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Milk production of pseudopregnant does

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ABSTRACT

The aim of the experiment was to examine the milk production and milk composition of presumably pseudopregnant does. In experiment 1 the milk production of regular nursing multiparous (MRN) and induced to ovulation nulliparous (NIO) (n=15) and multiparous (MIO) (n=20) does injected with GnRH (15 µg per animal) at 16.5 weeks of age or 11 days after parturition was examined. Compared to the MRN does NIO and MIO groups produced milk equal to 21 and 35% of the MRN does' production. In experiment 2 primiparous and multiparous pregnant, regular nursing (PRN and MRN) /RN/ does and two groups of presumably pseudopregnant nulliparous and multiparous does: non-pregnant /PNP and MNP/ and induced to ovulation /NIO and MIO/) were examined. The milk production of the PRN and MIO does reached 70 and 58% of the MRN does' production level (226 g milk/day). The average milk production of the MNP group was 75g but some does produced substantially higher or lower milk yield than average. The milk production of the PNP and NIO does was low. The milk composition of regular nursing does was similar to the groups of presumably pseudopregnant does. It was concluded that the MNP pseudopregnant does could be utilized as foster does.
(Keywords: rabbits, pseudopregnant, milk production, milk composition)

ÖSSZEFOGLALÁS

Az álvemhes anyanyulak tejtermelése

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A kísérlet célkitűzése az álvemhes anyanyulak tejtermelésének és tejösszetételének vizsgálata. Az első kísérletben többször fialt, rendesen szoptató (MRN), illetve 16,5 hetes korban, vagy a fialás után 11 nappal csak GnRH-val (15 µg/egyed) kezelt (indukált ovulációs), még nem fialt (NIO, n=15) és többször fialt (MIO, n=20) anyanyulak voltak. A második kísérletben egyszer (PRN) és többször fialt (MRN) rendesen szoptató és valószínűleg álvemhes még nem fialt és többször fialt anyák két csoportját: nem vemhesült (PNP és MNP), illetve GnRH-val ovuláltatott (NIO és MIO) vizsgáltuk. Az MRN csoporthoz (226 g tej/nap = 100%) képest a PRN és a MIO anyák tejtermelése 70 és 58% volt. A MNP csoport átlagos napi tejtermelése 75 g volt, de egyes anyák ettől is lényegesen több vagy kevesebb tejet termeltek. A PNP és a NIO anyák alig adtak tejet. A szoptató

anyák és a feltehetően álvemhes anyák tejösszetétele hasonló volt. Megállapítható, hogy az álvemhes MNP anyákat dajka anyaként lehetne hasznosítani.
(Kulcsszavak: nyulak, álvemhes, tejtermelés, tejösszetétel)

INTRODUCTION

Milk secretion capability of non-pregnant animals was demonstrated by several authors (*Dumon et al.,* 1993; *Zöldág et al.,* 1993). Hormonal background of pseudopregnancy was described by *Zöldág et al.* (1993) in dogs. In female dogs, with the decreasing blood progesterone concentration the prolactin level gradually increases (*Cowie et al*., 1980) and reaches its highest level during the last days of gestation (or pseudopregnancy). Biosynthesis of milk requires several other hormonal factors besides progesterone and prolactin, like oestrogen, STH and ACTH (*Karg and Mayer,* 1989). Because of the direct and indirect effects of STH and ACTH, the mammary gland and the lactiferous ducts are suitable for adequate milk yield.

The milk of the pseudopregnant rabbit does was used so far only for chemical and pharmaceutical analyses thus the rabbits were used as model animals (*Forcada et al.,* 1992; *Plantamura et al.,* 1996; *Ariona et al.,* 1997; *Schlegel et al.,* 1997). But it could also have a practical concern aspect. According to *Azard* (2006) in France 84.4% of rabbit farms (in 2005) use the method of single batch. In this case all does are inseminated on the same day, in most of the cases (91.3%) 11 days after parturition. They reach about 80% kindling rate (*Azard and Lebas,* 2006) so 20% of the females are inseminated but not conceived, they are probably pseudopregnant. If the milk production of pseudopregnant does is enough high they can be use as foster does. According to *Gyarmati* (2001) nulliparous pseudopregnant does which were injected with GnRH analogies at the age of 18.5 weeks produced milk. The application of prostaglandin (Enzaprost) on day 16 of pregnancy or oxytocin before each nursing could improve the milk production by 16 and 46%, respectively.

The aim of the study was to examine the quantity and composition of the milk produced by probably pseudopregant nulliparous and multiparous does. The objective of experiment 1 was to evaluate milk production of multiparous regular nursing and of nulliparous and multiparous induced to ovulation does. The objective of experiment 2 was to evaluate milk production and milk composition of primiparous and multiparous regular nursing does and that of the presumably pseudopregnant nulliparous, primiparous and multiparous does induced to ovulation or inseminated but non-pregnant does.

MATERIALS AND METHODS

The present analyses were based on data from Pannon White rabbits. The does were kept in a closed rabbitry, in flat-deck cages and were placed individually to wire net cages (850×350 mm) included the nest box (270×350 mm). The temperature in the rabbitry was ranged between 18 and 24 °C. The lighting period was 16L/8D. Rabbits were fed *ad libitum* with a commercial pellet for breeding does (10.3 DE MJ/kg, 16.8% crude protein, 14.1% crude fiber) and the water was available *ad libitum* from nipple drinkers.

In experiment 1, nulliparous (N, n=15) and multiparous randomly selected does (M, n=20) were injected with GnRH analogous (1.5µg/animal Ovurelin, Reanal) to induce to ovulation (IO) at the age of 16.5 weeks or 11 days after parturition, respectively, but they were not inseminated. A group of multiparous does were inseminated by fresh semen of individual bucks at the time of GnRH injection and were

pregnant, kindled and regular nursing (MRN). Kits (8 rabbits/litter) were nursed by their mothers at 8.00 am and by the presumably pseudopregnant (NIO or MIO) does at 5.00 pm. Milk production of the does was measured every 2 or 3 days using the weight-suckle-weight method (the weight difference of does before and after nursing) from the 31st day after the injection (corresponding at the moment of expected parturition). Apart from these events control nursing was applied. Weaning took place 5 weeks postpartum.

In experiment 2, milk production and milk composition of primiparous and multiparous regular nursing does and presumably pseudopregnant nulliparous, primiparous and multiparous does was evaluated. Eight nulliparous and fourteen multiparous randomly selected does were inseminated at the age of 16.5 weeks or 11 days after parturition, respectively. Part of them conceived and nursed regularly (PRN, MRN), the rest remained non-pregnant (PNP, MNP). Seven nulliparous and seven multiparous does were injected with 1.5 µg GnRH analogous (Ovurelin, Reanal) at the same time (16.5 weeks of age or 11 days after parturition) to induce to ovulation but were not inseminated (NIO and MIO).

The following groups were formed:

PRN = Primiparous, pregnant, kindled and regular nursing does (control group, n=7)
PNP = Primiparous, inseminated but not pregnant doe (n=1)
NIO = Nulliparous does, induced to ovulation (n=7)
MRN = Multiparous, pregnant, kindled and regular nursing does (control group, n = 7)
MNP = Multiparous, inseminated but not pregnant does (n=7)
MIO = Multiparous does, induced to ovulation (n=7)

On day 31 of effective gestation the pregnant does were injected with oxytocin (5 IU per animal). Litters were equalized according to the weight (8 kits in each litter). Each litter was nursed by two does. The does that were part of the trial were allowed to enter the nest boxes at 9.00 am for 30 minutes, other does kindled on the same day as the trial does could nurse the litters at 6.00 pm. The kits of the latter does were cross fostered to litters that were not part of this study. The milk production of the does was measured using the weight-suckle-weight method on days 3, 5, 8, 10, 12, 15, 17, 19, 22 and 24 of lactation.

In experiment 2, the does'milk composition was also determined (dry matter, protein, fat, ash, lactose). On days 9, 16 and 23 of lactation subsequent to 5 IU oxytocin injection milk samples were taken from one nipple of each doe by means of vacuum pump (totally emptying the mammary gland). Because of the small amount of milk samples produced by the does the chemical analysis was accomplished using the pooled milk samples.

The progesterone level was not measured in the experiment. This is why we used the term "presumably pseudopregnant" does.

Milk production measured at the given days in the six groups was compared by means of univariate analysis of variance applying SPSS 10.0 software package.

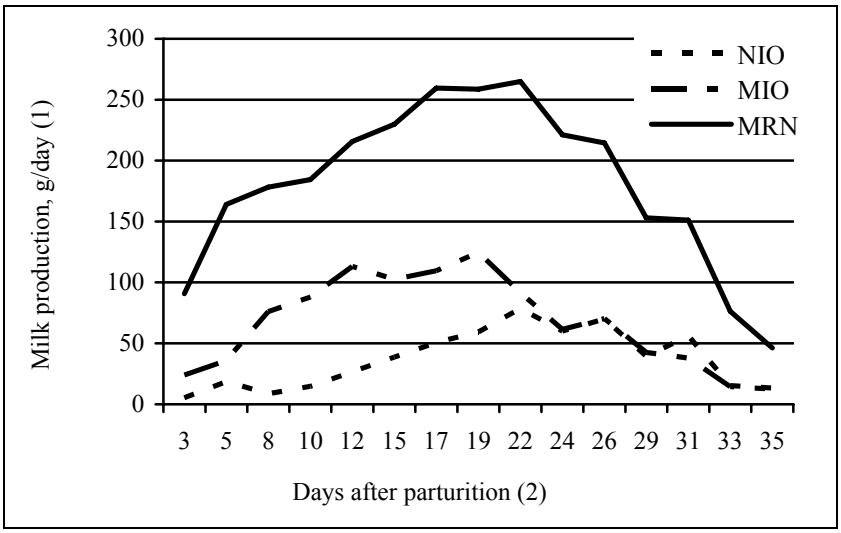
RESULTS AND DISCUSSION

Milk production of multiparous regular nursing and of nulliparous and multiparous induced to ovulation does (Experiment 1).

The milk production of regular nursing multiparous does (MRN), and nulliparous and multiparous, induced to ovulation does treated with GnRH at the time of insemination (NIO and MIO) is shown on *Figure 1.*

Figure 1

Milk production of regular nursing multiparous (MRN) rabbits does, nulliparous (NIO) and multiparous pseudopregnant (MIO) does induced to ovulation (Experiment 1)



1. ábra: A többször fialt, rendesen szoptató (MRN), valamint a még nem fialt (NIO) és a többször fialt GnRH-val kezelt álvemhes anyák (MIO) tejtermelése (1. kísérlet)

Tejtermelés g/nap(1), Fialás utáni napok száma(2)

The milk production of multiparous regular nursing does (MRN) was increased until the 22nd day of lactation. At the moment of weaning (35 days), the milk production of these animals was lower than 50 g/day. During the same period, the milk production of MIO group increased slowly until the 19th day. During the whole period, the milk production of MIO does reached 35% of the MRN does. The milk production of the NIO does showed a slow progress from 10 days after supposed parturition but the average milk production during the 5 weeks reached 21% of the MRN does'. At the third week after parturition (between day 14 and 21), which is the most important stage of lactation, these values were 39% and 15%, respectively. However, after day 22 the milk production was similar for MIO and NIO groups.

These production levels were obtained when the pseudopregnant does were closed in the nest boxes for the nursing period as they did not want to enter the box for nursing.

Milk production and milk composition of nulliparous, primiparous or multiparous regular nursing, inseminated but not pregnant and induced to ovulation does (Experiment 2)

During the whole trial significant differences of milk production were found between the groups (*Table 1*). The highest milk production was reached by the multiparous regular nursing does (MRN). Starting with 79 g/day milk yield on day 3, lactation peaked

between days 15–22 producing daily yields of 274–302 g with an average of 226 g/day for the whole lactation period. The primiparous regular nursing does (PRN) could reach only 70% of the former group's performance producing 158 g/day average daily milk yield. The difference between these groups was similar during the whole lactation.

Table 1

Milk production of nulliparous (N), primiparous (P) and multiparous (M), regular nursing (RN), non-pregnant (NP) and induced to ovulation (IO) does

Group	Day 3			Day 5			Day 8			Day 10			Day 12		
	Mean	SE	Sig	Mean	SE	Sig	Mean	SE	Sig	Mean	SE	Sig	Mean	SE	Sig
MRN	78.9	14.3	c	150.0	5.4	c	210.1	17.0	c	203.1	13.7	c	249.6	15.2	d
MIO	31.7	13.3	ab	39.1	10.5	a	115.4	20.1	b	122.4	20.5	b	159.9	19.7	bc
MNP	24.6	9.2	ab	41.1	8.9	a	82.7	27.0	ab	74.3	26.0	ab	85.1	30.1	ab
PRN	59.7	9.0	bc	111.4	13.4	b	137.7	22.7	bc	95.7	23.0	b	195.7	22.7	cd
NIO	4.6	2.0	a	4.4	2.4	a	6.6	2.1	a	9.3	4.3	a	10.4	5.1	a
PNP[1]	1.0			1.0			1.0			3.0			1.0		

Group	Day 15			Day 17			Day 19			Day 22			Day 24		
	Mean	SE	Sig	Mean	SE	Sig	Mean	SE	Sig	Mean	SE	Sig	Mean	SE	Sig
MRN	275.7	21.7	b	282.3	22.3	b	273.6	29.4	d	301.7	46.9	d	234.6	27.3	d
MIO	202.9	15.3	b	234.9	19.3	b	175.3	35.3	cd	148.1	18.7	bc	85.9	20.9	bc
MNP	87.6	31.3	a	84.4	35.2	a	63.3	26.4	ab	85.6	34.1	ab	123.4	25.2	ab
PRN	222.9	24.6	b	189.0	22.9	b	156.9	14.9	bc	229.9	18.6	cd	182.3	27.4	cd
PIO	20.1	10.6	a	0.9	0.1	a	19.4	10.2	a	28.1	13.6	a	18.6	10.1	a
PNP1	18.0			23.0			34.0			23.0			25.0		

Mean: Milk production measured on the given day, g; (*Átlag: A tejtermelést az adott napon mérték*); [1]The group consisted of a single doe; (*A csoportot egy anya alkotta*); [a,b,c,d]Different letters within a column show significant differences (*Azonos oszlopon belüli különböző betűk szignifikáns különbségeket jeleznek*) ($P<0,05$)

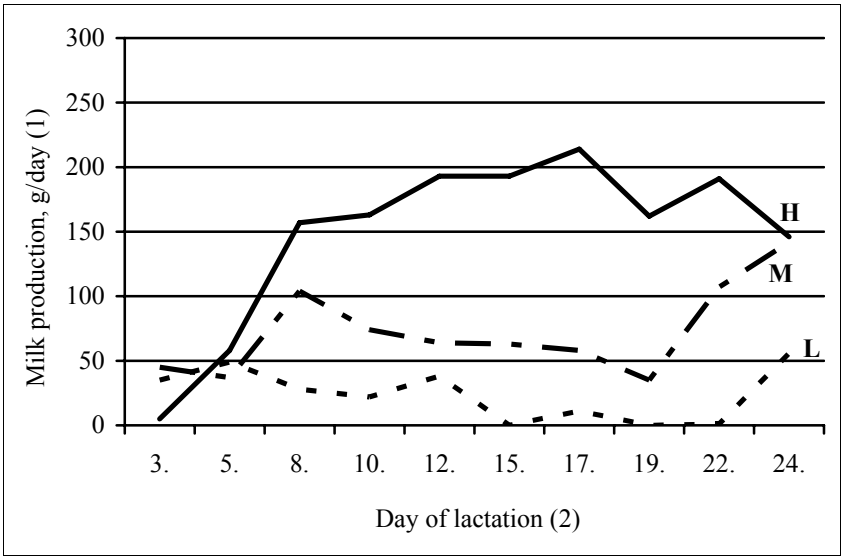
1. táblázat: Még nem fialt (N), egyszer fialt (P), többször fialt (M), szoptató (RN), nem vemhes (NP) és GnRH-val indukált ovulációs (IO) anyák tejtermelése

Multiparous induced to ovulation group (MIO) produced daily 132 g milk on average, reaching 58% of the MRN does' production. Milk production of the MIO group started from low level (32 g) and showed slow progress yet on day 17 of lactation, the daily milk yield reached 235 g then it quickly decreased. Milk production of multiparous does remained non-pregnant (MNP) was only one third that of the MRN group. The lactation of the MNP does initiated with low production levels which remained unchanged between days 8–22. The milk production level of the NIO group was low (13 g/day). The group of PNP consisted of a single doe, its production started on day 15 and reached 12 g/day on average.

The MNP group consisted of 7 does with the highest variability of milk production between days 8 and 17 (*Table 1*). One doe's production was negligible. Two does (L) started milk production, but on day 15 of lactation they were almost dried up (*Figure 2*). Two other does' milk production (M) showed only slight changes and its level increased only during the last days of lactation. However, two does (H) had normal lactation with 214 g highest daily milk yield.

Figure 2

Three groups of the multiparous non-pregnant (MNP) according to their milk production (Experiment 2)



H: does with high (*Nagy*-), M: medium, (*Közepes*-) L: low milk production, (*Kis tejtermelésű anyák*)

2. ábra: A többször fialt, nem vemhesült anyanyulakon belüli három csoport tejtermelése (2. kísérlet)

Evaluating milk composition (average of day 9, 16 and 23), lowest dry matter and ether extract content was found in the MRN group (29.2% and 14.1%, respectively) followed by the PRN groups (30.6% and 15.8%, respectively).

The MIO group produced milk containing 34.6% dry matter and 17.7% ether extract and the highest values (34.8% and 19.8%) were found in the MNP group (*Table 2*). At certain stages of lactation this tendency was not so obvious. But in most cases the highest values (dry matter or fat content) were found in the groups of MIO and MNP, and the lowest values in the MRN and PRN groups (*Table 2*).

Highest crude protein (13.4%) and ash (2.59%) and lowest lactose content were recorded in the MIO group (*Table 2*). Among the other groups negligible differences were found (crude protein: 11.4–11.6%, lactose: 1.1–1.2%).

In both experiments the same litters were nursed in the morning and in the afternoon by pseudopregnant and by regular nursing does. Theoretically the 9 hours difference between the first and second nursing could modify the milk production (the amount of milk suckled by the kits). According to our former results (*Szendrő et al*., 2000, 2002), using double nursing, it was established that the kits are able to suckle similar amount of milk in the morning and in the afternoon if the duration between two nursing was 9–12 hours. This phenomenon is justified by the present results, because in experiment 2 the "regular nursing does" (nursing in the afternoon) had higher milk production than that of the does in experiment 1 that nursed the kits in the morning.

Table 2

Milk composition of multiparous (M) (regular nursing /RN/, non-pregnant /NP/ and induced to ovulation /IO/) and primiparous regular nursing (RN) does (pooled samples)

Days after "kindling" (1)	Group of does (2)			
	MRN	MIO	MNP	PRN
Dry matter, % (3)				
9	32.2	27.6	36.3	33.5
16	25.4	37.3	34.2	24.0
23	30.0	38.8	33.9	34.4
Crude fat, % (4)				
9	16.2	12.7	20.2	18.5
16	11.1	19.4	20.0	9.5
23	14.9	21.1	19.3	19.2
Crude protein, % (5)				
9	12.3	11.7	12.1	11.6
16	11.0	14.2	11.0	11.1
23	11.6	14.2	11.7	11.4
Ash, % (6)				
9	2.05	2.16	2.01	2.05
16	2.19	2.72	2.04	2.40
23	2.58	2.88	2.75	2.68
Lactose, % (7)				
9	1.49	1.20	1.71	1.29
16	1.14	0.86	1.09	1.00
23	0.98	0.54	0.88	1.08

2. táblázat: Többször fialt (M) (szoptató /RN/, nem vemhes /NP/, GnRH-val indukált ovulációs /IO/) és egyszer fialt szoptató (RN) anyanyulak tejösszetétele (összevont minták)

Fialás utáni napok száma(1), Anyák csoportja(2), Szárazanyag(3), Nyerszsír(4), Nyersfehérje(5), Hamu(6), Laktóz(7)

The milk production of regular nursing does reached similar level to most data in the literature (*Maertens et al.,* 2006) but it was lower than that of some of the highly efficient hybrid does (*Fortun-Lamothe and Sabater,* 2003; *Xiccato et al*., 2005). Milk yield difference between the MP and PP groups was larger than those published by other authors (*Sabater et al*., 1993; *Xiccato et al*., 2004a), which may be attributable to the young age of the does at their first insemination (16.5 weeks). The pseudopregnant rabbits can also produce milk but their milk yield is lower than that of the regular nursing does (*McNitt and Lukefahr,* 1990; *Xiccato et al.,* 2004b) and it depends on their age (nulliparous or multiparous). The milk production of nulliparous pseudopregnant does was negligible, it has no practical interest.

Sometimes the lactation of pseudopregnant does reached the peak level earlier than that of the regular nursing does, which could presumably be connected with the progesterone level. The progesterone declines from 12 to 14 days after ovulation (*Scott*

and Rennie, 1970; *Browning et al*., 1980; *Boiti et al.,* 1999), which could explain the fall of milk production of these does.

Theau-Clément et al. (1990) evidenced that injecting 20 μg GnRH analogous induces the ovulation of 82.5% of treated does. *Eiben et al.* (1996) reported a higher value in Pannon White and lower one in Angora does (95.8–100% and 66.7–83.3%, respectively). On that basis, it can be supposed that some does were not pseudopregnant and could explain a part of variation in the milk production.

During the first stage of lactation the dry matter and fat content of the milk was high, it decreased and remained low for two weeks, then it increased again during the last weaning stage (*Lebas,* 1971; *Kustos et al.,* 1999). Thus low level of milk production coincides with high milk composition values. Our results justify the negative correlation between milk yield and dry matter and fat content: in the groups with lower milk yield (e.g. pseudopregnant does), these values were higher. The protein and ash content of the milk was found stable during lactation in the literature (*Lebas,* 1971., *Kustos et al*., 1999), which explained the small differences that were found among the groups.

All results of the present study were also justified for proven pseudopregnant does (*Szendrő et al*., 2010).

CONCLUSIONS

Pseudopregnant does are capable of milk production, but that of the presumably pseudopregnant nulliparous (induced to ovulation or inseminated but non-pregnant) does was low and practically cannot be used or valorized.

Milk production of the presumably pseudopregnant (inseminated but non-pregnant) multiparous does approximates to that of the nulliparous regular nursing does. As a result, the average milk yield of the pseudopregnant (non-pregnant) multiparous does is 83% of the nulliparous regular nursing does' performance.

Among the multiparous non-pregnant does (MNP) the milk production levels varied. Evaluating the progesterone levels during pseudopregnancy on a large group could determine the true pseudopregnancy rate and the milk production of the pseudopregnant does. The production level and the milk composition of true pseudopregnant does may be suitable for rearing small litters or could be used in the nursing system using two does. In the case of high prolificacy (e.g. larger total litter size than IO in some hybrids), the does are not able to rear up all of the kits. Using pseudopregnant (inseminated but non-pregnant) females as foster does, the culled and died kits can be decreased.

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