

## MILK PRODUCTION OF PSEUDOPREGNANT MULTIPAROUS DOES

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**ABSTRACT:** The aim of the experiment was to evaluate the milk production of pseudopregnant does. In this experiment, data of multiparous, inseminated, pregnant (IP, n=15) does and two groups of presumably pseudopregnant multiparous does: inseminated, non-pregnant (INP, n=17) and induced to ovulation by GnRH (1.5 µg per animal) at the day of insemination (11 d after parturition) (non-inseminated, ovulating: NIO, n=15) were analysed. The progesterone level was measured at the 12<sup>th</sup> d after treatment to determine if females were pseudopregnant. All IP and NIO does were pregnant and pseudopregnant, respectively. Within the INP group, 10 and 7 does were diagnosed as positive (INPO) or negative (INPNO) for pseudopregnancy. Two of the INPNO does perished during lactation. The average milk production of groups IP, INP and NIO was 212, 92 and 72 g/d, respectively ( $P<0.001$ ). The proportion of rabbit does reaching daily milk yields of <10, 10-50, 50-100, 100-160, >160 g in the various groups were: IP=0, 0, 0, 0 and 100%, NIO=20, 13, 27, 40 and 0%, INP=15, 15, 15, 55 and 0%, respectively. The daily milk yield of the 5 INPNO does was 2, 6, 27, 84 and 139 g, respectively. These results demonstrated that multiparous empty does, pseudopregnant or non-pseudopregnant, can produce milk, but in lower quantities than multiparous does after kindling.

**Key Words:** rabbits, pseudopregnant, milk production.

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## INTRODUCTION

Prior to puberty, the mammary tissue is influenced both by hormones and growth factors (Hovey *et al.*, 2002). In adults, the development of mammary gland and milk production is under hormonal control, mostly by the reproductive (oestrogen, progesterone, placental lactogen, prolactin, oxytocin), metabolic (GH, corticosteroids, thyroid hormones, insulin, GI hormones) and tissue hormones (GH, prolactin, parathyroid hormone-related peptide, leptin) (Neville *et al.*, 2002; Svennersten-Sjaunja and Olsson, 2005).

Pseudopregnancy in mammals is the appearance of clinical signs and symptoms associated with pregnancy when the animal is not pregnant. The clinical signs observed during pseudopregnancy in bitches are prenatal-like and maternal-like behaviours, nesting, digging, aggression, licking, mammary enlargement and distension, lactation and milk secretion (Gobello *et al.*, 2001 a,b; Concannon and Vestegen, 2005). Milk secretion capability of non-pregnant dogs was demonstrated by several authors (Dumon *et al.*, 1993; Zöldág *et al.*, 1993). Rood (1980) and Crell *et al.* (1991) observed spontaneous lactation in dwarf mongoose nursing the young of other females. Lactation of pseudopregnant females was also observed

in rats (Van der Schoot *et al.*, 1978; Montagnese *et al.*, 1987) and in ringtailed lemurs (Pereira and Izard, 1989). Non-puerperal lactation in humans was described in several cases (Briehl and Kulka, 1935; Sobrinho, 1998, 2003).

The hormonal background of reproduction and lactation was generally described by Neville (2006) and that of the rabbits was summarised by Nordio Baldiserra (1980) and McNitt (1992). These authors also showed the difference between pregnant and pseudopregnant rabbit does. In rabbits, functional luteolysis of corpus lutea is complete around 18 d of pseudopregnancy when the progesterone level declines to its basal level (Boiti *et al.*, 1999). At the end of pseudopregnancy, a maternal nest-making behaviour can be observed (Lebas *et al.*, 1986). The characteristic mammary gland growth during pregnancy and lactation was described by Ming Hsiung Lu and Anderson (1973). No difference was found for mammary gland weight at 16 d between pregnant and pseudopregnant does, but no further measurements were made. In a former experiment, Szendrő *et al.* (2000) observed that both nulliparous and non-lactating multiparous does were able to produce milk when their pseudopregnancy was induced by a GnRH analogous injection.

So far, the milk obtained from pseudopregnant rabbits was used only for chemical and pharmaceutical analyses and, in this context, rabbit was used as a model animal (Forcada *et al.*, 1992). However, this milk production could also have a practical application because, according to Theau-Clément *et al.* (1990), the ovulation rate of does was 82.5 %. The pseudopregnant rabbits that remained empty can be used as foster does if their milk production is satisfactory.

The aim of the study was to compare the milk quantity of regularly nursing does with that of does with induced ovulation (exogenous GnRH treatment (without insemination)) and with that of inseminated but non-pregnant does.

## MATERIALS AND METHODS

### *Animals, housing and diets*

The experiment was carried out at the Kaposvár University on Pannon White rabbits. The does were housed in a closed rabbitry, in flat-deck wire net cages (850 × 350 mm, including nest boxes: 270 × 350 mm). The rabbitry temperature ranged between 18 and 24°C. The lighting period was 16L/8D. Rabbits were fed *ad libitum* a commercial pellet (10.3 MJ DE/kg, 16.8% crude protein, 14.1% crude fibre) and water was available *ad libitum* from nipple drinkers.

### *Experimental groups*

In the experiment, multiparous lactating does (parity: 3-6, body weight: 4.4-4.8 kg) were used (n=100). Does were randomly sorted into two groups. Half of the does (n=50) were inseminated 11 d after parturition (17 remained empty), while the other part (n=50) was treated with GnRH (1.5 µg GnRH analogue, Ovurelin, Reanal) to induce ovulation (pseudopregnancy), but they were not inseminated. Three groups of randomly chosen rabbits were formed to examine the progesterone level and milk production:

IP=Inseminated, pregnant does (control group)

INP=Inseminated, non-pregnant does (n=17); within this group 10 ovulated (INPO), 7 non-ovulated (INPNO).

NIO=Non-inseminated (induced to ovulation by GnRH treatment), ovulating does

All of the INP (n=17) and randomly selected IP (n=15) and NIO does (n=15) were chosen for the experiment. Two of the INPNO does died during lactation (n=5).

The day of parturition was designated 0 d. Days preceding parturition (insemination, blood samples, etc.) were counted as “minus days”.

#### *Blood samples, progesterone level determination*

To detect pseudopregnancy, blood samples were taken from 15 randomly chosen animals at the beginning of the trial, at the time of insemination (baseline, -31 d; directly prior to GnRH treatment) then 12 d later (-19 d) blood samples were taken from all experimental rabbits (n=47) to measure progesterone concentrations.

Two mL of blood were collected from the central ear vein into EDTA vacutainer tubes. Blood samples were centrifuged for 15 minutes at 3000 g and plasma transferred into Eppendorf tubes and stored at -20 °C until assayed for progesterone concentrations to assess the functional status of the ovarian corpora lutea.

Plasma progesterone concentrations were determined in duplicate by RIA, using specific antibodies according to the procedure reported elsewhere (Boiti *et al.*, 1996). Progesterone was extracted from 0.1 mL of plasma with ethyl ether. The assay sensitivity was 0.08 ng/mL and intra- and inter-assay coefficients of variations were 5.3 and 10.2%, respectively. For the purpose of this work, progesterone levels below 2.0 ng/mL were considered as basal (Boiti *et al.*, 1996) and does were classified as non-pseudopregnant, while levels over 2.0 ng/mL were handled as indicative of pseudopregnancy.

#### *Nursing*

On 31 d of gestation (0 d), the pregnant does were injected with oxytocin (5 IU per animal) to induce parturition. Similar litters were formed and nursed by IP, INP and NIO does. Kits of these groups originated from does not enrolled in this experiment. Litters were equalised according to weight and number (8 kits in each litter). Each litter was nursed by two does. The does in study (IP, INP, NIO) were allowed to enter the nest boxes at 9.00 am. for 30 minutes, whereas other does (outside of the experiment) kindled on the same day (0 d) as the experimental does could nurse the litters at 6.00 pm. as described by Szendrő *et al.* (2002). The use of the second nursing does was justified by the uncertain nursing activity of the pseudopregnant does. The milk production of the does was measured using the weight-suckle-weight method (weight difference of does before and after nursing) at 2, 4, 8, 9, 11, 14, 16 and 18 d of lactation.

#### *Statistical analysis*

Average milk production (IP, INP, NIO) was compared by means of univariate analysis of variance with the SPSS 10.0 software package.

## RESULTS

Results of progesterone level measurement are summarised in Table 1. At the beginning of the trial (-31 d), progesterone level was low for all examined does. According to the hormone levels measured at -19 d, all IP and NIO does were indeed pregnant and pseudopregnant, respectively. Within the INP group, the proportions of the pseudopregnant and non-pseudopregnant does were 58.8 (10) and 41.2% (7), respectively.

All IP does showed a high milk yield. Their average daily milk yield between the 2<sup>nd</sup> and 18<sup>th</sup> d of lactation was 212±30 g; it was 112±30 g at 2 d and reached the lactation peak (277±64 g) at 18 d (Figure 1). The INP and NIO does' milk production showed a slow progress. As compared to the IP does, the average milk production of both the INP (92±56 g/d) and the NIO (72±52 g/d) groups was significantly lower ( $P<0.001$ ) at all measurement times. These rabbits could be sorted into 4 groups: individuals with

**Table 1:** Progesterone levels in the experimental rabbits.

	Progesterone level, ng/mL		
	No.	Mean	Standard Deviation
Baseline	15	0.57 <sup>a</sup>	0.28
IP	15	9.14 <sup>bc</sup>	3.01
NIO	15	7.88 <sup>bc</sup>	1.97
INP	17	6.14 <sup>b</sup>	5.43
INPO	10	10.04 <sup>c</sup>	3.38
INPNO	7	0.56 <sup>a</sup>	0.20

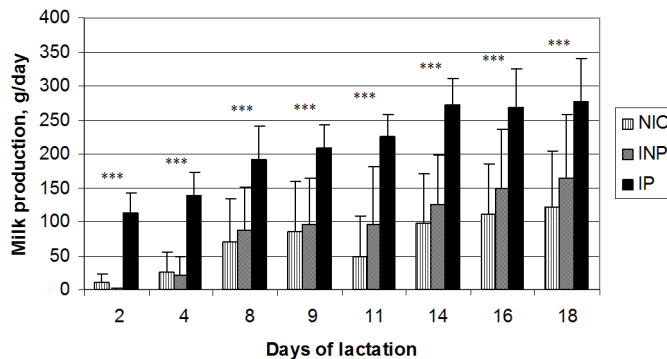
<sup>a,b,c</sup> Means within the column not sharing any superscript are significantly different at  $P < 0.001$

Baseline: Beginning of the experiment (-31 d) at the time of insemination, IP: inseminated, pregnant does (-19 d), NIO: non inseminated (induced to ovulation by GnRH), ovulating does (pseudopregnant) (-19 d), INP: inseminated, non pregnant does (total) (-19 d), INPO: inseminated, non pregnant, ovulating does (pseudopregnant) (-19 d), INPNO: inseminated, non pregnant, non ovulating does (non pseudopregnant) (-19 d).

negligible milk yield (average daily milk production  $< 10$  g); minimal milk yield (milk production: 10-50 g/d); low milk yield (milk production: 50-100 g/d) and moderate milk yield (milk production: 100-160 g/d). Within the INP and NIO groups 15, 15, 15, 55%, and 20, 13, 27, 40% of the does belonged to these categories, respectively. The average daily milk production of the 5 non-pseudopregnant INP does was 2, 6, 27, 84 and 139 g, respectively.

## DISCUSSION

In the experiment, the same litters were nursed in the morning and in the afternoon by experimental (IP, INP, NIO) does and by regular nursing does (out of the experiment). Theoretically, the 9 h difference between the first and second nursing could modify the milk consumption. In our former study (Szendrő *et al.*, 2002) using double nursing by two does, it was established that the kits are able to suckle similar amounts of milk in the morning and in the afternoon if the duration between the two nursings was 9-12 hours. This phenomenon was also confirmed here, as in this experiment the IP does reached similar milk



**Figure 1:** Milk production of does (mean±standard deviation). IP: inseminated, pregnant does, INP: inseminated, not pregnant does, NIO: non inseminated (induced to ovulation by GnRH), ovulating does. \*\*\*: The difference between groups IP and INP or NIO were significant at  $P < 0.001$  level.

production to that reported in the literature (Maertens *et al.*, 2006), although it was lower than that of some of the highly efficient hybrid does (Fortun-Lamothe and Sabater, 2003; Xiccato *et al.*, 2005).

Pseudopregnant does can also produce milk but their milk yield is lower than that of regular nursing does (McNitt and Lukefahr, 1990; Xiccato *et al.* 2004).

Theau-Clément *et al.* (1990) evidenced that injecting 20 µg GnRH analogue induces the ovulation of 82.5% of treated does. However, it largely depends on the genotype and the physiological status of the does at the moment of injection or insemination: parity order and lactation stage. Accordingly, Eiben *et al.* (1996) reported a higher value in Pannon White compared to Angora does (95.8-100% and 66.7-83.3%, respectively). Boiti *et al.* (1998) recorded 93.9% pseudopregnancy rate for rabbits treated with PMSG and GnRH.

In the experiment, all GnRH treated rabbits became pseudopregnant while only 59% of the inseminated but empty does were pseudopregnant. It must be noted that in the NIO group all does were treated with GnRH, in contrast to the INP group, where after AI only the empty does were examined.

A substantial difference was found concerning the milk production within the group of pseudopregnant does. Some does had a negligible yield (< 10 g) while other exhibited a moderate (100-160 g) daily yield. Therefore, it seems that pseudopregnancy is not strictly coupled with the does' milk yield. It can be seen that under our experimental conditions, the pseudopregnant females which did not have parturition at 0 d had weaned their previous litter a few days previously. Therefore, suckling by fostered new born rabbits can reactivate the prolactin secretion and initiate new milk production.

The decisive role of prolactin in regulating milk production is well known (Shin and Friesen, 1980). Prolactin injection in does during the decreasing stage of lactation (24 d) (Linzell *et al.*, 1972), or pseudopregnant (Bourne *et al.*, 1974) rabbit does with prolactin induced mammary gland development and milk production. There is a suckling-induced release of oxytocin and prolactin (Falconer and Vacek, 1980). For humans, mostly in primitive cultures, several reports were published describing the relactation of non-lactating, non-postpartum women (Brown, 1977). Successful induction of lactation can be achieved by breast suckling alone (Brown, 1977; Abejide *et al.*, 1997). Suckling stimulates nerve endings that cause the anterior pituitary gland to produce prolactin and indirectly the posterior pituitary gland, causing oxytocin release (Egli *et al.*, 1961). Milk production in females which did not have a previous parturition was observed in other species (König, 1997). Spontaneous lactation has been observed repeatedly in the dwarf mongoose (Crell *et al.*, 1991). Spontaneous lactation induced by the suckling stimuli of the kits may also be possible for non-pseudopregnant rabbit does. In the experiment, all litters were reared by two does (a regular nursing doe and a pseudopregnant doe), so the developmental stage of kits was suitable to induce hormone production.

Using a 42 d reproductive rhythm with a single batch production system, all does in a farm are inseminated 11 d after parturition. About 75-80% of them are pregnant (and will kindle) but 20-25% of the inseminated females did not conceive. Most of these non-pregnant does exhibit progesterone levels over 2 ng/mL and could be classified as pseudopregnant. If the milk production of these females is high enough, they could be utilised as foster-mothers. Due to the limited number of rabbits, this experiment could not evaluate this possibility.

## CONCLUSIONS

Pseudopregnant multiparous does are capable of milk production but in very variable amounts depending on the rabbits. Some non-pseudopregnant rabbits can also produce milk when stimulated by suckling kits.

Therefore, the possibility of using multiparous does not fertilised after insemination as foster dams should be investigated in further experiments.

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