

## GROUPING OF BREEDING RABBIT DOES AT DIFFERENT TIME POINTS: EFFECTS ON FERTILITY, MORTALITY AND WEIGHT

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**Abstract:** Semi-group housing in breeding does has been shown to reduce production and breeding success in comparison to single-housed animals. One reason for this reduction could be stress and aggression when grouping does only 2 d after artificial insemination. The aim of this study was to test different time points of grouping on fertility of does, body weight of both kits and does, and mortality rates. Hence, does were separated and housed individually one day before giving birth to their kits. The does were then artificially inseminated on day 10 postpartum (dpp) and regrouped according to the treatment (time point) on 12 (TG12), 18 (TG18), or 22 (TG22) dpp, respectively. In total, five trials with three groups pertaining to the three treatments (eight does per treatment group) were conducted. Non-pregnant does were replaced with pregnant does before each new trial (57 different does needed). Data were analysed with (generalised) linear mixed effect models and survival analysis. There were no significant differences in fertility, body weight or mortality of does among the treatments. The average fertility rate (number of kindling events/number of artificial inseminations×100) was low (40.92%) and seasonal effects may have partially masked treatment effects, as most trials took place during winter. Likewise, the survival rate of kits was not influenced by the treatment (survival test:  $\chi^2=2.3$ ,  $df=2$ ,  $P=0.3$ ). Body weight of the kits was also not affected by the time point of grouping (average weight: 447.70±46.42 g (TG12), 452.20±55.30 g (TG18) and 460.06±89.23 g (TG22);  $P=0.33$ ). In conclusion, grouping does at a later time point in the reproductive cycle did not show any significant improvement in the breeding or productive success in a Swiss semi-group housing system. An elongated separation from conspecifics did not enhance the welfare of semi-group housed rabbits.

**Key Words:** rabbit, reproduction, survival rate, semi-group housing, management, body weight.

## INTRODUCTION

The reproductive success of does is influenced by multiple factors like the insemination procedure (Bergonzoni *et al.*, 1994 in EFSA –European Food Safety Authority– 2005), breed (Al-Saef *et al.*, 2008) or lighting regime (Theau-Clément, 2000). Furthermore, the housing system has been shown to play an important role: continuous group housing has mostly low (Rommers *et al.*, 2006; Mugnai *et al.*, 2009a; Szendro *et al.*, 2109) and semi-group housing only slightly higher but still low reproductive performance compared to single housing (e.g. fertility rate: 76.2% and 82.8% for semi-group and single housing, respectively) (Dal Bosco *et al.*, 2019). In the current system found on “animal-housing-friendly” labelled European farms like in Switzerland and Belgium, does are separated shortly before giving birth until 11-12 d afterwards. Body temperature (Graf *et al.*, 2011) and corticosteroids (Andrist *et al.*, 2014) increase after regrouping and indicate a stress reaction. Mugnai *et al.* (2009b) described the possible effects of stress causing an increase in plasma prolactin (Manteca, 1998) which then, due to its antagonistic hormonal function, negatively affects the reproduction (Kermabon *et al.*, 1995). Likewise, Bench and Gonyou (2007) explained that stress may decrease the concentration of luteinising hormone (LH), leaving ovarian follicles without adequate stimulation

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and accordingly causing slower growing follicles. As the artificial insemination (AI) can take place only two days prior to regrouping at 10 days postpartum (dpp), stress shortly afterwards (12 dpp) may cause elevated embryo mortality, resulting in low overall fertility. Maertens and Buijs (2015) reported good reproductive results (fertility rate: 88.9%, kit mortality after regrouping: 2.1%) with semi-group housing where animals were regrouped later, namely at 18 dpp. Machado *et al.* (2019) compared single-housed with semi-group housed does, also grouped at 18 dpp, and found no significant differences in milk yield, litter size at birth and body weight of does. Thus, we assumed that a later regrouping with larger kits, when milk production was stable, would lead to less stress and to a better reproductive success, hence improving production. This study therefore focussed on production in a Swiss semi-group housing system using different time points of regrouping to investigate effects on fertility and mortality rate. As low fertility rates could also be related to a decreased body weight and a low social rank of the does (Machado *et al.*, 2019), we also took body weight and rank into account.

Group-housed does show shorter and less frequent nest visits than single-housed ones and fewer suckling events are reported in that system (Rommers *et al.*, 2012). We expected that later regrouping in the semi-group housing system would lead to higher growth rates of kits because the kits spent more time in close proximity to their mother.

Likewise, Dal Bosco *et al.* (2019) suggested that social stress may cause maternal failure affecting kit mortality. In fact, depending on the does' current pregnancy state (Hudson *et al.*, 2000, 1996), weaning of the kits naturally varies between 20 and 28 days of age, coinciding with the 22 dpp for regrouping. Therefore, the objective of this study was to see if a longer lasting single housing phase until 22 dpp may positively affect the survival rate of kits, as they are more developed and less dependent on their mothers' care during regrouping.

## ANIMALS, MATERIALS AND METHODS

### *Animals and housing*

The study was conducted on a commercial Swiss rabbit breeding farm using 57 does of the Hycole hybrid. The does were multiparous (at least two previous parturitions). The experiment was performed in five consecutive trials from September 2018 until March 2019. The does were kept in groups of eight per pen according to a Swiss label programme for animal-friendly housing (BTS, <https://www.blw.admin.ch/blw/de/home/instrumente/direktzahlungen/produktionssystembeitraege/tierwohlbeitraege.html>, last accessed on 4-10-2019). The semi-group housing pen consisted of eight individual cages, which were closed at the top with a grid one day before parturition. The area

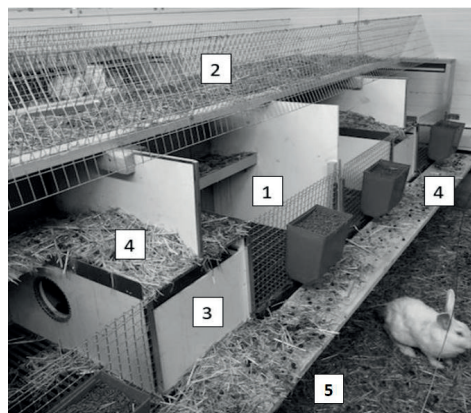


Figure 1: One-sided pen outlay: Individual cage (1), closable grid (2), one nest box per cage (3), platforms (4) and common area (5).

of every cage was approximately 1.6 m<sup>2</sup>. Each cage included a nest box (0.30×0.40 m), a straw bedded platform, a feeder and a nipple drinker. During the regrouping phase, the grids were opened and the does had access to a shared area (3.20×2.20 m) (Figure 1). Air temperature and relative humidity were recorded during the entire experiment (HOBO® Datalogger U10-003, Onset Computer Corp., Bourne, MA 02532) at the height of the animals and varied between 8.4 and 20.1°C and 26 to 67% humidity, respectively. There was natural daylight in the barn, supplemented with artificial light during feeding and early working times during winter. Additionally, 5 d before until 2 d after AI, there was a 16/8 lighting regime. The does had *ad libitum* access to commercial rabbit pellets (UFA 925, UFA AG, Herzogenbuchsee, Switzerland), containing 10.0 MJ digestible energy, 170 g crude protein, 145 g crude fibre, 35 g crude fat and 95 g crude ash per kg, as well as water and hay.

### **General procedure and treatments**

The does were kept on a 41-day reproductive cycle and were artificially inseminated at 10 dpp. Afterwards, three different treatments were applied: the first group was regrouped at 12 dpp (TG12), the second one at 18 dpp (TG18) and the third one at 22 dpp (TG22). Before AI, doe-litter separation (24 h) was carried out in all treatment groups to improve fertility rates (Arias-Álvarez *et al.*, 2010). Non-pregnant or sick does were removed before each new trial and replaced with pregnant does, as is common practice on farms. Initially, groups were randomly allocated to treatments. After the first trial, each group was switched to another treatment in the following trial in a randomised way. All does were individually marked with ear tags and livestock spray. Due to low fertility rates, there were not enough pregnant does available for trials 2 and 3. In these cases, non-pregnant does were added to keep a constant group size of eight does per pen. However, these animals were not included in the analyses for weight and rank. During the group housing phase, the kits were mixed and could no longer be assigned to a particular doe. Therefore, the values collected from the kits refer only to their specific pen and treatment group. At the day of kindling, no data was collected to provide a quiet environment for the does.

### **Body weight**

All does were weighed individually at 8 dpp (2 d before AI) and at 25 dpp (day of weaning). The kits were only weighed at weaning in groups of 10 ("weight group") and their average weight was calculated per treatment group. In trial 2, the kits unfortunately had been weaned by the farm personnel before they were weighed, so we could only consider the weight from trials 1, 3, 4 and 5 for our analyses.

### **Mortality rate**

The mortality rate of does and kits was monitored during the entire trial (birth until weaning). As the cages stayed open at the top (Figure 1) after regrouping, kits were able to leave or fall out into the common area. However, they had the possibility to regain access to one of the cages by a ramp. As this cage was not necessarily their original one, kits could not be traced back to their mothers. The mortality rate for kits was therefore calculated per treatment group. No pathology was performed on deceased animals. However, they were inspected for obvious external causes of death by a trained veterinarian.

### **Fertility**

The fertility rate (number of kindling events/number of artificial inseminations $\times$ 100) (Mugnai *et al.*, 2009b) of the does was documented at the level of each treatment group. The influence of rank and treatment on the pregnancy status ("pregnant", "not pregnant") was analysed. The litter size of each doe was recorded after kindling. The litter sizes were not standardised.

### **Rank**

To determine the rank, we used the Elo-rating method, which is based on the number of individual wins and losses against the other members of a group (Albers and de Vries, 2001).

The agonistic behaviour was based on the ethogram of Graf (Graf *et al.*, 2011). The animal directing the behaviour was considered the dominant subject and the recipient of the behaviour the submissive animal. The rank for each animal in the treatment group was determined from one being the highest to eight being the lowest ranked. For more details see Munari *et al.* (2020).

### **Ethical approval**

This study was approved by the Cantonal Office of Aargau (No. 30611) and met all cantonal and federal regulations for the ethical treatment of experimentally used animals.

### Statistical evaluation

The data were analysed using the statistics program R (“R Core Team: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing” 2019). The mortality rate of the kits was analysed by a survival test using the packages “survival” by (Therneau, 2019) (version 2.38, <https://cran.r-project.org/web/packages/survival/index.html>, last assessed 11-9-2019). The weight difference (gain/loss) of the does between 8 dpp and 25 dpp was analysed using a linear mixed effects model with treatment as a fixed and trial as a random factor (“lme4” package by (Bates *et al.*, 2019). The difference in kits’ weight between the treatments was analysed using a linear mixed effects model with weight group nested in trial as a random factor. A generalised linear mixed effects model with binomial link function was executed to test the influence of the treatment and rank, as main effects, on the pregnancy status (“pregnant”, “not pregnant”). Doe identification was considered as a random term. Estimates and confidence intervals of the model were calculated using the boot package by (Canty and Ripley, 2019) and the pbkrtest package by (Højsgaard, 2017). Ranks were calculated using the “EloRating” package (Kulik, 2019); version 0.46.8, <https://cran.r-project.org/web/packages/EloRating/index.html> last accessed 9-9-2019). All means are given as  $\pm$  standard deviation. *P*-values below 0.05 were considered significant.

## RESULTS

### Fertility of does

The fertility rate after insemination was low in all three treatment groups, with an average of 40.92% (TG12 = 35.4  $\pm$  48.3%, TG18 = 41.7  $\pm$  49.8%, TG22 = 45.8  $\pm$  50.4%). The treatment did not affect the pregnancy status ( $\chi^2 = 0.209$ , *df* = 1, *P* = 0.65).

### Mortality

#### Kits

During five trials, a total of 854 kits (TG12 = 264; TG18 = 303; TG22 = 287) was born. The survival test showed no significant effect of treatment on kit mortality ( $\chi^2 = 2.3$ , *df* = 2, *P* = 0.3). The average kit mortality rate was at 8.42  $\pm$  0.54%. Two cases of infanticide (bite marks on kit) were documented. They were most certainly caused by the biological mothers as no other doe had access to the nest box at that time.

#### Does

In total, three does died during the experiment (one in TG18, two in TG22) due to unknown causes. However they showed no external lesions that could have been associated with their specific treatment.

### Weight

#### Kits

The results on the mean body weight at weaning, as well as the number of offspring per treatment group are shown in Table 1. There was no significant effect of treatment on body weight ( $\chi^2 = 2.2$ , *df* = 2, *P* = 0.33).

**Table 1:** Means  $\pm$  standard deviation of total litter weight (kg), number of kits/litter and average kit weight (g) for three treatment groups across five trials at weaning.

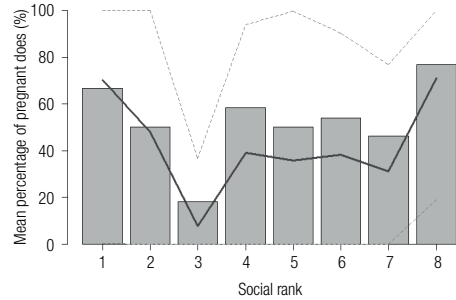
Treatment Group	Total litter weight (kg)	No. of kits/litter	Average kit weight (g)
TG12	22.6 $\pm$ 4.8	7.86 $\pm$ 0.8	447.1 $\pm$ 40.2
TG18	27.4 $\pm$ 4.7	9.05 $\pm$ 0.6	452.2 $\pm$ 47.9
TG22	27.4 $\pm$ 3.5	8.64 $\pm$ 1.0	460.1 $\pm$ 77.3

**Does**

Neither weight gain in pregnant-lactating animals nor weight loss in non-pregnant-lactating does were influenced by the treatment (pregnant-lactating:  $\chi^2=1.08$ ,  $df=2$ ,  $P=0.58$ , lactating:  $\chi^2=0.24$ ,  $df=2$ ,  $P=0.89$ ). Body weight was also not correlated with the specific rank of the animals. (Spearman correlation:  $r=0.036$ ,  $P=0.727$ ) (Table 2).

**Rank**

The rank was not related to the pregnancy status of the animals ( $\chi^2=0.076$ ,  $df=1$ ,  $P=0.78$ ) (Figure 2) and was highly variable for individuals across trials (data not shown).



**Figure 2:** Mean percentage of pregnant does (%) in relation to the social ranks (bars 1 to 8, 1 being the highest; 8 being the lowest rank). Solid line = model estimates. Dash lines = 95% confidence intervals.

**DISCUSSION**

Contrary to our hypotheses that a later time point of regrouping in a semi-group housing system would improve the reproductive success of the does, as well as the body condition of kits, we did not find effects on the fertility rate of does, the survival rate in kits or the weight change in kits and does.

**Does**

The fertility did not improve with later regrouping. In fact, the overall fertility rate in all our treatment groups was low (average: 40.92%), which is comparable to results from continuous group housing studies with a fertility rate of 40.8% (Mugnai *et al.*, 2009b) and 45.6% (Szendro *et al.*, 2013a), respectively. Other studies working with semi-group housed does reported higher fertility rates: 76.2% in Dal Bosco *et al.* (2019) or 83.4% in Maertens (2015).

The larger timespan between AI and the regrouping event, and thus the delay of the social stress due to grouping for the treatment groups TG18 and TG22, was supposed to reduce early implantation failure (Pratt and Lisk, 1991), compared to TG12. However, based on the low fertility rates in all treatment groups, high foetal mortality might still have occurred for these two treatment groups, or the conception rate was generally low. A study on Golden Hamsters (*Mesocricetus auratus*) found low progesterone levels connected to social stress caused foetal resorption (Huck *et al.*, 1988). Rommers *et al.*, 2006 came to a similar conclusion in group-housed does. In the present work, high progesterone levels were only found in the group housing system. Progesterone concentrations higher than 2 mg/mL were proven to negatively affect the kindling rate in these does. However, no stress response in rabbit does during regrouping, as estimated by body temperature measurements, was detected during this study (Braconnier *et al.*, 2020). Others reported similar results in the past (Andrist *et al.*, 2012; Buijs and Maertens, 2015). But see Rommers *et al.* (2006), Graf *et al.* (2011) and Szendro *et al.* (2013b). Furthermore, from a biological standpoint, though fighting for nesting sites and rank appear to be a stressful social activity, it is a natural behaviour in rabbits and they should be adapted to it (Albonetti *et al.*, 1990, in DiVincenti *et al.*, 2016). Thus, other factors concerning semi-group housing,

**Table 2:** Difference and means±standard deviation of does' body weight (kg) at 8 d postpartum (dpp) and at weaning.

Status	TG	Body weight [kg]		Difference (kg)
		At 8 dpp	At weaning	
pregnant-lactating	12	4.80±0.57	4.98±0.51	0.18
pregnant-lactating	18	4.99±0.46	5.21±0.39	0.22
pregnant-lactating	22	4.95±0.53	5.11±0.56	0.16
lactating	12	4.75±0.45	4.69±0.46	-0.06
lactating	18	4.78±0.56	4.75±0.55	-0.02
lactating	22	4.90±0.71	4.88±0.74	-0.03

such as artificial insemination, might be more stressful and decrease fertility. Furthermore, AI on 10-11 dpp may not be biologically ideal, as the does are in a phase of hormonal antagonism and energy deficiency due to concurrent pregnancy and lactation (Cardinali *et al.*, 2008). This could cause a reduced fertility, contrary to the day of partum or the day of weaning, where the sexual receptivity of the does is high (Harned and Casida, 196; Castellini *et al.*, 2003). However, unlike Switzerland with a 41-d interval, most other countries apply a 42-d interval.

Possibly, the season could also have contributed to the low fertility, as most of the trials took place in autumn and winter and the does were mostly kept under natural light and temperature conditions. Even though rabbit does are induced ovulators, their ovarian activity decreases with a decreasing photoperiod (Vella and Donnelly, 2012). However, we only looked at does on one farm, so our findings for the semi-group housing system may not be generalisable.

We did not find a relationship between rank and pregnancy status in our treatment groups. This result supports Stauffacher *et al.* (1986) (in Graf, 2010) and Machado *et al.* (2019), who reported that the rank is of low importance for reproductive performance in breeding does.

However, in wild European rabbits, a good body condition is related to a higher rank and leads to a better reproductive performance (Holst *et al.*, 2002). In our study on domestic breeding does, we could not confirm this. The body weight did not correlate with the rank of the animals. However, as our animals were fed *ad libitum*, their general body condition was more equal than in their wild conspecifics, which may mask the effect (Chu *et al.*, 2004). Additionally, the farmed does were probably more homogenous in parity and age, contrary to wild living groups, which may increase the competition and instability within groups. In the wild, one of the older does is the dominant doe in a group (Rödel *et al.*, 2008). This, as well as the regular replacement of does in the groups, as it is commonly done on farms, may be a reason why the rank was highly variable within our individuals between trials. Generally, in the wild and in domestic groups without varying change of group members, the rank remains stable for a few months once established (EFSA, 2005).

As only three animals died during the experiment, we were not able to test the influence of rank on mortality, unlike in previous studies (Holst *et al.*, 2002; Gerencsér *et al.*, 2019), in which a higher survival rate was partially linked to a higher rank.

### **Kits**

The kits' body weight was not affected by treatment. Though in close proximity to their kits for a longer time (TG18 and TG22), the does did not seem to increase their nursing frequency. In fact, although single-housed does visit the nest more frequently than does in group housing (Rommers *et al.*, 2012), they do not nurse their kits after every entry (Seitz *et al.*, 1997 in Schlolaut *et al.*, 2013; Coureaud *et al.*, 2000). On the other hand, potentially less nursing in TG12 might be compensated by an earlier intake of regular feed due to the earlier access to fermented grass (silage) disposed in the common area. The power of the analyses might have been too low to detect differences because weaning weights could only be assessed for the entire pen due to the mixing of kits in the common area.

Furthermore, it has been hypothesised that kits in TG12 may face higher mortality, as their mothers are in a possibly stressful situation during regrouping, while the kits are still depending on their care. However, no stress response was detected (Braconnier *et al.*, 2020), maternal failure (causing starvation of kits) did not occur and kit survival was not influenced by the time point of grouping. Kits in TG12 may not have been physically able to leave their cage at regrouping (12 dpp), and therefore could not have become malnourished and/or hypothermic. Another explanation for the lack of differences in mortality may be the ability of the kits to return to a nest box when falling into the common area per accident at an early age. Furthermore, kits were also observed nursing at different does in the common area (personal observations).

## **CONCLUSION**

A delay of 6 or 10 d for regrouping after 12 dpp did not improve the breeding or productive success in a Swiss semi-group housing system.

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