Proceedings 4th World Rabbit Congress, 10-14 October 1988, Budapest Hungary, Vol. 2,510-518

PLASMA PROGESTERONE LEVELS IN PREGNANT DOE RABBITS : RELATION WITH PATHOLOGY OBSERVED IN MATERNITY WARD

Françoise VIARD and Françoise PROVOT with the technical assistance of Mauricette BOIVIN;

I.N.R.A., Station de Pathologie Aviaire, Laboratoire de Pathologie du Lapin, Centre de Recherches de Tours-Nouzilly, 37380 Monnaie, France.

INTRODUCTION

In standardized rabbit breedings, a large amount of doe rabbits die before the weaning of their first or second litter, and 16 to 20 per cent of youngs die before weaning.

Our previous experiments (Viard-Drouet et al. 1983) showed that this pathology of doe rabbits, especially death of youngs before weaning, found expression in metabolic disorders and an early modification of plasma prolactin patterns observed during pregnancy.

Horrobin (1977) expressed the hypothesis that a defect of balance between prolactin, progesterone and cortisol might explain the preeclampsia syndrom in woman. An abnormal progesterone secretion is frequently implicated in spontaneous pathology observed at the beginning of pregnancy in woman (Gerhard and Runnebaum 1982 ; Aspigalla et al . 1983) or in pathology experimentally induced in doe rabbits (Jost 1986).

At last, Coppola et al. (1979) showed a relation between plasma progesterone levels and fertility of doe rabbits.

Therefore, we looked for a possible relation between plasma progesterone levels and death of youngs or death of does and with productive capacity of the females.

MATERIALS AND METHODS

1). Experimental procedure

76 nulliparous doe rabbits 120 to 128 days old were dispatched in 3 groups according to the mating day. Matings were separated by 2 days between each group.

In each group, four non-mated females were used as control animals. Blood drawing was done so that we obtained data for every day of the first pregnancy. The day G0 of gestation was the mating day.

Pregnancy was diagnosed by palpating the animals 13 days post-mating. After parturition the females were not mated again.

At weaning, females were divided in 4 groups : does that weaned all or part of their litter, does that died before parturition, non-pregnant females though mated, non-mated females. In our experiment, we did not observe abortions and no female died during lactation contrary to previous observations.

Blood was collected on lithium heparinate, immediately refrigerated and centrifuged (15 mn, 1100 g) ; plasma was stored at -20°C.

Does were weighed before each blood sampling.

2). Animals and breeding conditions

We used 1067 INRA does (crossing between Californian and New-Zealand breeds) produced by J. Ponceau (37370 Epeigné-sur-Dême, France). The animals received ad libitum water and a pelleted feed "Lapin Repro" from Coopaliment (86360 Chasseneuil-du-Poitou) which is composed as follows : crude protein 16.5%, lipids 2%, cellulose 15.5%, minerals 10%, water 14%, spiramycine 16 ppm, Bifuran 150 ppm, Robenidine 50 ppm.

The does'quarter was composed of 72 cages arranged in three-storied batteries (air speed 0.25 m/s ; temperature 15-18°C ; light 8 hours/day until the 3rd day preceding the first matings and 16 hours/day afterwards).

3). Progesterone assay

The assays were done by Laboratoire de Dosages of the Station de Physiologie de la Reproduction, INRA, CR de Tours-Nouzilly, 37380 Monnaie.

Progesterone is assayed by radioimmunoassay from a 0.1 ml plasma sample. The assay, adapted from the method of Saumande et al. (1985) is enough reliable so that a single assay per blood sample could be done (D. André, unpublished). The first antibody was produced in a rabbit immunized with 11-α-hemisuccinate : BSA and the second antibody was produced in sheep. The detection limit was 6.25 pg/sample and sensitiveness of the assay was 0.2 ng/ml. The precision of the assay was 15% for a mean value of 1.5 ml (n = 21) and was 9.3% for a mean value of 5.9 ng/ml (n = 21).

4). Statistical analysis

Zootechnical data (prolificity, mortality, weight) and plasma progesterone levels of the four groups of females (pregnant does, does which died, non pregnant does and non-mated females) are compared with a four levels unifactorial analysis of variance (ANVARU program ; Bachacou, Masson, Millier, 1981). The correlation coefficients between daily plasma progesterone levels and zootechnical data of doe rabbits alive at weaning are calculated with STAT 1 program (Bachacou, Masson, Millier, 1981).

RESULTS

1). Plasma progesterone patterns in pregnant rabbits. Relation with the death of does.

In pregnant does, two patterns were observed within the first three hours following mating (fig. 1) : in some females, progesterone level was high (10 to 15 ng/ml) and in others it was very low (0.1 to 0.4 ng/ml). This difference was neither linked with what happened to the litter, nor to the doe (death of youngs or death of the doe).

Figure 2 and table 1 show the average evolution in the four groups of females. There was a sharp increase in progesterone level during the first week of pregnancy. Then, the level was approximatively constant up to the 20th day of gestation. From G21, there was a gradual decrease and a sharp decrease 1 to 2 days before parturition or just before death. This evolution was common to all pregnant rabbits whether they were to die or not. Only the maximum value was variable but this value could not be related with the pathological state of the female.

2). Evoluion of plasma progesterone in non-pregnant rabbits, mated or not

In mated rabbits which were considered non pregnant when examined the 13th day after mating we observed :

- the day of mating, the two patterns of response observed in the does that became pregnant.
- from day G1 : in some of the females there was a fast increase of progesterone level just as in pregnant rabbits, followed by a sharp decrease from G15 to G20 (fig. 3a), in two females we noted a delayed increase of progesterone, after G8, and a late decrease, posterior to G20 (fig. 3a) ; then in other females, progesterone levels were constantly low (fig. 3b).

Most of non-mated rabbits (fig. 4) had very low progesterone levels during all the experiment. But three of them had a high level respectively on days G1, G6, G10.

3). Relation between progesterone levels and productivity of the female

In does that weaned all or part of their litter, we calculated the correlation coefficients between plasma progesterone level of a day and the different zootechnical parameters. Among all the data, the progesterone level was best correlated with the total number of rabbits born per litter and with their weight at birth (table 2). From G29, plasma progesterone level was well correlated with the number of youngs born alive, with their weight and with the number of youngs weaned by the females.

4). Relation between progesterone level and weight of the female before mating

The correlation coefficients between weight of doe before mating and progesterone levels were low and constantly negative between G2 and G25 except for G15 though not significant.

DISCUSSION

1). The general pattern of progesterone levels observed in pregnant rabbits is much similar to what is frequently described. The values observed are similar to those noted by Challis et al. (1973), Browning et al. (1980), Lau et al. (1982), Munsel et al. (1982), and are slightly more elevated than those observed in the middle of pregnancy by Harrington and Rothermel (1977), Enbergs (1979), Browning and Wolf (1981). These differences are light and probably due to the assay method.

2). The two patterns of progesterone evolution observed in the few hours following mating (peak in some females, lack of peak or delay of apparition in other animals) are in agreement with the results Goodman and Neill (1976) obtained with α-dihydroprogesterone : they observed a rise in this steroid 1 to 4 hours after mating, and that, in part of the females studied. Khan-Dawood and Dawood (1984) and Mills and Gerardot (1984) noted a progesterone peak level two hours after mating ; six hours post-coïtum the level was already low while Fredericks et al. (1982) observed a peak level six hours after mating and basal values twenty four hours after mating.

None of these authors mentionned a difference in the post-coïtum evolution of progesterone ; Harrington and Rothermel (1977) do not mention any difference between their animals what can be explained because the blood drawing was made just after mating.

We found also these two patterns as well in non-pregnant females, though mated as in control females. It is possible that progesterone peak levels observed at G0 and G4 were the consequence of the stimulus resulting from the squeak of males when they are mated, but this hypothesis cannot explain the late peak levels observed after G14.

None of the females was mated after the first week of our experiment but, after this time, we observed high progesterone levels during several days or even during a period as long as pseudogestation, in non-pregnant rabbits and even in non-mated females. These progesterone peak levels could be observed again in the same females during the 2nd month of the experiment. It is likely that a rather important number of females have spontaneous ovulation as described by Donovan and Harris (1956) or even sexual cycles ; to conclude it would be necessary to follow these females for a period over 3 months. In these cases a high progesterone level at the moment of mating could prevent ovulation. Indeed, Mills and Gerardot (1984) think that the high plasma progesterone level that characterises pregnancy could be accounted for the fact that mating has no effect on ovulation and on LH, FSH or progesterone levels in blood. It is likely, though we did not check it, that some of the mated females that did not become pregnant did not ovulate, that others ovulated and developped a corpus luteum of pseudopregnancy after a spontaneous or induced ovulation and that some of them aborted. In a few does, the increase in progesterone level after G2 is slow. In these cases, the corpus luteum might not work properly and could induce early abortions.

3). The strength of the preovulatory peak does not seem to modify the working of corpus luteum as we do not see any connection between this peak and stillbirths or abortions (seen or suspected when the females were mated). Otherwise, we do not clearly acertain the results of Coppola et al. (1979). We find as they do, a negative correlation between the number of youngs per litter and progesterone levels of the does at G16, 17, 18 but none at G30, 31. It appears that, as Polidoro and Black (1970) and Enbergs (1979), there is not a strong relation between plasma progesterone level and the number of corpus luteum, the number of implantation sites and the number of youngs born alive.

On the contrary, we noted a significant negative correlation between progesterone levels at G29, 30 and the number of youngs weaned. As there is antagonism between progesterone and prolactin during 24 to 48 hours preceding parturition on the onset of lactation, our result acertains the hypothesis that a bad endocrine working in the female can cause death of youngs during lactation. This observation must be confirmed before studying its mechanism.

CONCLUSION

Plasma progesterone levels are not directly linked with the pathology we observed at the end of gestation and during lactation. It would be interesting to study more precisely the relation existing between embryonic death, abortion and progesterone.

REFERENCES

Aspigalla M.O., Whittaker P.G., Grey C.E., Lind T., (1983). Endocrinologic events in early pregnancy failure. Am. J. Obstet. Gynecol.147 : 903-908.

Bachacou J., Masson JP., Millier C.,(1981). Manuel de la programmathèque statistique AMANCE. 1 vol., 516p., Copédith, Paris.

Browning J.Y., Keyes P.L., Wolf R.C., (1980). Comparison of serum progesterone, 20 -dihydroprogesterone and estradiol-17 in pregnant and pseudopregnant rabbits : evidence for postimplantation recognition of pregnancy. Biology of reproduction, 23 : 1014-1019.

Browning J.Y., Wolf R.C., (1981). Maternal recognition of pregnancy in the rabbit : effect of conceptus removal. Biology of Reproduction, 24 : 293-297.

Challis J.R.G., Davis I.J., Ryan K.J., (1973). The concentration of progesterone, estrone and estradiol-17 in the plasma of pregnant rabbits. Endocrinology, 93 : 971-976.

Coppola A., Zicarelli L., Bernini G.P., Gasperi M., Franchi F., Pellegrini S., Luisi M., (1979). Reproductive rythm and ovarian activity in rabbits. Zbl. Vet. Med. A, 26 : 590-597.

Enbergs Von H., (1979). Radioimmunologische untersuchungen zum verlauf des progestronspiegels im blut von trangenden kaninchen unter berücksichtigung der jahreszeit und der zahl der neugeborenen. Berl. Münch. Tierärztl. Wschr. 92 : 32-34.

Jost A. (1986). New data on the hormonal requirement of the pregnant rabbit : partial pregnancies and fetal anomalies resulting from treatment with a hormonal antagonist, given at a sub-abortive dosage. C. R. Acad. Sc. Paris, t. 303, Série III, n°7, 281-282.

Gerhard I., Runnebaum B.,(1982). -hCG, progesterone and estradiol-17 in women with early pregnancy disorders. Acta Endocrinologica. Suppl. 246. 1.

Goodman A.L., Neill J.D., (1976). Ovarian regulation of postcoïtal gonadotropin release in the rabbit : reexamination of a functional role for 20 -dihydroprogesterone. Endocrinology, 99(3) : 852-860.

Harrington F.E., Rothermel J.D., (1977). Daily changes in peripheral plasma progesterone concentrations in pregnant and pseudopregnant rabbits. Life Sciences, vol 20 : 1333-1340.

Khan-Dawood F.S., Dawood M.Y., (1984). Implantation of the rabbit blastocyst : sequential changes in estradiol and progesterone and their receptors. American Journal of Obstetrics and Gynecology, 150(1) : 450-455.

Lau I.F., Saksena S.K., Salmonsen R., (1982). The concentration of progesterone, 20 - dihydroprogesterone, testosterone, oestrone and oestradiol-17 in serum, amniotic fluid and placental tissue of pregnant rabbits. Acta Endocrinologica, 99 : 605-611.

Mills T.M., Gerardot R.J., (1984). Dissociation of copulation from ovulation in pregnant rabbits. Biology of Reproduction, 30 : 1243-1252.

Munsel Von M., Elsaesser F., Paufler S., (1982). Endokrinologische untersuchungen der peripartalen periode und des puerperiums bei kaninchen. Zuchthyg. 17 : 165-171.

Polidoro J.P., Black D.L., (1970). Peripheral plasma progesterone and the number of foetuses in normal and superovulated rabbits. J. Reprod. Fert. 22 : 493-498.

Saumande J., Tamboura D., Chupin D., (1985). Changes in milk and plasma concentrations of progesterone in cows after treatment to induce superovulation and their relationships with number of ovulations and of embryos collected. Theriogenology, 23, (5) : 719-731.

Table 1 - Plasma progesterone levels during pregnancy in doe rabbits.

Day of pregnancy	Pregnant females alive at weaning			Pregnant females which died before parturition			Non pregnant females			Control females		
	X̄	s	n	X̄	s	n	X̄	s	n	X̄	s	n
0	4.4	4.5	11	4.8	1.3	2	9.6	5.2	6	12.8	*	1
1	0.2	0.1	11	0.4	0	2	6.9	7.5	6	10.3	*	1
2	1.6	0.6	13	2.0	0.8	3	2.8	5	6			
3	3.3	0.7	13	3.9	0.2	3	3.9	6.3	6			
4	5.7	1	11	5.7	1.8	3	5.6	5.6	7	2.9	4.8	3
5	8.4	1.8	11	8.9	1.8	3	6.3	5.5	7	3.6	5.9	3
6	10.4	2.3	11	10.7	1.1	3	6.4	5.8	7	2.8	4.5	3
7	11.8	2.2	11	13.3	0.6	2	3.0	4.1	6	0.7	*	1
8	11.9	2.8	11	15.5	1.4	2	2.8	3.9	6	0.7	*	1
9	12.3	4.7	13	9.7	0.8	3	5.1	4.7	6	3.5	4.1	2
10	13.3	5.6	13	11.3	2.6	3	5.4	4.7	6	4.2	5.2	2
11	12.1	2.5	12	10.4	2	3	4.4	4.1	6	3.5	4.5	2
12	15.6	4.7	11	12.4	0.4	3	5.4	6.9	2	0.5	0.3	3
13	14.2	3.7	11	12.5	0.2	3	5.5	6.9	2	0.4	0.1	3
14	13.1	3.1	11	19.2	9.5	2	6.1	4.4	3	0.9	*	1
15	12.3	3.5	11	13.9	5.4	2	5.9	4.7	3	0.7	*	1
16	12.5	3.9	13	9.5	6.9	3	4.5	5.8	2	0.7	0.0	2
17	9.9	2.2	12	9.7	2.5	2	5.7	6.7	2	0.6	0.0	2
18	10.0	4.1	12	9.7	0.8	2	5.5	7.2	2	0.7	0.2	2
19	11.1	3.8	10	8.9	0.6	3	6.7	9.2	2	0.5	0.1	3
20	10.2	3.1	11	10.4	4	3	5.0	6.5	2	0.2	0.1	3
21	7.4	1.9	11	6.9	*	1	2.6	3.7	3	0.3	*	1
22	8.1	2.8	11	3.5	*	1	1.5	1.5	3	0.3	*	1
23	7.9	1.7	13	8.0	*	1	0.6	0.4	2	0.6	0.0	2
24	8.7	1.8	12	8.1	*	1	0.4	0.1	2	0.5	0.0	2
25	9.6	3	11	7.8	0.3	3	5.3	7	2	0.2	0.1	3
26	9.9	2.3	11	5.2	5.2	3	5.2	7	2	0.2	0.2	3
27	9.3	2.7	11				3.0	4.5	3	0.3	*	1
28	8.2	2.5	11				3.9	5.4	3	0.3	*	1
29	5.0	2.4	11				4.4	4.6	3	0.2	*	1
30	2.1	1	13				0.5	0.1	2	0.4	0.1	2
31	0.5	0.6	13				0.3	0.1	2	0.4	0.1	2
32	0.9	*	1									

* 1 female

Table 2 : Correlation between plasma progesterone level of a day of pregnancy with productivity data of healthy females (° P(5% ; * P(1% ; ** P(1%.).

Day of pregnancy	Nb of couples	Nb of youngs born	$\bar{X}$Weight of youngs born	Nb of living neonates	Weight of living neonates	$\bar{X}$Weight of living neonates	Nb of weanlings	Weight of weanlings	$\bar{X}$Weight of weanlings	Nb of dead sucklings	Weight of doe before mating
0	12	-0,34	0,51°	-0,29	-0,13	0,58*	-0,20	0,05	0,54°	-0,41	0,59*
1	12	0,05	0,20	0,09	0,19	0,24	0,24	0,19	0,13	-0,38	0,22
2	13	-0,62*	0,18	-0,54°	-0,36	0,22	-0,26	0,03	0,41	-0,45	-0,25
3	13	0,07	-0,18	-0,31	-0,29	0,06	-0,26	-0,25	0,03	-0,10	-0,56*
4	11	0,52°	-0,35	-0,26	-0,07	0,23	-0,24	-0,32	0,15	0,29	-0,35
5	11	0,66*	-0,48	0,05	-0,16	-0,18	-0,14	-0,36	-0,33	0,52°	-0,25
6	11	0,49	-0,14	0,34	0,21	-0,17	0,43	0,18	-0,64*	-0,09	-0,32
7	12	-0,05	0,22	0,07	-0,1	0,10	0,16	0,21	0,16	-0,22	-0,27
8	12	0,3	-0,18	0,43	0,03	-0,25	0,45	0,30	-0,12	0,13	-0,15
9	13	-0,37	0,09	-0,29	-0,16	0,18	-0,13	-0,01	0,17	-0,25	-0,29
10	13	-0,46	0,12	-0,49	-0,35	0,17	-0,33	-0,12	0,33	-0,26	-0,12
11	13	-0,34	0,15	-0,15	0,03	0,21	0,03	-0,09	-0,15	-0,27	-0,33
12	11	-0,55°	-0,24	-0,01	0,09	0,08	0,16	-0,12	-0,47	0,06	-0,45
13	11	0,83**	-0,71**	-0,13	-0,32	-0,12	-0,27	-0,55	-0,21	0,57*	-0,08
14	12	-0,09	0,24	0,04	-0,02	0,04	0,14	0,36	0,11	-0,29	-0,04
15	12	0,15	0,04	0,21	0,09	0,06	0,36	0,13	-0,05	-0,34	0,23
16	13	-0,51°	0,03	-0,40	-0,23	0,27	-0,30	-0,15	0,24	-0,17	-0,39
17	13	-0,44	-0,05	-0,43	-0,36	0,13	-0,34	-0,22	0,20	-0,15	-0,31
18	13	-0,50°	0,01	-0,36	-0,32	0,07	-0,21	-0,02	0,26	-0,23	-0,63*
19	11	0,62*	-0,42	-0,31	-0,13	0,19	-0,19	-0,39	-0,18	0,37	-0,27
20	11	0,44	-0,44	-0,55°	-0,28	0,30	-0,40	-0,51	0,11	0,41	-0,34
21	12	0,39	-0,16	0,51°	0,36	-0,58	0,63*	0,42	-0,31	-0,13	-0,19
22	12	0,34	-0,14	0,44	0,42	-0,27	0,61*	0,34	-0,40	-0,32	-0,08
23	13	-0,28	0,10	-0,38	-0,37	-0,02	-0,24	0,07	0,41	-0,24	-0,38
24	13	-0,09	-0,48°	-0,26	-0,52°	-0,23	-0,19	-0,29	-0,10	0,10	-0,9**
25	11	0,67*	-0,50	0,04	-0,14	-0,15	-0,01	-0,32	-0,41	0,30	-0,27
26	11	0,44	-0,64*	0,04	-0,53°	-0,47	-0,29	-0,49	-0,23	0,59°	0,25
27	12	0,13	0,05	0,25	0,31	-0,12	0,42	0,26	-0,23	-0,38	-0,33
28	12	-0,11	0,19	-0,03	-0,14	0,17	0,08	0,05	0,15	-0,33	0,33
29	12	-0,60*	0,61*	-0,55°	-0,40	0,54°	-0,52°	-0,25	0,54°	-0,34	-0,11
30	13	0,01	-0,10	-0,43	-0,01	0,61*	-0,73**	-0,62*	0,19	0,41	-0,03
31	13	0,01	0,02	-0,41	0,18	0,79**	-0,47	-0,53	-0,06	0,06	0,24

Fig. 1 : Plasma progesterone levels in two pregnant rabbits alive at weaning (13 youngs born and 12 youngs weaned)

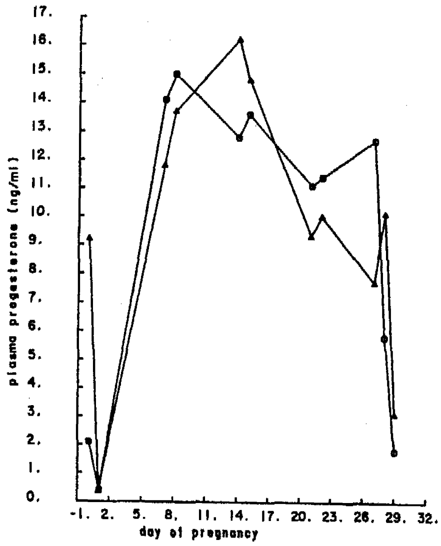
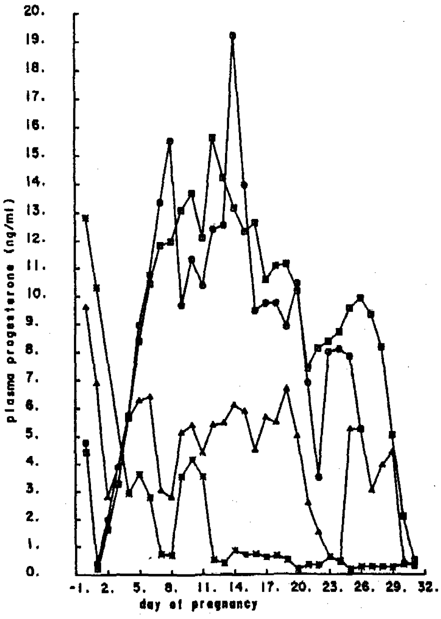


Fig. 2 : Average evolution of plasma progesterone in the following four groups of females :

- □ females alive at weaning
- ○ females that died during pregnancy
- ▲ non pregnant females
- ✱ non mated females



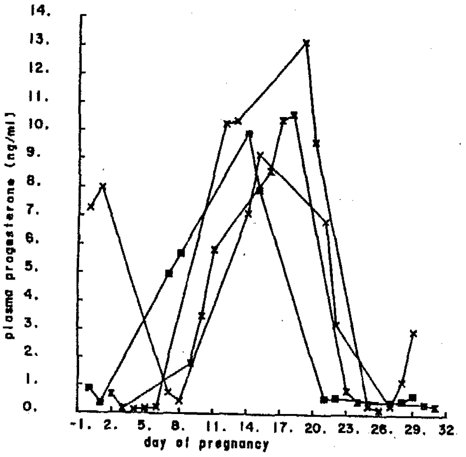


Fig. 3a : Plasma progesterone levels in four non pregnant rabbits

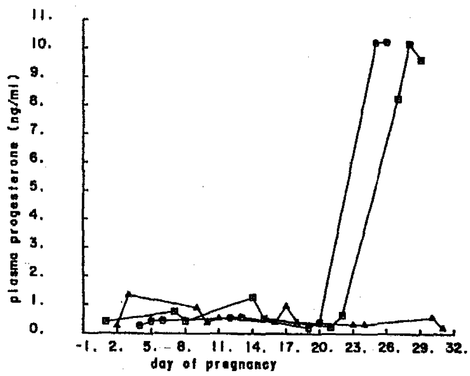


Fig. 3b : Plasma progesterone levels in three non pregnant rabbits

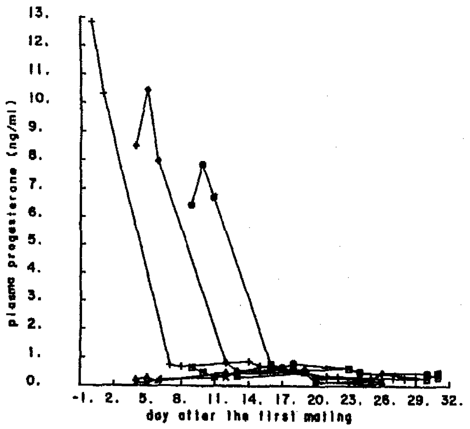


Fig. 4 : Plasma progesterone levels in six non mated rabbit does

SUMMARY

PLASMA PROGESTERONE LEVELS IN PREGNANT DOE RABBITS : RELATION WITH PATHOLOGY OBSERVED IN MATERNITY WARD.

Françoise VIARD and Françoise PROVOT with the Technical Assistance of Mauricette BOIVIN.
I.N.R.A., Centre de Recherches de Tours-Nouzilly, 37380 Monnaie, FRANCE.

The progesterone concentration in plasma was measured everyday during pregnancy in nulliparous rabbit does.

Results were analysed according to the becoming of the animals (death of female, death of the litter) and according to zootechnical datas (number of newborns, number of weaned rabbits in the litter, weight of the litter,...).

It appears that progesterone levels are neither directly linked with the pathology of doe rabbits: death of females before parturition, stillbirths or death of the litter before weaning nor with productivity of females.

RESUME

EVOLUTION DU TAUX DE PROGESTERONE PLASMATIQUE CHEZ LA LAPINE EN GESTATION : RELATION AVEC LA PATHOLOGIE OBSERVEE EN MATERNITE.

Françoise VIARD et Françoise PROVOT avec la collaboration technique de Mauricette BOIVIN.
I.N.R.A., Centre de Recherches de Tours-Nouzilly, 37380 Monnaie, FRANCE.

L'évolution de la progestéronémie a été suivie au cours de la gestation de lapines nullipares.

L'analyse des résultats a été faite d'une part en fonction de la pathologie des femelles (infécondité de la femelle, mortalité des lapereaux à la naissance et pendant la période d'allaitement), d'autre part en fonction des critères zootechniques de productivité (nombre de lapereaux nés vivants, nombre de lapereaux sevrés, poids de la portée,...).

Il semble que la progestéronémie n'ait de lien direct ni avec la pathologie étudiée notamment avec la mortalité des femelles, ni avec la productivité des lapines.