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Secretaría de Desarrollo Agropecuario del Gobierno del Estado de México, Secretaría de Agricultura, Ganadería, Desarrollo Rural, Pesca y Alimentación, Consejo Mexiquense de Ciencia y Tecnología



REVIEW [REVISIÓN]

HOW CAN RABBIT RESEARCH IN THE LABORATORY CONTRIBUTE TO CUNICULTURE ON THE FARM?

[¿CÓMO PUEDE LA INVESTIGACIÓN SOBRE CONEJOS, REALIZADA EN EL LABORATORIO, CONTRIBUIR A LA CUNICULTURA EN LA GRANJA?]

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SUMMARY

Rabbit research coming from the laboratory can have a profound impact on Cuniculture, performed on the farm, and vice versa. This bi-directional communication is scarce at present but, by finding issues of common interest, an effective interaction between these two niches can be promoted. I will present five examples from Reproductive Neuroendocrinology where I have identified evidence that research in one niche has had (or can have) an impact on the other one, specifically: 1) distinguishing between pregnancy and pseudopregnancy; 2) preventing death of kits due to deterioration of the maternal nest and/or loss of maternal behavior; 3) facilitating the management of groups of does mated according to the “biostimulation” method; 4) increasing the success of the “biostimulation” method; 5) improving the welfare of rabbits housed in the laboratory and on the farm. Promoting communication between “the lab and the farm” will lead to new ways of exploring key scientific questions and to better management practices on the farm.

Keywords: rabbit; pregnancy; lactation; pseudopregnancy; mating; cuniculture; animal welfare.

RESUMEN

La investigación sobre el conejo, generada en el laboratorio, puede tener un impacto profundo sobre la Cunicultura, realizada en la granja. Esta comunicación bi-direccional es escasa actualmente pero, identificando tópicos de interés común, puede promoverse una interacción efectiva entre estos dos nichos. Se presentarán cinco ejemplos, derivados de la Neuroendocrinología Reproductiva, en los que se tiene evidencia de que la investigación en un nicho ha tenido (o puede tener) un impacto en el otro, específicamente: 1) distinguir entre gestación y pseudogestación; 2) prevenir la muerte de gazapos debida al deterioro del nido y/o la pérdida del comportamiento materno; 3) facilitar el manejo de grupos de conejas apareadas conforme al método de “bioestimulación”; 4) incrementar el éxito del método de bioestimulación; 5) mejorar el bienestar de los conejos alojados en el laboratorio y en la granja. Promover la comunicación entre estos dos ámbitos llevará a nuevas maneras de explorar preguntas científicas importantes y, a la vez, mejorará el manejo del conejo en la granja.

Palabras clave: conejo; gestación; lactancia; pseudogestación; apareamiento; cunicultura; bienestar animal.



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INTRODUCTION

Rabbits have been studied for many years in the laboratory and on the farm. Their physiology (e.g., digestion, reproduction), disease susceptibility (to pathogens or toxic substances), behavior (aggressive, social, reproductive), or use (of their meat and fur) have been the subject of numerous investigations published in journals specialized in the corresponding field or devoted specifically to rabbit research. Sadly, this rich information has been separated into two areas

that constitute the niches of scientists working in the laboratory ("basic" research) or on the farm (agronomic research). Usually, scientists from one niche read the publications, attend the meetings, and interact with the colleagues from their own area and are unaware that similar research lines are being pursued by investigators from "the other" niche. This division is, of course, impoverishing to science as the strategies used in the laboratory and on the farm are complementary to each other; the models used in each field can reveal new ways of approaching the same

González-Mariscal, 2012

scientific question. As emphasized in earlier publications (González-Mariscal and Roselli, 2007; González-Mariscal et al., 2007) it is necessary to promote a larger interaction between "the lab and the farm". Thus, it is the objective of this article to present specific examples that illustrate how these two fields can converge and interact effectively to improve rabbit production and to generate new scientific knowledge.

WHAT ISSUES OF COMMON INTEREST TO REPRODUCTIVE NEUROENDOCRINOLOGY AND CUNICULTURE HAVE WE IDENTIFIED?

Before attempting to bring together two fields that are related but operate independently one needs to identify common interests. It is necessary to find out if a given scientific question is relevant to both areas and if it is (or has been) investigated by researchers working in the laboratory or on the farm. In the following sections five specific topics will be presented that meet these criteria; the main findings and missing information identified in each one will be described.

Distinguishing between pregnancy and

effectiveness of the "palpation" method depends on the experience of the person performing the procedure. We found in our laboratory that the frequency of a particular form of scent-marking, consisting of the rubbing of the rabbit's chin on any solid object in the environment ("chinning"), is directly related with the degree of sexual receptivity of a doe (González-Mariscal et al., 1990). Thus, unmated females kept under a long photoperiod and given adequate nutrition are sexually receptive (Edey and Casida, 1972) and show a high frequency of chinning (González-Mariscal et al., 1990). Pregnant does, by contrast, are not sexually receptive and show low chinning frequencies. What about pseudo-pregnant rabbits? Interestingly, their chin-marking activity is indistinguishable from that of truly pregnant does but only during the first 14 days post-mating. From then onwards chinning frequency increases steadily in pseudopregnant animals, to reach levels characteristic of estrous does (Figure 1; González-Mariscal et al., 1990). Thus, monitoring chinning frequency for about ten days (starting at around 8-10 days post-copula) can be a reliable, inexpensive, and simple method for determining if a mated female is truly pregnant.





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Distinguishing between pregnancy and pseudopregnancy.

Rabbit does are induced ovulators, i.e., they ovulate only in response to copulation (Ramirez and Beyer, 1988). Although, theoretically, this would "ensure" that all mated does became pregnant and gave birth to young in reality a variable proportion of such rabbits enter a peculiar endocrine state known as pseudopregnancy. This is characterized by an endocrine profile similar to the one of rabbits in early pregnancy, i.e., high levels of progesterone (P) in blood, produced by the corpus luteum (Erskine, 1999). This ovarian structure, however, has a shorter life span in pseudopregnant (14 days) than in pregnant (around 30 days) does. As a consequence P levels drop in the former about two weeks earlier than in the latter (Niswender and Nett, 1994) and pseudopregnant does -of course- do not produce a litter. The mechanisms underlying the occurrence of pseudopregnancy -rather than pregnancy- following mating in rabbits have been little explored. However, it is known that vaginocervical stimulation received during mating reaches the central nervous system to promote surges of prolactin that maintain the corpus luteum (Erskine, 1999). For the "lab scientist" the occurrence of pseudopregnancy is a fascinating research area in Reproductive Neuroendocrinology; for the professional trying to breed tens to hundreds of rabbits in the farm pseudopregnancy entails an important financial loss. Experienced rabbit producers can distinguish between pregnant and pseudopregnant does by palpating their ventrum at around 14-20 days post-copula. However, this method can give false negatives if pregnant does are carrying few embryos and the

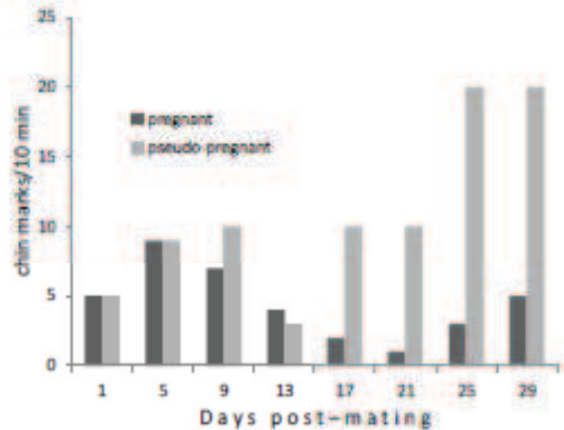


Figure 1. Chin-marking (chinning) frequency does not significantly differ between pregnant and pseudopregnant rabbits for the first 14 days post-mating. From then onwards, chinning frequency increases steadily *only* in pseudopregnant females (modified from: González-Mariscal et al, 1990).

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Preventing death of kits due to deterioration of maternal nest and/or loss of maternal behavior.

Some mammals that give birth to a single offspring (like sheep and goats) nurse only their own young - which they identify largely by smell- and refuse to allow suckling from other lambs or kids, respectively. This is called exclusive nursing (for review see: González-Mariscal and Poindron, 2002). By contrast, mammals that deliver a litter (such as sows, rats, and bitches) can nurse even young that are not their own.





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To determine whether something similar occurs in rabbits we explored: a) if does accepted suckling from another doe's kits and b) whether placing either the mother's own or an alien litter inside a nest different from their own modified their willingness to nurse (González-Mariscal and Gallegos, 2007). As shown in Fig. 2 placing the mother's litter inside a nest different from her own (i.e., made with synthetic or male hair) significantly increased her latency to enter the nest box for nursing. This effect was also evident when alien kits were provided, although the magnitude of the difference was smaller.

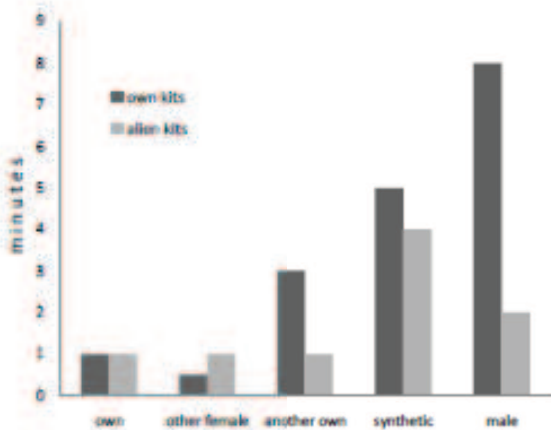


Figure 2. Latency to enter nest box of mothers provided with their own or foster kits in various types of nests (modified from: González-Mariscal and Gallegos, 2007).

Yet, once the does entered the nest box provided, they all nursed the litter placed inside it (i.e., their own or an alien one) for the usual amount of time (around 3 min; Table 1).

of primiparous does). Under such conditions a recommendation can be made -based on scientific evidence- that new nests (made from fresh materials, including synthetic hair) be provided and/or kits be relocated to other lactating does. The latter may be particularly important in cases where -for a variety of reasons- some kits from a litter die within the first days of life. As shown in Fig. 3 the maintenance of maternal behavior across lactation relies heavily on the number of suckling kits, particularly for primiparous does. Nursing only one or two young leads to a gradual loss of the mother's willingness to nurse. This observation, combined with the fact that lactating rabbits consume more feed than unmated or pregnant ones (González-Mariscal et al., 1994, 2009a), emphasizes the need to recommend the relocation of kits to "alien" mothers for optimizing rabbit production on the farm.

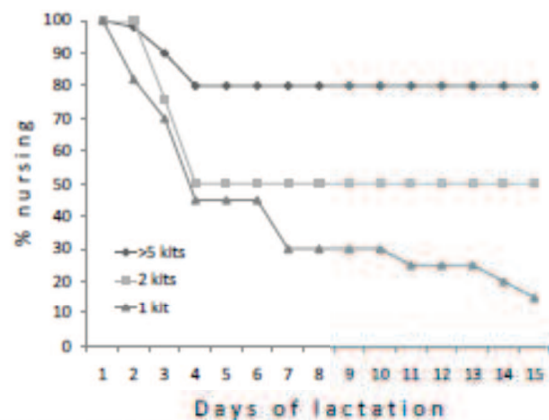


Figure 3. Number of suckled young determines maintenance of maternal behavior across lactation days 1-15.





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Table 1. Time (min; mean±s.e.) does spent inside different types of nest boxes nursing their own or foster kits (modified from: González-Mariscal and Gallegos, 2007).

Type of kits	Type of nest box where kits were placed				
	own	another female	another own	synthetic	male
own	3±1.0	3±1.0	4±1.0	4±1.0	3±1.0
foster	3±0.1	4±0.3	3±0.3	4±0.4	4±0.3

These results confirm that rabbits *do not* show exclusive nursing and that their willingness to nurse is not dependent on the type of nest in which the kits are placed. These findings, obtained in the laboratory, can be used as a managing strategy on the farm in cases where: a) the mother's original nest deteriorates or b) she loses her maternal behavior (especially in the case

Facilitating the management of groups of does mated according to the "biostimulation" method.

To maximize the number of kits produced/doe/year rabbit breeders have adopted a mating strategy commonly known as "biostimulation". This method takes advantage of the fact that rabbits can sustain lactation concurrently with pregnancy. Following parturition does have a so-called post-partum estrus, lasting several hours, during which they are sexually receptive. If mated at such time they can become pregnant without compromising the nursing of the newborn litter. This capacity for concurrent pregnancy and lactation has allowed that, in nature, rabbits leave a large progeny in their rather short lives. In the laboratory the endocrine profile and milk output have been compared between does mated or not at post-partum estrus. Such studies have revealed that: a) progesterone concentrations in blood are smaller in pregnant-lactating does than in pregnant-only rabbits

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across gestation days 7-17 and b) milk output declines precipitously from lactation day 20 onwards *only* in does that are concurrently pregnant-lactating (Fig. 4; Fortum et al. 1993; González-Mariscal et al, 2009a; Lebas, 1972). This difference in milk production has the unwanted consequence that kits suckling from pregnant-lactating does gain less weight than litters nursed by lactating-only rabbits. However, the fact that milk output declines in concurrently pregnant-lactating does only from day 20 onwards implies that if such females are mated on lactation day 10 (rather than at parturition), the litter can obtain a normal amount of milk for a full 30 days.

individually). This is a major problem for breeders wanting to use the biostimulation strategy to mate lactating does because, if nursing did not occur, kits will not survive a 48-h fast. We recently found that, under a photoperiod of 14 h light:10 h darkness (lights on at 0700 h), rabbits spontaneously nurse the litters, kept inside the nest box and placed within their home cages, at around 0215 h. The rigorous statistical analysis used to determine this and the large number of suckling episodes recorded (20 lactating does across 15 days of lactation) allowed us to obtain a level of confidence of $p < 0.001$ (González-Mariscal et al, 2009b). This finding can allow breeders to safely assume that nursing has, indeed, occurred before the litters are removed in the morning for "biostimulation" without having to verify individual kits.





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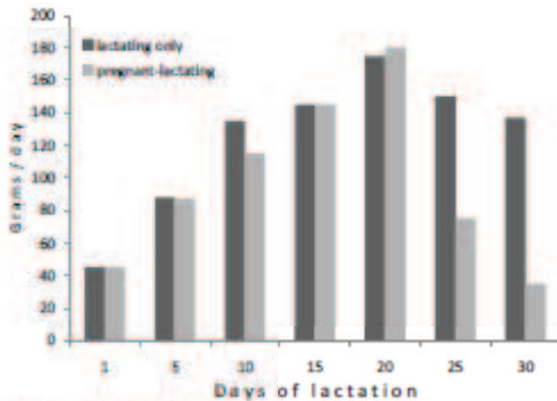


Figure 4. Milk production in lactating only vs concurrently pregnant-lactating rabbits (modified from: González-Maniscal et al, 2009).

This possibility, however, has the obstacle that lactating does are *not* sexually receptive (Beyer and Rivaud, 1969). Yet, a number of studies have revealed that cancelling a single nursing bout in early lactation (usually between days 8 to 10) leads to the restoration of estrus 24 h later and the consequent pregnancy following natural or artificial insemination (Alvaríño et al, 1998; Castellini et al, 1998; Theau-Clément and Mercier, 1999). This “biostimulation” mating strategy has been adopted worldwide to maximize productivity on rabbit farms. Nonetheless, for this method to be effective it is essential that: a) nursing is prevented on a specific day; b) nursing has occurred before the litter is removed or the door leading to the maternal nest is closed (otherwise the kits risk 48 h of milk deprivation and can die). Verifying individual kits for the presence of milk in their stomachs is, of course, a time-consuming, labor-demanding process. In small farms lacking automatic systems -that open and close the doors to the nest at a pre-programmed time- it is common that litters stay in the maternal nest throughout lactation and does have round-the-clock access to the kits. Under such conditions breeders cannot be sure whether a nursing bout occurred or not on a given day (unless they inspect the kits

without having to verify individual kits.

Increasing the success of the “biostimulation” method.

Although cancelling a suckling episode in early lactation indeed restores estrus in lactating rabbits the proportion of females that do become pregnant following natural mounting or artificial insemination can vary greatly (Alvaríño et al, 1998; Bonanno et al, 2002; Theau-Clément and Mercier, 1999). The sources of this variability are hardly known and, in fact, the factors underlying the effectiveness of the “biostimulation” method have been little explored. To our knowledge only the concentrations of prolactin and estradiol in blood have been compared between lactating and “biostimulated” does in early lactation. In general, the latter show higher prolactin levels before insemination (as a nursing bout was cancelled) and higher estradiol concentrations after insemination relative to control does in which nursing was not interrupted (Fig. 5; Ubilla et al, 2000).

However, the relevance of these differences for determining the likelihood of a successful pregnancy after insemination are unknown. In addition, external factors, such as: photoperiod duration, feed composition, and temperature have been reported to impinge on the relative success of the “biostimulation” method through as yet unidentified ways (Gómez et al, 2004; Marai et al, 2002; Szendrő et al, 2004; Theau-Clément et al, 1998). For the neuroendocrinologist working in the laboratory exploring the mechanisms by which steroid hormones, peptides, metabolic products, and environmental factors act on specific brain regions and peripheral organs to regulate sexual receptivity, fertility, and pregnancy is an intriguing research challenge. For rabbit breeders, employing a variety of management strategies and housing conditions, information coming from the laboratory may provide a solid basis for adjusting specific parameters to improve the relative success of the “biostimulation” method.





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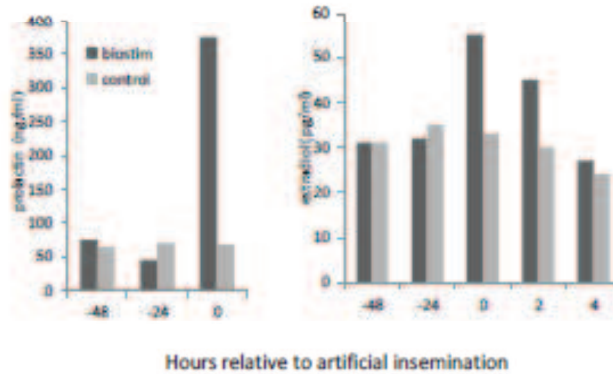
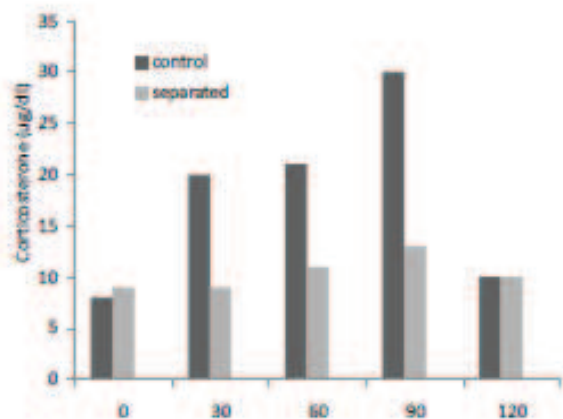


Figure 5. Effect of biostimulation on prolactin and estradiol concentrations in blood (modified from: Ubilla et al, 2000).

Improving the welfare of rabbits housed in the laboratory and on the farm.

The physical and psychological well-being of animals kept under laboratory or farm conditions is a major concern worldwide. Needless to say the requirements of food, space, social interaction, tranquility, duration of photoperiod, etc., vary greatly across mammals and, of course, among vertebrate classes (for review see: Moberg and Mench, 2000). Moreover, the ways by which information can be obtained regarding the presence of stress, the malaise provoked by sickness, the perception of pain, or the occurrence of complex mood states (such as anxiety and boredom) is largely dependent on the species under study. That is, animals are variable not only in the ways they react to the environment; they also differ in the types of responses (e.g., motor, endocrine) they can show under a specific setting. Thus, to design methods that effectively measure welfare/stress it is essential to know what types of responses a "normal" animal -of a particular species (age and sex)- can provide. Consequently, we should ask: what behavioral, physiological, and endocrine responses can we measure in rabbits that vary as a consequence of their physical and psychological well-being? Moreover, we need to inquire whether the regular management practices used in the laboratory and on the farm (regarding type of cages, feed, temperature, photoperiod, transportation, etc.) have an impact on the different categories of rabbits (i.e., bucks, juveniles, pregnant/lactating does) that usually occupy breeding

of both sexes and that group-housing does has no impact on their blood corticosterone levels, especially after several weeks of lodging under this condition. By contrast, a major difference in the responsiveness to a specific stressor in adult females was noted between those raised "normally" (i.e., that received a single daily nursing bout throughout a 30-day lactation) and the ones that missed a single nursing episode on lactation day 10 (as a consequence of the "biostimulation" procedure their mothers were subjected to). As shown in Figure 6 injecting saline in the thigh provoked a large increase of corticosterone in blood, evident from 30 to 90 min post-injection, but only in the control group: rabbits that were separated from their mothers for 48 h in early lactation showed a blunted response to stress (Brecchia et al, 2009).





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pregnanolactating does) that usually occupy breeding facilities. As the main endocrine indicator of stress is the responsiveness of the hypothalamus-pituitary-adrenal axis to a variety of conditions, some works have measured the concentration of corticosterone or cortisol in blood in relation to: exposure to heat, cold, noise or a social mix (De la Fuente et al. 2007), group vs single housing (Whary et al. 1993), and rearing conditions in early life (Brecchia et al. 2009). The main findings from these works indicate that heat, followed by loud noise, is the main stressor for adults

Figure 6. Impact of mother-young separation (days 11) on stress reactivity as adults (modified from Brecchia et al. 2009).

Additional evidence for the impact of manipulation during the early neonatal period on the permanent modification of the stress response comes from a study in which kits were handled by humans at different

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times around nursing during the first week of lactation. When tested at weaning (day 28) rabbits that were handled at 15 min before or 30 after nursing showed a smaller latency to approximate a human and a larger number of approaches in the 5-min test than control (i.e., non-handled) kits (Fig. 7; Pongrácz and Altbäcker, 1999).

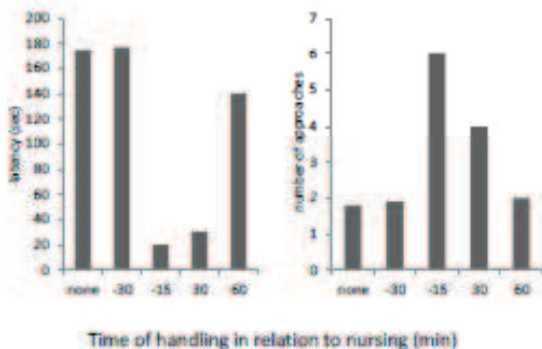


Figure 7. Effect of handling kits at different times before or after nursing on their reactions to humans at weaning (modified from Pongrácz and Altbäcker, 1999).

behavior; 2) the generation of new scientific questions amenable to investigation; 3) better management practices on the farm. Such consequences are desirable because: a) the rabbit is a unique model for exploring key issues in several fields of "basic" science (e.g., chronobiology, behavioral neuroendocrinology, developmental psychobiology, energetic metabolism); b) as the world faces new challenges to feed the human population (Paillard et al. 2011) rabbit production has many advantages over that of other domestic animals. New ways to promote a constant exchange of ideas between the scientists of the laboratory and the researchers working on the farm are needed. The education of students needs to be modified according to the vision that the scientific study of rabbits is richer as it is broader. It is hoped that this article contributes to achieve such goals.

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weaning (modified from: Pongracz and Altbacker, 1999).

Other works have studied pregnant rabbits, subjected to vibrations that simulate transportation or to noise: neither the duration of pregnancy nor the number of liveborn kits was affected by such environmental factors (only a slight increase in the number of dead young was noted; Stephens and Adams, 1982). By contrast, pregnant or lactating does housed collectively showed fewer “stereotyped” behaviors (like biting the cage bars), more sniffing of the environment, and fewer rearings than did females housed individually. However, reproductive parameters (e.g., sexual receptivity, fertility, milk production) were practically unaffected by the type of lodging condition (Dal Bosco et al. 2004). Taken together, the above findings show that important contributions are being made in the laboratory that reveal the sensitivity of rabbits to management practices and specific characteristics of the environment. They also indicate that more research is needed in this regard to explore other indicators of welfare/stress and to determine the long-term effects of particular procedures on the responses of rabbits of different breeds, ages, sex, and reproductive state.

CONCLUSIONS

Contact between the researchers devoted to studying Reproductive Neuroendocrinology and those working on Cuniculture can lead to: 1) a richer understanding of complex phenomena of rabbit physiology and

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