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DESCRIPTION OF THE OVARIAN STATUS AND FERTILISING ABILITY OF PRIMIPAROUS RABBIT DOES AT DIFFERENT LACTATION STAGES

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ABSTRACT

One hundred and seventy lactating primiparous rabbit does were inseminated 1, 4, 12, 19 days *postpartum* or 2 days post-weaning. They were sacrificed 24 hours later in order to study the ovarian status and fecundity components. At ovary observation, 135 does showed fresh *corpora lutea* (CL), 35 other does had 2 CL generations (few hours fresh CL and prominent CL). For does having only one CL generation, the percentage of receptive does, the number of preovulatory follicles, CL and the fecundity components (ovulation rate, fertility, number of segmented ova) greatly varied according to the lactation stage and lead to important differences in the productivity index : 7.0, 7.0, 12.1, 13.3 and 13.6 segmented ova/ insemination realised for 1, 4, 12, 19-day lactating and non-lactating does, respectively. The level of peripheral progesterone remained close to a basal level (0.9 ng/mL). Only 8 of the 35 does having 2 CL generations were receptive (22.9 %), all of them ovulated but only one was fertile and had segmented ova. Since the level of progesterone was high (9.4 ng/mL), it was concluded that these does were pseudopregnant at insemination. Moreover, none of 1-day lactating does evidenced 2 CL generations, therefore the authors suggest that spontaneous ovulation occured. The frequency and the causes of this phenomenon have to be studied, since these does were unable to produce, as long as they were secreting progesterone.

INTRODUCTION

Artificial insemination (A.I.) has been largely developed during the nineteen on European rabbit farms. Nevertheless, several studies have pointed out an antagonism between lactation and the reproduction functions especially for sexually non-receptive does at the moment of insemination (THEAU-CLÉMENT and ROUSTAN, 1992; CASTELLINI 1996; CASTELLINI and LATTAIOLI, 1999). Moreover, the intensity of this antagonism varies according to the lactation stage. The aim of this study was to describe the ovarian status and the fertilising ability of lactating primiparous does (receptive or not) at different lactation stages.
Primiparous does were chosen as a model since CHMITELIN *et al.* (1990) and POUJARDIEU *et al.* (1995) showed the difficulty of such does to simultaneously assume their first lactation and carry out their second pregnancy. The studied lactation stages were : day 1, 4, and 12 *postpartum (p.p.)*, corresponding to the most commonly used reproduction rhythms, day 18-19 *postpartum* (pick of lactation) and day 31 *postpartum* (48 hours after weaning for non-lactating does).

MATERIAL AND METHODS

Animals. A total of 179 INRA A1067 rabbit does and 36 males (receptivity tests), were used. Does under an 8L:16D photoperiod were fed *ad-libitum* with a commercial diet containing 16.5% protein and 15.5% fibre. Water was provided *ad-libitum*.
Experimental design. Two groups of 18 week old does (2 series of AI at an interval of 3 weeks : 65 and 114 nulliparous) were inseminated with Hyplus buck semen (Grimaud frères).

Heterospermic pools (sent by the postal services and packed in flasks) were used for insemination using a bent glass pipette. The moment of littering was precisely recorded (2 observations per day : 9 a.m. and 17 p.m.) and the litters were immediately homogenised to 9 kids. Only lactating primiparous does having at least 5 young were included in the experiment.

At the first littering, the does were divided into 5 identical groups, taking into account the moment of littering, the total number of young born and their genealogy. Does inseminated from 16 to 36 h, 72 to 112 h, 12 to 13 days or 19 to 20 days after parturition were considered as 1, 4, 12 and 19-day lactating does, respectively. Since weaning of young rabbits of the 5th group of does took place when they were 27 or 28 days-old, AI was performed 2 days post-weaning (p.w), so does were non-lactating.

Prior to insemination, sexual receptivity of the does was tested in the presence of two bucks. A doe was considered as receptive if she took a lordosis position with at least one of the two bucks. Immediately after the test (at 9 a.m.), the does were inseminated. Soon thereafter, blood samples were collected by the marginal ear vein followed by i.m. injection of 20 μg of GnRH (Receptal, HOECHST ROUSSEL VET, Romainville, France) to induce ovulation. No other hormone was used in this experiment.

One day later (at 9 a.m.), the does were slaughtered in the same order as the inseminations. To define ovarian status, the ovaries were removed so that, the number of preovulatory follicles (MARIANA *et al*., 1989), hemorrhagic follicles, fresh *corpora lutea* (CL), and old CL remaining from previous pregnancy were counted, if their diameters were greater than 1 mm. Both of the oviducts and uterine horns were flushed with 5 mL of saline solution until at least one oocyte or segmented ova was found. All collected oocytes and segmented ova were counted and observed under a microscope (x 50). Plasma progesterone was evaluated by RIA according to the procedure described by BOITI *et al*. (1996).

Registered parameters. The following parameters were analysed : percentage of receptive does at AI, progesterone concentrations, ovarian status as previously defined, and fecundity components (ovulation frequency and intensity, fertility and fertilisation rate).

Statistical analysis. In a first part, the results of does having only one CL generation were studied. Analysis of variance took into account the fixed effect of the series (2 levels), the lactation stage (5 levels : 1, 4, 12, 19 days, and 2 days after weaning) and the interaction series by lactation stage. Another analysis of variance examined the fixed effect of receptivity (2 levels) to study the relation with the studied ovarian structures and fecundity components. Ovulation frequency, fertility (number of does having at least 1 segmented ova/number of inseminated does), fertilisation rate (on ovulating does : number of segmented ova/number of CL) were considered to be Bernoulli variables (range 0-1). In a second part, since the number of does having two CL generations was limited, only general means were considered.

RESULTS

Of the 170 does examined *postmortem* 24 hours after insemination, 135 showed fresh CL (from 1 to 21), with red and turgescent stigma. Thirty-five other rabbits had two CL (from 11 to 33), a first generation of CL corresponding to the fresh category and a second one, with big and prominent CL (or big and turgescent stigma for 4-day lactating does).

1 Does having only one CL generation 24 hours after insemination

1.1 Effect of lactating stage on the ovarian status and fecundity components (table 1)

Receptivity. The sexual receptivity of does significantly (P = 0.0036) varied according to lactation stage. Four-day lactating does were less receptive than 1-day, 19-day lactating does and non-lactating does.

Table 1. Description of the ovarian status and reproductive performance of rabbit does at different lactation stages, 24 hours after insemination.

	Number	Receptivity (%)	Preov. follicles ∅<1mm	Hemorrhagic follicles	Old corpora lutea	Ovulation (%)	Corpora lutea	Fertility (%)	Segmented ova	Fertilisation (%)	Progesterone ng/ml
Does having only one corpora lutea generation 24 hours after insemination *:* results of variance analysis (least square means (standard error))											
General mean	*135*	88.8	9.96	0.9	10.5	97.0	12.6	83.7	10.7	80.9	0.9
R^2		*0.11*	*0.51*	*0.08*	*0.06*	*0.07*	*0.44*	0.07	*0.41*	*0.13*	*0.06*
Series :		NS	NS	NS	NS	NS	NS	NS	***	**	NS
1	*51*	90.5 *(4.4)*	10.7 *(0.7)*	0.5 *(0.3)*	11.2 *(0.5)*	98.9 *(0.1)*	12.3 *(0.4)*	82.2 *(5.3)*	8.9 *(0.7)*	71.2 *(4.8)*	0.6 *(0.3)*
2	*84*	86.1 *(0.1)*	9.4 *(0.5)*	1.1 *(0.2)*	10.0 *(0.4)*	96.1 *(0.1)*	13.3 *(0.3)*	84.7 *(4.1)*	12.0 *(0.5)*	86.1 *(3.7)*	1.1 *(0.2)*
Lactation stage		**	***	NS	NS	T	***	T	***	T	NS
1 day *p.p*	*36*	97.5^{a} *(5.1)*	8.8^{ac} *(0.8)*	0.4^{a} *(0.4)*	11.6 *(0.6)*	100^{a} *(2.8)*	9.7^{a} *(0.5)*	77.6^{ab} *(6.1)*	7.0^{a} *(0.8)*	73.4^{a} *(5.7)*	1.39 *(0.32)*
4 days *p.p*	*26*	70.3^{b} *(5.8)*	16.3^{b} *(0.9)*	0.9^{ab} *(0.4)*	10.3 *(0.7)*	88.8^{b} *(3.2)*	10.1^{a} *(0.6)*	70.4^{a} *(7.0)*	8.0^{a} *(0.9)*	66.9^{a} *(6.9)*	0.46 *(0.37)*
12 days *p.p*	*19*	83.3^{ab} *(7.4)*	10.8^{a} *(1.3)*	0.7^{ab} *(0.5)*	10.2 *(0.9)*	100^{a} *(4.1)*	14.5^{b} *(0.7)*	81.9^{abc}*(8.9)*	11.9^{b} *(1.2)*	76.6^{ab} *(7.9)*	1.25 *(0.47)*
19 days *p.p*	*24*	92.6^{a} *(6.3)*	7.4^{c} *(1.1)*	0.4^{a} *(0.5)*	9.7 *(0.8)*	100^{a} *(3.5)*	14.7^{b} *(0.6)*	91.2^{bc} *(7.6)*	11.8^{b} *(1.0)*	85.6^{ab}*(6.7)*	0.45 *(0.39)*
2 days *p.w*	*31*	97.6^{a} *(5.5)*	7.1^{c} *(0.9)*	1.8^{b} *(0.4)*	10.9 *(0.7)*	97.3^{ab}*(3.1)*	14.8^{b} *(0.6)*	96.4^{c} *(6.6)*	13.4^{b} *(0.9)*	90.7^{b} *(6.2)*	0.77 *(0.35)*
SeriesxLact. st.		NS	***	NS	NS	NS	NS	NS	*	NS	NS
Does having two corpora lutea generations 24 hours after insemination *:* general means (standard deviation)											
General mean	*35*	22.9 *(42.6)*	12.4 *(4.6)*	1.8.*(2.0)*	8.1 *(3.2)*	100 *(0.0)*	23.1 *(5.2)*	2.9 *(17.0)*	0.4 *(2.5)*	1.6 *(9.4)*	9.4 *(4.0)*
Lactation stage											
4 days *p.p*	*5*	40.0 *(54.8)*	18.6 *(5.5)*	2.4 *(2.5)*	10.2 *(3.1)*	100 *(0.0)*	15.2 *(6.4)*	0.0 *(0.0)*	0.0 *(0.0)*	0.0 *(0.0)*	3.0 *(2.5)*
12 days *p.p*	*16*	25.0 *(44.7)*	13.0 *(3.4)*	2.2 *(2.3)*	8.6 *(3.1)*	100 *(0.0)*	22.4 *(2.9)*	0.0 *(0.0)*	0.0 *(0.0)*	0.0 *(0.0)*	10.6 *(2.3)*
19 days *p.p*	*11*	9.1 *(30.2)*	9.5 *(3.4)*	1.2 *(1.5)*	7.4 *(2.9)*	100 *(0.0)*	26.6 *(3.0)*	9.1 *(30.2)*	1.4 *(4.5)*	5.1 *(16.8)*	11.0 *(3.2)*
2 days *p.w*	*3*	33.3 *(57.7)*	9.3 *(1.2)*	1.0 *(1.0)*	4.7 *(3.8)*	100 *(0.0)*	27.3 *(3.1)*	0.0 *(0.0)*	0.0 *(0.0)*	0.0 *(0.02)*	8.2 (*6.5)*

[3]

NS: P>0.05, T: P<0.10, *: P<0.05, **: P<0.01, ***: P<0.001. Within columns, means with different letters (a,b,c,) are significantly different P<0.05

Ovarian status. The number of preovulatory follicles (e.g. non-ruptured follicles following GnRH injection), was significantly (P = 0.0001) related to lactation stage. Four-day lactating does have a greater number of unruptured follicles than does of the other lactation stages. There was a significant interaction between the series and the lactation stage due to a higher number of preovulatory follicles in series 2 on 1-day lactating does. Inversely, there was a higher number of follicles in series 1 on 4-day lactating does. Does had an average 0.9 hemorrhagic follicles and 10.5 old CL remaining from the previous pregnancy. The frequency of these ovarian structures did not vary significantly according to lactation stage.
Fecundity components. The percentage of ovulating does significantly differed (P = 0.05) in relation to the lactation stage. Four-day lactating does ovulated less frequently (88.8 *vs* at least 97.3 % for the other ones) and had a tendency (P = 0.0625) to be less fertile than 19-day lactating and non-lactating does (70.4 *vs* 91.2 and 96.4, respectively). The intensity of ovulation and the number of segmented ova greatly differ (P = 0.0001) in relation to lactation stage. One-day and 4-day lactating does ovulate less intensively than 12-, 19-day lactating and non-lactating does (9.7 and 10.1 *vs* 14.5, 14.7 and 14.8, respectively). As a consequence, the number of ova shed followed the same trend (7.0 and 8.0 *vs* 11.9, 11.8, 13.4, respectively). A series effect and a series-lactation stage interaction have to be underlined, due to a greater number of segmented ova in series 2 for 4-day and 19-day lactating does (10.4 *vs* 5.5 and 15.3 *vs* 8.3, respectively). For ovulating does, the percentage of segmented ova had a tendency (P = 0.0740) to be lower for 4-day and 1-day lactating does, compared with non-lactating does (66.9 and 73.4 *vs* 90.7 %, respectively).
Progesterone titrations. The concentration of progesterone (general mean = 0.94 ng/mL) did not vary according to lactation stage.
A productivity index measured 24 hours after insemination greatly varied according to lactation stage : 7.0, 7.0, 12.1, 13.3, and 13.6 segmented ova/insemination for 1, 4, 2, 19-day lactating and non-lactating does, respectively.

1.2 Relationship between sexual receptivity, ovarian status and fecundity components

The oestrus behaviour of does was not related to the number of suckled young at insemination. Receptive does had fewer preovulatory follicles than non-receptive does (9.4 *vs* 14.3, P = 0.0057), they ovulated more frequently (99.2 *vs* 80.0 %, P = 0.0001), had a greater number of corpora lutea (12.9 *vs* 9.7, P = 0.0055), and were consequently more fertile (89.1 *vs* 40.0 %, P = 0.0001), with a greater number of segmented ova (11.2 *vs* 4.8, P = 0.0005) leading to a greater productivity (11.0 *vs* 4.1 segmented ova/insemination, P = 0.0001). For does having only one corpora lutea generation, the progesterone level did not vary according to their sexual receptivity at insemination.

2 Does having two corpora lutea generations 24 hours after insemination (table 1)

In this experiment, 20% of primiparous does had 2 CL generations but the frequency depended on lactation stage. None of the does inseminated 1 day *postpartum* presented this phenomenon *vs* 16 %, 46 %, 31 % and 9 % for 4, 12, 19-day lactating does and non-lactating does, respectively.

Receptivity. The percentage of receptive does was very low (22.9 %) and varied according to lactation stage from 9.1 to 40.0 % (19 days *p.p. vs* 4 days *p.p.*, respectively).
Ovarian status. The number of preovulatory follicles, hemorrhagic follicles, and old corpora lutea tended to decrease when the interval between littering and insemination increased. Compared with does having one CL generation, these irregular does had more preovulatory (12.4 *vs* 10.0) and hemorrhagic follicles (1.8 *vs* 0.9) and less old CL (8.1 *vs* 10.5).
Fecundity components. All the does ovulated. Out of a total mean of 23.1 CL, only 11.8 corresponded to a recent ovulation (red and turgescent stigma). Only 2.9 % of does were fertile, moreover the fertilisation rate was 1.6 % leading to an average of only 0.4 segmented ova. For these does, progesterone levels were high (9.4 *vs* 0.9 ng/mL for does having only 1 CL generation).

DISCUSSION

These results show that the lactation stage greatly influences ovarian status and the does' reproductive ability in early development (24 h after insemination) and consequently rabbit doe productivity. No experiment has previously quantified contemporaneously these effects.

Does having only 1 corpora lutea generation. When does are inseminated 1 day after parturition, they are highly receptive (BEYER and RIVAUD, 1969 ; HARNED and CASIDA, 1969; MAERTENS and OKERMAN, 1987) and have few unruptured or hemorrhagic follicles. The number of old CL remained high since their regression was recent and could explain the high, even though non-significant concentration of peripheral plasma progesterone. As previously evidenced by LAMB *et al*. (1991), the number of fresh CL is low, as well as the number of segmented ova. Four-day lactating does are less receptive (THEAU-CLÉMENT *et al*., 1990), have the greatest number of unruptured follicles (since not totally brought to maturity) and a poor ovulation frequency (despite GnRH injection) and intensity. Moreover, the fertilisation rate is the lowest. These two last lactation stages are often in opposition with longer intervals between littering and insemination. When does are inseminated 12 days after parturition, corresponding to the most used reproduction rhythm in European rabbit farming, their receptivity, ovarian status (less unruptured follicles) fertility and prolificacy components are at a good level. Nevertheless, on 19-day lactating does, the fecundity components seemed to be higher but remained non-significant. Whatever the studied variable, non-lactating does have the best reproductive potentialities, illustrating an antagonism between lactation and reproductive functions. Moreover, oestrous behaviour at the moment of insemination is related to the reproductive potentialities of the does (THEAU-CLÉMENT and ROUSTAN, 1992) : receptive primiparous does have less unruptured follicles, ovulate more frequently and intensively and have a higher fertilisation rate.

Does having 2 corpora lutea generation 24 hours after insemination. No explanation was found for the spontaneous ovulation occurring in 4-day lactating does (no animal manipulation before insemination, no known particular environmental stress, no relationship with buck proximity). These does were characterised by a low receptivity and a high level of peripheral plasma progesterone at the moment of insemination. All of them ovulated, but fertility and fertilisation were very low. In fact, only one doe, 19-day lactating doe was fertile (15 segmented ova) and 12 old CL (of unknown age) together with 15 fresh ones were observed. At insemination, the peripheral plasma progesterone concentration was only 1.2 ng/mL. Since functional luteolysis of CL normally begins on approximatively day 12 to 14 of pseudopregnancy and is completed around day 18 when progesterone declines to basal values (BROWNING *et al*., 1996), it can be supposed that this doe was at the end of a pseudopregnancy, allowing a new normal reproduction cycle. The distribution of does having abnormally high progesterone levels is similar to that reported by BOITI *et al*. (1996, 1998), who studied progesterone profiles in the early *postpartum* period. We confirm in agreement with these authors, that ovulation is not influenced by high levels of progesterone. We demonstrate in this study that fertilisation is generally inhibited.

BOITI *et al*. (1999) demonstrated that uterine infections increase the life span of a CL and could explain high levels of progesterone at insemination. Since none of the 1-day lactating does of that experiment was pseudopregnant at insemination, this suggests that spontaneous ovulation occurs in primiparous does. The causes, unknown today, have to be investigated.

CONCLUSION

This study confirmed the influence of the lactation stage on rabbit does early reproductive potentialities and more generally the antagonism between lactation and reproductive function. Moreover, 20 % of does were pseudopregnant at insemination and consequently had high levels of progesterone. This study suggests spontaneous ovulations which do not prevent ovulation but

further block the fertilisation process. The frequency and the causes of this phenomenon have to be studied since these does are unable to produce as long as they are secreting progesterone.

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