand, the changes of cage (REBOLLAR et al., 1995) and the separation of doe from her litter during different time intervals are biostimulation methods, to improve sexual receptivity of lactating does as an alternative technique to hormonal treatments. When biostimulation is applied, fertility improves, especially when the separation of doe from litter is over 36 hours (ALVARIÑO et al., 1998). Recent studies demonstrate that a transient doe-litter separation may induce several endocrinological changes and a different response of hypothalamus-pituitary axis in such a way that when these animals were inseminated the pulse of LH is greater than the pulse observed in control females (UBILLA et al., 2000). Biostimulation

methods and hormonal treatments have to be applied at least 48 hours prior to artificial insemination, so it is difficult to manage in large rabbit farms and need additional manpower costs.

The aim of this trial was to study the ovary response and the implantation rate, in lactating multiparous does artificially inseminated on day 11 post-partum, after a biostimulation or after a treatment with PMSG or saline solution.

MATERIALS AND METHODS

The trial included 50 multiparous females of the Californian x NZW breed, housed in individual cages under controlled light/dark cycles (16h/8h) and fed *ad libitum* with a commercial pelleted diet. All females were on day 9 post-partum and lactating more of 7 pups per litter. Three experimental groups were composed with fourteen randomly allocated females treated with 0.5 ml of saline solution (i.m.)(CONTROL group), with 25 UI of PMSG (i.m.) (PMSG group) or separated from her litter during 48 hours (BIOS group). On day 11 post-partum all females were inseminated with fresh semen collected with artificial vagina from several males. Semen was diluted with MA-24 diluent (Lab. Ovejero, León, Spain) in such a way that was obtained a final sperm concentration of 25 million spermatozoa/seminal dose. Biostimulated does were inseminated shortly after suckling their young. Ovulation was induced with 20 µg of GnRH (Inducel GnRH, Laboratorios Ovejero, León, Spain). Sexual receptivity by means of observation of turgidity and vulva colour on day 9 and 11 post-partum was determined (UBILLA and REBOLLAR 1995). Fifteen days after AI, each female was anesthetized with 5 mg of Diazepam (Valium, Lab. Roche, Madrid Spain) and 30 mg of Ketamine Clorhidrate (Imalgène, Lab. Merial, Madrid, Spain) (i.v.) using the marginal vein. Then, by means of a laparotomy, the corpora lutea present in each ovary and the embryos in each uterine horn were counted.

On the other hand, six multiparous lactating (more than 7 pups) females in the same conditions (2 BIOS, 2 PMSG and 2 CONTROL) were killed on day 11 post-partum with intravenous pentobarbitone sodium and their ovaries were removed immediately and compared with ovaries of two does killed on day 9 post-partum. Number of follicles higher than 1mm of diameter and morphology and size of oocytes recovered were determined (LORENZO et al. 1996). Animals used in this study were treated according to the CEE Council Directive (86/609, 1986) for the Care of Experimental Animals.

Statistical Analysis was performed using the Statistical Analysis Sistem program (SAS version 6.12). A non-parametric procedure (CATMOD) was employed to identify significant effects of treatments on the variables that did not approximate to a normal distribution (Sexual receptivity, Ovulation rate, Conception rate and Fertility). A GLM procedure was used to compare corpora lutea and embryos followed by Duncan's multiple range test (SAS, 1990).

RESULTS AND DISCUSSION

Sexual receptivity of all females was similar on day 9. On day 11 post-partum this subjective observation showed a higher proportion of does with red and pink vulvas ($p < 0.05$), but in the same way in all groups (table I). Previously the increase on sexual receptivity after a PMSG treatment or biostimulation method has been observed already (MAERTENS et al. 1983; MAERTENS 1998; ALVARIÑO et al., 1998; BONANNO et al. 1999). The best method to determine sexual receptivity after apply a synchronization method is the acceptance to mate, nevertheless BONANNO et al. (1999), note that this change is not the effect of biostimulation only but the contact with the male could stimulate receptivity.

In accordance with previous results (ALVARIÑO et al., 1998), in spite of 48 hours separation of biostimulated females from her litter, the viability of the pups was not affected. Lactating rabbits nurse their litters once daily and the pups suckle during 2-5 min. uninterrupted (McNITT 1992), for this reason does only were submitted to one suckling suppression.

Ovulation rate, Conception rate and Fertility are shown in table I. Fifteen days after AI and administration of 20 µg of GnRH, the corpora lutea in each ovary of ovulated does were developed and in relation to implantation day, embryos of pregnant females were a size and an adequate appearance. The macroscopic observation of genital tracts was similar in all animals independently of treatment administered. No significant influence of biostimulation, PMSG or saline solution treatment has been observed on ovulation rate, conception rate and fertility. ALVARIÑO et al., (1998) described an effect more clear when the synchronization methods were applied on day 4 post-partum because the increase of fertility is remarkable with only a separation for 24 hours or with 25 UI of PMSG in relation to a control group. Nevertheless, on day 11 post-partum, the transient doe-litter separation for 48 hours showed similar results to treatment with PMSG or control group in accordance with our results.

On the other hand, our control group has to be considered like a group of rabbit submitted to a intervention two days before AI because all does were injected with saline solution. This manipulation could be similar to observed by LUZI and CRIMELLA (1998), who indicate a increase of fertility when lactating does with their litter were transferred to another cage 2 days before AI.

Table I. High Sexual Receptivity (HSR) (percentage of females with red or pink vulvas), Ovulation rate (percentage of females with corpora lutea in her ovary in relation with total inseminated females), Conception rate (percentage of pregnant females in relation to ovulated females) and Fertility (percentage of pregnant females in relation to inseminated females) of rabbit does separated 48 hr from her litter (BIOS), treated with 25 UI of PMSG (PMSG) or with 0.5 ml of saline solution 48 hr prior AI.

GROUP	HSR (%) DAY 9	HSR (%) DAY 11	OVULATION RATE (%)	CONCEPTION RATE (%)	FERTILITY (%)
BIOS n=14	57.1	92.8*	85.7	75	64.3
PMSG n=14	58.6	92.8*	92.8	84.6	78.6
CONTROL n=14	50	71.4*	85.7	83.3	71.4

*= p<0.05.

The results of number of corpora lutea and embryos are shown in table II. Number of corpora lutea was similar to observed by RODRIGUEZ et al. (1988) and STRADAIOLI et al. (1997). In accordance with previous results the mean number of corpora lutea observed with 25 UI of PMSG indicate a normal ovary response without superovulatory effect. No statistical differences were observed in the results obtained. One hour after exogenous GnRH and AI, higher plasma LH concentrations than control animals has been observed in biostimulated does (UBILLA et al. 2000a), for this reason we expected a different response in this kind of animals. The study of number and aspect of follicles of multiparous lactating does on day 9 post-partum and of biostimulated, PMSG treated or control multiparous lactating does on day 11, could explain the favorable results of these methods. According to our appreciation the number of follicles greater than 1 mm of diameter on day 11 post-partum is higher in the two biostimulated females (23 and 14, respectively) and the two PMSG does (12 and 13,

respectively) compared with the two control does (7 and 7, respectively), at the same day and compared with two does on day 9 (7 and 6 respectively). No differences were observed in oocyte quality. This trial has been made with a limited number of animals and will be necessary additional experiments.

Table II. Corpora lutea and Embryos implanted in does separated 48 hr from her litter (BIOS), treated with 25 UI of PMSG 48 hr prior to AI (PMSG) or treated with 0.5 ml of saline solution 48 hr prior AI.

GROUP	CORPORA LUTEA (MEAN ± S.E.M.)	EMBRYOS (MEAN ± S.E.M.)
BIO	9.5 ± 0.5 (n=12)	9.1 ± 0.6 (n= 9)
PMSG	11.7 ± 0.7 (n=13)	11.3 ± 0.7 (n=11)
CONTROL	10 ± 0.8 (n=12)	8.8 ± 1.4 (n=10)

CONCLUSIONS

Our results suggest that AI on day 11 post-partum is a practice which can be carried forward with good results without sophisticated treatments. Only a small manipulation on day 9 post-partum could replace the transient doe-litter separation and the hormonal treatments with their residues. Nevertheless the endocrinological and morphological results of the ovaries suggest a study in a long-term practice.

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