# INFLUENCE OF A PHOTO-STIMULATION ON OVARY AND EMBRYO RECOVERY IN NULLIPAROUS RABBIT FEMALES

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## ABSTRACT

The goal of the present experiment was to estimate the effect of a photo-stimulation following previous one month maintenance of the young rabbits within short-day (8L:16D) conditions and applied during 10 days before artificial insemination (AI). Forty 17-weeks old nulliparous rabbit does were divided into four experimental groups (10 does/group). Group L was kept in constant long-day (16L:8D) photoperiod until insemination, imitating commercial situation. Groups S+4, S+6 and S+8 were first submitted to a short photoperiod (8L:16D) then the duration of the light was increased with 4, 6 and 8 hours, respectively, 10 days before insemination. AI was performed with standard techniques using fresh heterospermic pools. Doe rabbits were sacrificed at 48 hours after AI. Ovarian function was evaluated, oviducts were flushed to collect embryos and evaluate them after 48 hours in *in vivo* conditions. Increasing daylight hours did not influence receptivity, as assessed by the vulva state at the moment of insemination, nor weight of the two ovaries and average ovary length. Only one doe did not ovulate in group S+4. The mean number of corpora lutea in ovulated females was 13.2, 5.7, 10.6, 12.0 in groups L, S+4, S+6, S+8, respectively ($P<0.001$). Proportion of embryo donor does was different ($P<0.10$) being 1.0 the highest value in the group S+6 and 0.71 the lowest in the group S+4. The highest number of collected embryos for inseminated does was in group S+6 with 9.2 and the lowest in S+4 with 3.4 per doe ($P<0.001$). Embryo recovery rate was the highest (0.89) in S+6 group and this was significantly ($P<0.001$) different from the results of the other groups (0.56, 0.64 and 0.52, respectively for S+4, S+8 and L). Proportion of 96 hours old good quality embryos within the total washed amount was the highest in group L and S+8 (0.84 and 0.87, respectively) and lower in S+4 and S+6 (0.69 and 0.71, $P<0.05$). Ovarian follicle anomalies (follicle cysts and haemorrhagic follicles) were present in each group: the average value (1.4 per inseminated doe) was the lowest in group S+8 and was more than 3 times higher in group L (4.7 per inseminated doe, $P<0.001$). Higher proportion of the receptive females gave an embryo and the embryo recovery rate was also higher in these does compared to the non-receptive females. It is concluded that switching light hours from 8 to 14 per day leads to the same proportion of the embryo donor does, embryo recovery rate, number of collected embryos and similar number of blastocysts appropriate for transfer per inseminated female in comparison with the constant long day illumination. Smaller change of daylight hours is probably insufficient. Long days either by 8 hours supplemental lighting or constant however can result in lower embryo recovery rate.

**Key words**: Photo-stimulation, Ovary, Embryo, Rabbit.

## INTRODUCTION

In transgenic rabbit production and embryo cryopreservation programs young female rabbits are often used as zygote donors to obtain high numbers of embryos suitable for transfer. Embryo quality is however often poor as revealed by the small number of embryos capable to survive and undergo segmentation till 96 hours of age and then vitrified or transferred (Carney and Foote, 1990; Stradaioli *et al.*, 1997). This could create a bottleneck of transgenesis and breed preservation or colony management vitrification programs such as reconstitution of population and infection control (Vicente *et al.*, 2003; Suzuki *et al.*, 1990).

Lifting of light hours/day has been investigated in a number of studies (Maertens and Luzi, 1995; Schuddemage *et al.*, 2000; Quintela *et al.*, 2001; Mattaraia *et al.*, 2005; Gerencsér *et al*., 2006) with regards to the reproductive performance of rabbit does at different parity numbers and lactating or not, at the moment of mating or artificial insemination. Within these only very few presented data on the effect of treatments on ovary function, possible because rabbit ovary is not tactile by vaginal route, can't be monitored by ultrasound, laparoscopy needs equipments and technical skills, and extermination without further disposal of embryos is expensive. None of these studies investigated the effect of the photo-stimulation on the embryo development *in vitro*.

The goal of the present experiment was to study the effect of a photo-stimulation applied for 10 days before insemination, on the ovarian response, the number of embryo produced and their quality in *in vitro* conditions in nulliparous rabbits.

## MATERIALS AND METHODS

### Animals and experimental design

Contemporary Grimaud hybrid females (n=40) used in the experiment were raised up in a commercial rabbitry under a 16L:8D photoperiod. When 17 weeks old, they were allocated to one of the four experimental groups (Table 1) and placed into four rooms (one rabbit per cage). Feed and water was provided *ad libitum*. Group L was kept in constant long-day (16L:8D) photoperiod until insemination, imitating commercial situation. Group S+4, S+6 and S+8 were first supplied with short (S) photoperiod (8L:16D for 30 days) then the duration of the light was suddenly increased with 4, 6 and 8 hours, respectively 10 days before AI. The choice for the timing of photo-stimulation is based on the study of Hudson and Distel (1990) who found that short changes of daylight hours can affect the female rabbit's reproductive performance within 2–3 weeks. Receptivity of does was assessed by vulva colour and turgency, red and turgent considered as receptive. Ovulation was induced with intramuscular injection of 0.8 µg GnRH (Receptal, Hoechst). At the same time insemination was performed with standard techniques using freshly collected pooled semen containing more than $20\times10^6$ motile spermatozoa/ml.

**Table 1**: Light duration (hours/day) in the acclimation and stimulation periods

| Light hours/day in the period of | | Group label | Inseminated females | |
|---|---|---|---|---|
| Acclimation | Stimulation | | Number | Body weight* (kg, mean±s.e.) |
| 8 | 12 | S+4 | 10 | 4.03±0.05 |
| 8 | 14 | S+6 | 10 | 3.95±0.05 |
| 8 | 16 | S+8 | 10 | 3.93±0.06 |
| 16 | 16 | L | 10 | 4.25±0.06 |

*Doe body weight was measured on the day of AI.

### Ovary function, embryo collection and culture

Does were sacrificed by stunning and exsanguination from 48 to 50 hours after insemination. Reproductive tract was removed, ovaries dissected from the surrounding tissue and weighed. The numbers of corpora lutea, haemorrhagic and cystic follicles (Ø>1.5 mm, filled with blood or serous fluid) were recorded. Oviducts with one-third of the uterine horns were excised and exhaustively flushed in retrograde direction with PBS. Good quality embryos which were in correct morphological stage of development (8 cells) with 30-40 µm thick intact mucin layer and integrity were taken into 50 µl (10-15 per drops) of the MEM and RPMI-1640 medium supplemented with extra amino acids, vitamins, nucleotides, sugars and growth factors (Sigma). In vitro culture was continued up to 90 hours post insemination under mineral oil at 38.5°C with 5% $CO_2$ in air.

**Statistical Analysis**

All statistical evaluation was performed with GenStat Release 8.1 software (Lawes Agricultural Trust, 2005). The occurrence of oestrus, ovulation, and fertilization were expressed as Bernoulli variables (range 0-1). The effect of photo-stimulation on the receptivity was evaluated in the GLM module. Other traits were evaluated with receptivity and photo-stimulation as effects using ANOVA or GLM process.

## RESULTS AND DISCUSSION

Following ten days of light stimulation the proportion of receptive females among the inseminated ones was the lowest in S+4 group (0.22) and the highest in groups S+8 and L (0.60 and 0.60) but the difference was not significant (Table 2). Likewise the supplemental lighting in the study of Mattaraia *et al*. (2005) with 24 does per treatment increased the receptivity by 20% but the effect was not significant. Based on the presence of corpora lutea all does ovulated except one in the S+4 group. At least 1 embryo was collected from each doe in group S+6, whereas the embryo production was weaker in S+4, S+8 and L groups (0.71, 0.87 and 0.75, respectively, P<0.10) but the difference statistically was not significant. Higher proportion of receptive does became embryo donors than non receptive (1±0.0 vs. 0.67±0.1,respectively, P<0.002).

**Table 2**: Influence of the lighting program on the sexual behavior, the ovulation frequency and the percentage of embryo donors (mean±s.e.)

| | Photo-stimulation | | | | *Chi*$^2$ |
|---|---|---|---|---|---|
| | S+4 | S+6 | S+8 | L | Prob. |
| Receptive[1] | 0.22±0.14 | 0.56±0.17 | 0.60±0.15 | 0.60±0.16 | n.s. |
| Ovulated[2] | 0.93±0.10 | 1±0.0 | 1±0.0 | 1±0.0 | n.s. |
| Embryo donor[3] | 0.71±0.10$^a$ | 1±0.0$^b$ | 0.87±0.11$^a$ | 0.75±0.12$^a$ | <0.10 |

[1]Females with red and swollen vulva; [2]females with at least 1 corpus luteum; [3]females with at least one recovered segmented embryo. Different letters in the same row stands for statistical difference at P<0.05 level

The effect of photo stimulation on ovarian macroscopic morphology is presented in Table 3. The ovary weight tended to increase with the light duration (0.43, 0.50, 0.50 and 0.61 grams in groups S+4, S+6, S+8 and L, respectively). Similarly, Mattaraia *et al.* (2005) evidenced heavier ovaries under a supplemental lighting program compared to a control group (0.41 vs. 0.23). In contrast, the number of corpora lutea per ovulated doe is lower (P<0.001) for group S+4 than for groups S+6, S+8 and L (5.63 vs. 11.0, 12.5 and 13.76, respectively). Similar values were reported by Mocé *et al*. (2004), Mehaisen *et al*. (2006) and Mattaraia *et al*. (2005) for receptive nulliparous females. The number of corpora lutea however was not affected by light supplementation when referred only to the ovulating does (Mattaraia *et al*., 2005). Morphological anomalies of follicles such as haemorrhage and cystae were also observed on ovaries. This number was significantly lower (P<0.001) in groups S+8 and S+6 compared to S+4 and L groups (1.4, 2.6 vs. 3.5, 4.7, respectively). This is in agreement with the numbers observed for nulliparous receptive but non-stimulated does in other studies (Stradaioli *et al*., 1997; Mehaisen *et al*., 2006). The follicular anomalies in the lack of hormonal stimulation could be also caused by the GnRH used for artificial insemination as reported by Viudes *et al*. (1995).

**Table 3**: Influence of the lighting program on the macroscopic morphology of the ovary

| | Photo-stimulation | | | | Prob. |
|---|---|---|---|---|---|
| | S+4 | S+6 | S+8 | L | |
| Ovary length (mm) | 8.84±0.49$^{ab}$ | 7.88±0.48$^a$ | 8.46±0.45$^{ab}$ | 9.51±0.45$^b$ | <0.1 |
| Ovary weight (mg) | 0.43±0.05$^a$ | 0.50±0.05$^{ab}$ | 0.50±0.05$^{ab}$ | 0.61±0.04$^b$ | <0.1 |
| Corpora lutea/doe[1] (n) | 5.63±0.80$^a$ | 11.0±1.09$^b$ | 12.5±1.11$^b$ | 13.76±1.16$^b$ | <0.001 |
| Follicle anomalies/doe[2] (n) | 3.51±0.67$^b$ | 2.64±0.54$^a$ | 1.38±0.37$^a$ | 4.71±0.69$^b$ | <0.001 |

[1] number of ovulated does; [2] number of inseminated does. Significant differences (p<0.05) of means stand with different letter in the same row

The number of collected embryos (Table 4) per inseminated females was significantly lower (P<0.001) in group S+4 (3.39 vs. 7.31, 6.64 and 9.17) than for groups S+8, L and S+6, respectively. Higher number of embryos were recovered from receptive than from non-receptive does (8.43±0.65 vs. 4.86±0.53, P<0.001). The embryo recovery rate was 0.89 in group S+6 and significantly differed (P<0.001) from the other groups (0.56, 0.64 and 0.52 found in group S+4, S+8 and L, respectively). Embryo recovery rate was higher in case of receptive compared to the case of non-receptive does (P<0.001, 0.83±0.03 vs. 0.46±0.03). On group S+6, only 85 % of does had 8 cells embryos of good quality (P<0.001). In group S+6, the high recovery rate was accompanied by a lower number of follicular abnormalities similarly as it has been reported by Garcia-Ximénez and Vicente (1992). The frequent presence of these anomalies in each treatment was however striking, and there is no clear explanation for that. Mocé *et al*. (2004) found similar recovery rate compared to the group S+6 in our experiment for receptive but non-stimulated does and Stradaioli *et al*. (1997) slightly lower for PMSG stimulated does. The amount of recovered embryos in the experiments of Mocé *et al.* (2004), Stradaioli *et al.* (1997) who counted abnormal+normal+oocytes altogether and related it to the number of the ovulated does was like to those that we found in group S+6. Considering group S+4 the small number of corpora lutea and the weak embryo recovery rate together led to a poor number of collected embryo per inseminated doe compared to that of other groups .

**Table 4**: Influence of the lighting program on embryo production

| | Photo-stimulation | | | | Prob. |
|---|---|---|---|---|---|
| | S+4 | S+6 | S+8 | L | |
| Collected embryo/doe[1] (n) | 3.39±0.68[a] | 9.17±1.0[b] | 7.31±0.84[b] | 6.64±0.84[b] | <0.001 |
| Embryo recovery rate[#] | 0.56±0.06[a] | 0.89±0.03[b] | 0.64±0.04[a] | 0.52±0.04[a] | <0.001 |
| Rate of good quality 8 cell embryo[§] | 1.0±0.0[b] | 0.85±0.04[a] | 1.0±0.0[b] | 0.97±0.02[b] | <0.001 |
| Rate of good quality blastocysts[§] | 0.69±0.09[a] | 0.71±0.05[a] | 0.87±0.04[b] | 0.84±0.04[b] | <0.05 |
| Good quality blastocysts/doe[1] | 2[a] | 6.67[b] | 6.1[b] | 5.9[b] | <0.05 |

[1]Number of inseminated does; [#]number of recovered normal and abnormal 8 cells embryos per number of corpora lutea; [§]good quality embryos and blastocysts per normal and abnormal embryos together recovered. Significant differences (P<0.05) of means stand with different letter in the same row

Good quality (8 cells) embryos represented nearly all collected and segmented embryos in groups S+4, S+8 and L (1.0, 1.0, and 0.97, respectively). But a significantly smaller proportion of good quality 8 cell embryos was found in group S+6 (0.85, P<0.001). Further degeneration of embryos was observed during the next 48 hours of *in vitro* incubation leading to decreased (P<0.05) proportion of good quality blastocysts also in group S+4 compared to groups S+8 and L (0.69 vs. 0.87 and 0.84, respectively). The number of good quality blastocysts per inseminated doe was significantly lower in group S+4 compared to other groups (2.0 vs. 5.9, 6.1 and 6.67, in groups L, S+8 and S+6 respectively, P<0.05). The number of good quality blastocysts per donor was 3.6, 6.67, 7.44 and 7.38 in groups S+4, S+6, S+8 and L respectively.

## CONCLUSIONS

Compared to the constant long day lighting at least the same proportion of the embryo donor does, embryo recovery rate, number of collected embryos and good quality blastocysts per inseminated doe can be accomplished by switching light hours from 8 to 14 per day. Smaller change of daylight hours is probably insufficient. Long days either by 8 hours supplemental lighting or constant however can result in lower embryo recovery rate.

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