Interaction of reproduction rhythm, suckling and parity on mating incidence, ovarian dynamics and estrogen secretion in the rabbit doe

Maria Laura Marongiu

Dipartimento di Scienze Zootecniche, Università di Sassari, Italy

Corresponding author: Maria Laura Marongiu. Dipartimento di Scienze Zootecniche, Università di Sassari. Via De Nicola 9, 07100 Sassari, Italy - Tel. +39 079 229304 - Email: marongiu@uniss.it

ABSTRACT - The effects of suckling and remating interval (11 or 21 d *post partum*) were studied in primiparous (P=25) and multiparous (M=23) hybrid rabbits. In P rabbits, suckling had no significant effect on mating incidence; however, a higher percentage of females accepted the male on d 11 than on d 21 (P<0.01). In M rabbits, no significant differences in mating performance due to either day, suckling or their interaction were found. A higher proportion of nonsuckled than suckled P rabbits ovulated (P<0.01). Ovulation incidence was not affected significantly by day of mating, suckling or their interaction in M rabbits. A comparison of the day influence in P and M rabbits on mating incidence showed a greater effect in the P (P<0.05) while the suckling effect on ovulation performance showed an insignificant difference in the two parity groups. Ovulation rate, as assessed by ultrasound, was not influenced significantly by day or suckling in either P or M rabbits. The follicle distribution was marked by fewer large follicles and lower plasma estrogen levels were detected in suckled than in nonsuckled P and M rabbits (P<0.05). Further research is needed to elucidate these phenomena also when AI is performed.

Key words: Reproduction rhythm, Suckling, Parity, Ovarian dynamics.

Introduction – Control of the interval from parturition to subsequent conception is crucial in obtaining the optimal reproductive rate for a species. Even though rabbit does can be mated just after kindling and be concurrently pregnant and lactating, their reproduction efficiency varies considerably with parity order, physiological state (lactating or not, stage of lactation) and sexual receptivity at mating (Theau-Clément, 2007).

The present study was therefore designed to investigate various aspects of reproduction as affected by suckling and interval since parturition in primiparous and multiparous rabbits. An additional purpose was to examine follicular dynamics in the postmating rabbit ovary by means of ultrasound scanning. A particular attention was drawn to the population of large-sized follicles (LF) present at day 6 of pregnancy, since this follicular category, being steroidogenically active, should be crucial to the survival of the developing corpora lutea (CL) and then to the reproduction efficiency.

Material and methods – Primiparous (P=25) and multiparous (M=23) hybrid rabbits does were studied in a 2x2 factorially designed experiment. The factors under focus were day of attempted mating, whether on day 11 or day 21 following parturition, and whether or not young were suckled (S or N) after the day of parturition. The length of time a female was exposed to a given male was 60 to 120 seconds. If copulation occurred, the female was removed immediately and classed as mating. A nonmating rabbit doe was one which had been exposed to 6 different males for a total period of about 12 minutes. The ovarian dynamics (CL and follicle population) of the mated females were examined on day 6 *post coitus* which was day 17 postpartum (groups 11-N and 11-S) and day 27 *post*

partum (groups 21-N and 21-S) by means of transabdominal real time B-mode ultrasound scanning (Marongiu and Gulinati, 2008). Blood samples were obtained at day 6 *post coitus* from the ear central artery (Marongiu *et al.,* 2007) and plasma was stored at -30°C until analyzed for estradiol-17α (E_2) by a direct competitive heterologous enzyme-linked immunosorbent assay (ELISA) developed with rabbit polyclonal antiestradiol antibodies. Statistical analysis was carried out using the SAS statistical package (2001). A mixed procedure was used according to an auto-regressive model to analyze repeated measures, including the effects of reproductive rate, suckling, parity order and their interaction with respect to mating and ovulating incidence, ovarian dynamics and estrogen secretion. Means were compared using a protected t-test and differences were considered significant when $P<0.05$.

Results and conclusions – The mean values for various reproductive characteristics of both P and M postmating rabbits are listed in Table 1. Results from the statistical analyses are shown in Table 2. Smaller proportions of P rabbits mated on day 21 than on day 11 *post partum* ($P<0.01$). No significant suckling effect or interaction of suckling with *post partum* day was detected in the analysis. Mating performance in the M rabbits was not influenced significantly by suckling or day or their interaction. In P rabbits, ovulating percentages were depressed due to suckling ($P<0.01$) but were not affected significantly by day or by the suckling x day interaction. The incidence of ovulation, like mating, in M rabbits was not affected significantly by suckling, day or their interaction. A comparison of the effect of day of mating in P and M rabbits on the proportion of does that mated showed a greater effect in the P as compared with the M animals ($P<0.05$) (not included in any table). A comparison of the suckling effect on ovulation incidence in the two parity groups showed an insignificant difference. The occurrence of mating without ovulation in some subjects may indicate the different effect exerted by suckling upon hypothalamic centres regulating sexual receptivity and LH release.

No comparisons were significant in the analysis of the number of CL on both ovaries (ovulation rate) in P rabbits. However, groups 11-N and 21-N had means of 8.0 and 10.3 CL, respectively. CL number was not affected significantly by day, suckling or their interaction in M rabbits. The follicle distribution was marked by fewer LF in S than in N-P and N-M rabbits ($P<0.05$). In contrast, the number of LF was not affected by day or interaction of day and suckling. The lower population of LF found in the S group might be responsible of the lower plasma E_2 levels of the same group compared with the N one. Since E_2 concentrations have been commonly used to assess follicular growth and steroidogenic capacity (Ubilla *et al.,* 1995), the lower hormonal levels in the S rabbits may reveal poorer ovarian activity which could result in reduced reproductive parameters.

Table 1. Mean values for various reproductive characteristics of both P and M rabbits.

Group	Number of rabbits			Mean CL no.	Mean LF no.	Mean E_2 (pg ml^{-1})
	Total	Mating	Ovulating			
Primiparous						
11-N	6	5	5	8.0	20.3	14.8
11-S	6	4	3	7.9	14.1	10.3
21-N	7	4	4	10.3	20.3	14.7
21-S	6	1	1	...[a]	...[a]	...[a]
Multiparous						
11-N	6	6	4	10.0	21.1	15.3
11-S	5	5	4	10.0	15.6	11.3
21-N	6	6	3	11.3	22.1	16.0
21-S	6	5	3	9.9	13.8	10.0

[a]*Group 21-S was omitted from the analysis because only one rabbit ovulated. Orthogonal comparisons were used to the remaining three groups.*

Table 2. Least squares means for various reproductive characteristics of both P and M rabbits. Effect of suckling, day of mating and their interaction.

Source of variation	Primiparous rabbits					Multiparous rabbits				
	Incidence of mating	ovulation	CL	LF	E_2	Incidence of mating	ovulation	CL	LF	E_2
Suckling	0.48	15.41**	3.9	137.8*	100.5*	0.0003	0.12	8.2	163.3*	119.0*
Day	2.42**	0.04	16.1	0.002	0.001	0.02	0.12	8.2	0.3	0.2
Interaction	0.25	0.06	2.7	2.2	1.7	0.20	0.19	8.2	6.8	4.9
Error	0.19	0.12	4.4	23.7	17.4	0.09	0.22	5.2	25.3	18.5

**=P<0.05; **=P<0.01.*

In nursing rabbits, as in other species, sexual receptivity and fertility appear to be depressed during the period of lactation and the existence of a partial antagonism between lactation and reproduction, reflecting the corresponding hormonal antagonism between PRL and release of gonadotropins, has been widely reported (Boiti, 2004). In the present research suckling had a depressive effect on both ovulation incidence and LF number in P rabbits. Estrogens have been shown to play a key role in the maintenance of CL in the rabbit, prompting Hilliard and Eaton (1971) to refer to estradiol as the "ultimate luteotropin" in this species. The rabbit CL becomes an estradiol-dependent tissue by day 6 of pregnancy and pseudopregnancy, and afterwards adeguate E_2 is critical to the continued development of the CL (Marongiu and Gulinati, 2008). The luteotropic E_2 is supplied by the LF present in the ovary and their destruction leads to immediate failure of the CL and termination of pregnancy and pseudopregnancy, as reviewed by Niswender *et al.* (2000).

Data resulting from the present experiment could support additional research aimed to elucidate these phenomena also when AI is performed, since ovulating response and fertility after AI are high in rabbits that accept mating and significantly lower when they are not receptive to the male (Theau-Clément, 2007). The real-time ultrasonography, being feasible *in vivo,* is confirmed a non-invasive, rapid and reliable method for studying several reproductive functions also in the rabbit.

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