NEUROENDOCRINOLOGY APPLIED TO RABBIT BREEDING

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Abstract: Successful rabbit production relies heavily on the use of adequate practices that enhance specific aspects of reproduction, such as mating, ovulation and lactation. Regardless of the type of production unit or strain of rabbits used, these processes rely on a complex chain of neuroendocrine steps that include particular hormones, peripheral stimuli and activation of discrete brain regions. Such is the case, for instance, of reflex ovulation, which occurs in response to copulation but is inhibited throughout lactation. Little is known about the mechanisms mediating lactational anoestrus and the restoration of oestrus following the cancellation of a single suckling episode (biostimulation). Nevertheless, the latter procedure (adopted worldwide to accelerate reproduction) has unwanted consequences for the doe and her litter. After successive episodes of biostimulation, the former shows a loss of fertility and body mass. In the kits, alterations are observed in their neuroendocrine response to mildly aversive stimulation in adulthood, as well as reductions in sexual behaviour. In addition to milk intake, a good nest is essential for normal litter growth and development. If this is not available, or if it deteriorates, rabbit caretakers can easily (re) build one from hair sheared off other rabbits or using synthetic material. Lactating does will nurse equally well their own or 'alien' young, placed inside the nest. It is crucial to have a minimum of six suckling kits in the nest, as the doe relies on this stimulation to maintain a normal nursing behaviour, i.e., only once a day throughout lactation. Recent work is revealing the similarities and differences in the responsiveness to mating among oestrous, lactating and biostimulated does. The relevance of these findings for the likelihood of reflex ovulation and the additional contribution of factors contained in the semen warrant more in-depth research. New insights on these issues, essential to reproductive neuroendocrinology, can emerge by fostering a richer interaction between academic laboratories and rabbit production settings worldwide.

Key Words: rabbits, mating, lactation, ovulation, stress, welfare.

INTRODUCTION

Rabbit breeding is successfully performed under a variety of conditions, ranging from small production units associated with human dwellings to high-tech farms with different breeds, environments or feedstuffs. Despite these differences, rabbit production can be successful *within* each of these two approaches, although the *absolute values* regarding fertility, meat production, cost-benefit relationship, etc. can vary widely. Much of this variation stems from the use (or not) of findings derived from scientific research performed on areas as diverse as nutrition, feeding, pathology and reproduction. Such studies have been conducted for many years, in several countries —in specialised farms and academic laboratories— and have provided the backbone for the recommendations made to rabbit breeders to improve production and to treat or prevent disease. Nonetheless, innovation is continuously required to deal with the unexpected, to make a 'traditional' process more efficient and to correct obsolete or outright inadequate practices. Innovation in the agronomic sciences requires solid scientific research which, in turn, can enrich its inquiries and interpretations by interacting with workers, staff, and technicians who breed animals under a variety of conditions.

From my interaction with the Rabbit Sciences over the last 16 yr I have identified specific issues concerning productivity on the farm that are directly related with reproductive neuroendocrinology, e.g.: the underpinnings of 'biostimulation', the concurrency of pregnancy and lactation, the differences between pregnancy and pseudopregnancy, the likelihood of mating-induced ovulation, etc. These topics are 'big themes' in themselves and they are also a challenge for anyone interested in 'bridging over" from the laboratory to the farm (and back). It is the purpose of this presentation to show results that illustrate specific examples of this outlook.

BIOSTIMULATION: IMPACT ON DOES AND KITS

Doe rabbits show lactational anoestrus (Beyer and Rivaud, 1969) despite the fact that —unlike in most mammals— suckling bouts are brief (ca. 3 min) and occur only once a day (González-Mariscal *et al.,* 2016). This state is characterised by a lack of sexual receptivity and ovulation, effects presumably provoked by the neuroendocrine conditions of lactation and maintained by the suckling young (Mc Neilly, 2006). Indeed, oestradiol concentrations in blood (Ubilla *et al.,* 2000), sexual receptivity, scent-marking (chinning) and ambulation in an open field are reduced as a consequence of nursing. Interestingly, these behavioural traits are not equally sensitive to the inhibitory effects of suckling: more kits are required to reduce the lordosis quotient than to antagonise chinning and ambulation (García-Dalmán and González-Mariscal, 2012; Table 1).

Yet, separating lactating does from their litters for 48 h (rather than the usual 24 h) restores oestrus in all studied breeds, kept under a variety of environmental conditions (Alvariño *et al.,* 1998; Theau-Clément and Mercier, 1999; Virag *et al.,* 1999; Bonanno *et al.,* 2002). Consequently, this so-called 'biostimulation' procedure has been adopted worldwide to accelerate production in rabbit farms. It has drawbacks, however, for both the doe and the kits. After repeated cycles of concurrent pregnancy and lactation, does decrease their fertility and lose body mass (Bonanno *et al.,* 2002; Eiben *et al.,* 2008). The metabolic mechanisms underlying this 'unavoidable' physical deterioration are not clearly understood. Clearly, more scientific research is warranted in this area to ensure the welfare of breeding does while simultaneously promoting a profitable productivity on the farm.

From the litter's point of view, the 48-h fast and separation from the mother has specific neuroendocrine consequences that persist into adulthood. Brecchia *et al.* (2009) clearly documented a massive secretion of corticosterone following a 48-h mother-litter separation in early lactation. This single, acute response, however, permanently modified the rabbit's neuroendocrine reactivity to mildly aversive stimulation: in contrast to the 'non-deprived' group, *adults* whose mothers were 'biostimulated' showed a blunted corticosterone secretion together with more abundant concentrations of glucocorticoid receptors in the dorsomedial hypothalamus. This, in turn, would support a more intense negative feedback by corticosterone.

Recently, we undertook a broader investigation of this issue by exploring the behavioural and neuroendocrine responses of adult male and female rabbits, which were the progeny of 'biostimulated' or control mothers (García-Fernández *et al.,* 2019). Regardless of time of day or sex, we found no differences between control and 'deprived' litters in any of the six behavioural tests used, previously documented in rabbits as indicative of stress/anxiety (Table 2).

Table 1: Decrease in lordosis, chinning frequency and ambulation provoked by nursing a variable number of kits (lactation day 14; modified from García-Dalmán and González-Mariscal, 2012).

h post-suckling	Chinning marks/10 min; median (iqr)			Ambulation crossings/10 min; median (iqr)			Lordosis quotient median (iqr)		
	suckling kits			suckling kits			suckling kits		
	1	5	10	1	5	10	1	5	10
Baseline	32 (23,29)	2 (2,1)	0 (0,0)	35 (22,20)	28 (15,11)	50 (9,9)	100 (31,0)	65 (31,34)	30 (31,0)
0	2 (13,2)	0 (0,0)	0 (0,0)	30 (22,18)	27 (11,9)	23 (15,8)			
10	13 (13,14)	4 (7,3)	0 (0,0)	32 (7,9)	20 (11,13)	22 (15,13)			

iqr: interquartile range.
Baseline: before nursing.

Table 2: Responses of control and deprived litters in specific behavioural tests presumably measuring stress-anxiety in rabbits (median [iqr]). (Modified from García-Fernández *et al.,* 2019).

	Females		Males	
	Control (n=28)	Deprived (n=24)	Control (n=17)	Deprived (n=25)
Righting reflex (s)	41.5 (74,19)	39.5 (65,21)	33 (59,17)	40 (95,23)
Contacts with human	3 (5,2)	2 (5,1)	3 (6,2)	3 (5,1.5)
Latency to exit dark box (s)	30 (69,17)	27.5 (180,19)	36 (58.5,18)	48 (135,19.5)
Ambulation (lines crossed/min)	8.2 (9.6,5.7)	6.9 (8.1,5.3)	7.7 (8.7,6.3)	7.6 (9.1,6.4)
Chinning (marks/min)	1.2 (2,0.2)	1.4 (3.1,0.2)	6.6 (8,4.1)	7.9 (9.5,0.9)

iqr: interquartile range.

In contrast, sexual behaviour was altered in both sexes when comparing *non-kin* animals: 'deprived' males showed a significantly larger (P=0.002) 'miss rate' (indicative of a large number of mounts not culminating in ejaculation) and the lordosis quotient of 'deprived' females was reduced (this difference, however, did not reach statistical significance; P=0.21; Figure 1).
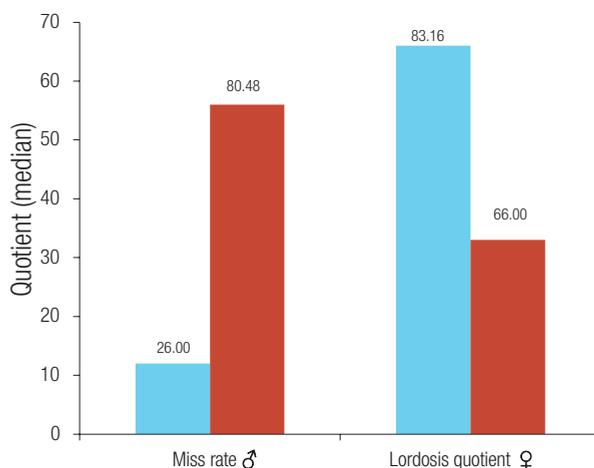

Figure 1: Effect of canelling one nursing episode in early lactation on the sexual behavior of non-kin adult ale and female rabbits. Reductions in male and female sexual behaviour of 'deprived' *vs.* control adult non-kin rabbits. Numbers above bars indicate interquartile ranges. ■ : control; ■ : deprived.

The injection of intramuscular saline in adulthood increased (relative to baseline) the secretion of corticosterone and cortisol in all animals. However, the magnitude of these responses was modified by maternal deprivation, time of day and sex (Figure 2).

Taken together, our results show that the massive corticosteroid secretion that occurs in suckling rabbits as a consequence of a 48-h fast and a brief maternal separation has profound, long-lasting effects on reproductive behaviour and reactive corticosteroid secretion in adulthood. Is there any relevance of these findings for the way rabbits are raised under 'high-tech' or 'low-tech' conditions? Before attempting an answer, I believe one must first reflect on issues like: how is fertilisation of does achieved (i.e., natural mounts or artificial insemination)? At what time of day (i.e., morning or afternoon) are animals commonly subjected to potentially stressful procedures (e.g., tattooing, transport to slaughterhouse, medication)? Is any sex preferred for a particular use (e.g., shearing, shipping to local market, sacrificing *in situ*)? Discussing these points, with consideration to different rearing conditions, strains of rabbits
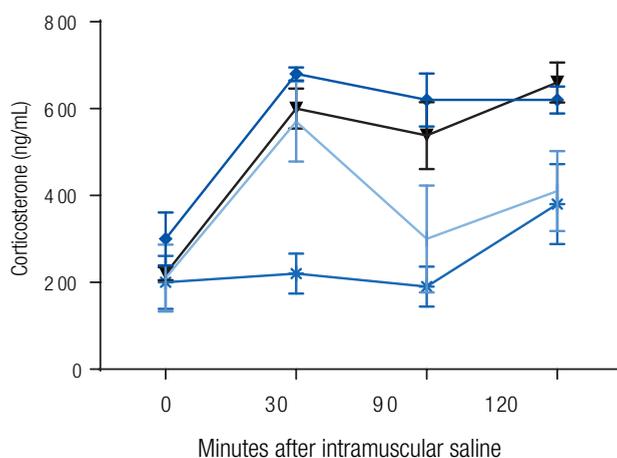
Figure 2: Reactive corticosterone secretion of control and 'deprived' adult male and female rabbits, sampled in the afternoon. Data show means±standard error. ◆ Control ♂; ─┼─ deprived ♂; ─✳─ control ♀; ▼ deprived ♀.

and interests of the farmers is essential for a constructive, bi-directional collaboration between neuroendocrinology and livestock science.

NURSING: ONCE A DAY IS BEST

Rabbits have evolved a unique mothering system in which the direct contact between nursing does and kits is restricted to a brief, single, daily nursing bout per day (González-Mariscal *et al.,* 2016). In contrast, does compensate for this 'absentee' mothering style by building an elaborate maternal nest (of straw and body hair) from early pregnancy to parturition (González-Mariscal *et al.,* 1994). This nest acts like a 'proxy' of the mother, as it provides thermal insulation and protection for the litter and also guides the kits' feeding preferences after weaning (Altbäcker *et al.,* 1995). Thus, a low-quality nest leads to death of littermates and alterations in the mother's nursing behaviour. We have found that the number of suckling young plays a major role in determining the circadian periodicity of nursing (González-Mariscal *et al.,* 2013a). Whereas does suckling six kits display a single nursing bout (at around 03:00 h), females given one, two, or four young enter the nest box many times a day (Figure 3).

Additionally, an insufficient suckling stimulation provokes multiple entrances into the nest box, which leads to accidental hurting of the young and also to a gradual loss of nursing behaviour (González-Mariscal *et al.,* 2013a; Figure 4).

These findings emphasise the need to ensure that: a) a good quality nest is maintained, especially in early lactation; b) nursing does always have at least six kits to suckle. Depending on the type of setup used and the environmental characteristics surrounding the farm, maternal nests can deteriorate over time; additionally, some does (especially primiparous ones) do not always build good nests. These drawbacks can be easily overcome by adding straw or hay to the female's home cage that she can then carry into the nest box. Additionally, hair of different types (e.g., synthetic, or shaved from other female or male rabbits) can also be placed in a container and the doe will collect it and line the straw nest with it (González-Mariscal *et al.,* 1998).

Still, for a number of reasons (e.g., delivery outside the nest box, low birth weight, insufficient milk intake), kits can die in the first days postpartum. To maintain a minimal litter size of six suckling kits these can be exchanged among does
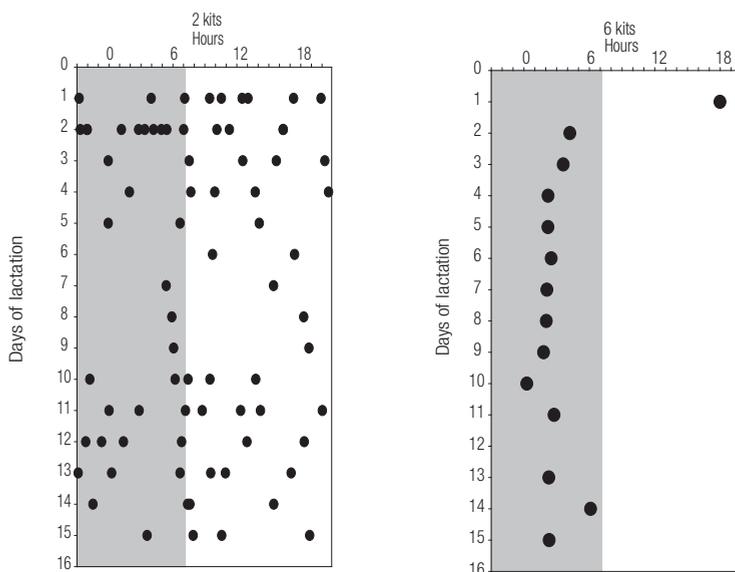
Figure 3: Nursing bouts displayed across lactation days 1-15 by does suckling two or six kits (Modified from González-Mariscal et al., 2013a).

without compromising the doe's maternal behaviour. Rabbits do not show exclusive nursing; rather, they will nurse any kit inside the nest box (provided it is of a similar age as their own; González-Mariscal and Gallegos, 2007; Table 3).

REFLEX OVULATION: NOT AN EASY MATTER

Most oestrous rabbits ovulate in response to mating. Despite its brief duration (Contreras and Beyer, 1979), copulation provides the tactile, olfactory, acoustic and visual stimuli necessary and sufficient to induce the release of hypothalamic GnRH (Yang *et al.,* 1996), followed by a massive secretion of pituitary LH (Ramírez and Beyer, 1988). In
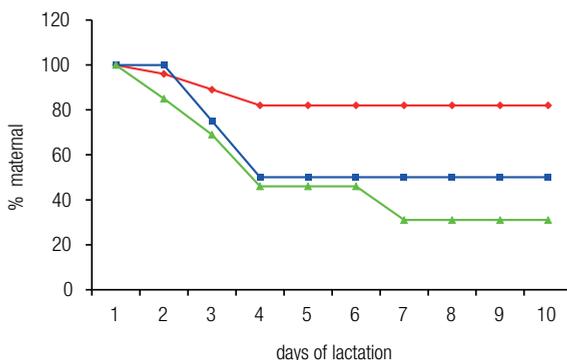

Figure 4: Number of suckling kits impacts maintenance of maternal behavior. Gradual loss of nursing behaviour in does suckling small litters; more than five kits are required to maintain maternal behaviour throughout lactation. ◆ > 5 kits; ■ 2 kits; ▲ 1 kit.

Table 3: Time (min; mean±standard error) spent nursing own or alien young, placed within nests made of straw and different types of hair, across early lactation. (modified from González-Mariscal and Gallegos, 2007).

Type of litter	Type of nest			
	Own	Other doe	Synthetic hair	Male hair
Own	3.0±1.0	3.0±1.0	4.0±1.0	3.0±1.0
Alien	3.0±0.1	4.0±0.3	4.0±0.4	4.0±0.3

addition, following copulation, slow waves appear in the EEG of the cerebral cortex, hypothalamus, and hippocampus (Sawyer and Kawakami, 1959) and scent-marking plus lordosis are immediately inhibited (González-Mariscal *et al.,* 1997; Figure 5).

However, the adequate, predictable operation of this neuroendocrine reflex in oestrous rabbits (plus its associated behavioural consequences) requires that does have an adequate concentration of oestrogens in blood (Hilliard and Eaton, 1971), a sufficient food intake (Brecchia *et al.,* 2006) and a daily photoperiod of 12-14 h (Hudson *et al.,* 1994). In contrast, suckling does show lactational anoestrus, presumably as a consequence of their lower oestradiol concentration in blood together with suckling stimulation *per se* (García-Dalmán and González-Mariscal, 2012). Nonetheless, lactational anoestrus is abolished by cancelling a single nursing bout (biostimulation). To further understand the similarities and differences in the responsiveness to mating among oestrous, lactating and biostimulated does, we recently compared their expression of the c-FOS protein in specific brain regions known to play a crucial role in rabbit reproduction (González-Mariscal *et al.,* 2015). The c-FOS protein, detected by means of immunohistochemistry, has been reliably used as a proxy for neuronal activation. We found that in the preoptic area (POA; rich in GnRH neurons) and the paraventricular nucleus (PVN; rich in oxytocin-producing cells) of oestrous does, copulation markedly increased the number of c-FOS-immunoreactive cells, compared to unmated rabbits. This rise, however, was not seen in the POA of lactating or biostimulated females. Moreover, in the PVN of these groups, mating reduced c-FOS expression (Figure 6).
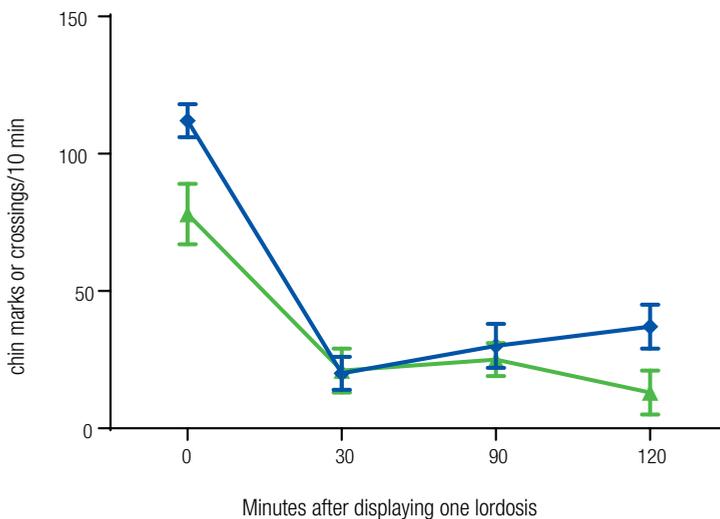

Figure 5: Mating-induced inhibition of chinning and ambulation. Data show means±standard error. ◆ chinning; ▲ ambulation.
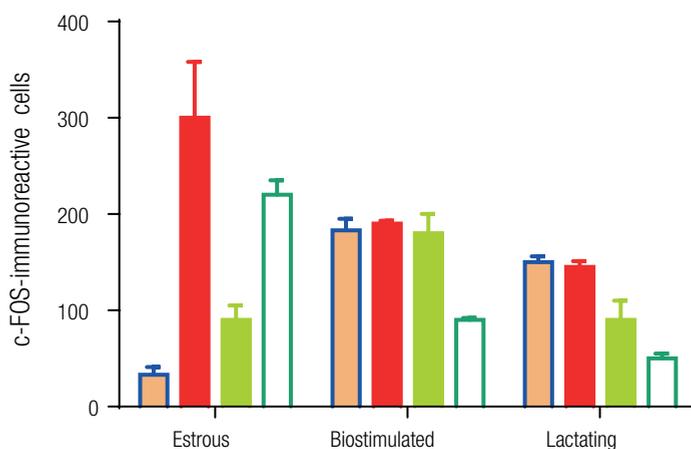
Figure 6: Differential expression of the c-FOS-protein in the POA and PVN of oestrous vs biostimulated vs lactating rabbits. Data show means±standard deviation. ☐ POA, unmated; ■ POA, mated; ■ PVN, unmated; ☐ PVN, mated.

These findings are beginning to reveal that the brain's differential reactivity to the stimulus of mating is finely modulated by as yet undetermined factors associated with the doe's reproductive stage. Future studies are warranted to establish the degree to which the probability of reflex ovulation following copulation depends upon such differences.

In addition to the above, recent evidence obtained by a multinational team (Maranesi *et al.,* 2018), has revealed that the intravaginal deposition of semen is an important additional factor that promotes ovulation. Such complex biological fluid has, of course, myriad proteins, but we found evidence that one of them (nerve-growth factor, NGF), by acting on its cognate receptors in the ovary, facilitates ovulation. Additionally, NGF —synthesised by the uterus— may act on its pituitary receptors to promote LH secretion.

CONCLUSION

Taken together, the results shown in the preceding sections are opening new avenues where the academic setting of neuroendocrinology laboratories can meet findings coming from the animal sciences. We hope that a greater collaboration between these two spheres of endeavour will ultimately lead to a better handling of animals, improved procedures to enhance reproduction and a reconciliation between profitability and care for the environment.

REFERENCES

Altbäcker V., Hudson R., Bilkó Á. 1995. Rabbit-mothers' diet influences pups' later food choice. *Ethology, 99: 107-116.* *https://doi.org/10.1111/j.1439-0310.1995.tb01092.x*

Alvariño J.M.R., Del Arco J.A., Bueno A. 1998. Effect of mother-litter separation on reproductive performance of lactating rabbit females inseminated on day 4 or 11 post partum. *World Rabbit Sci., 6: 191-194. https://doi.org/10.4995/wrs.1998.341*

Beyer C., Rivaud N. 1969. Sexual behaviour in pregnant and lactating domestic rabbits. *Physiol. Behav., 4: 753-757.* *https://doi.org/10.1016/0031-9384(69)90112-7*

Bonanno A., Di Grigoli A., Alabiso M., Boiti C. 2002. Parity and number of repeated doe-litter separation treatments affect differently the reproductive performances of lactating does. *World Rabbit Sci., 10: 63-70.* *https://doi.org/10.4995/wrs.2002.477*

Brecchia G., Bonanno A., Galeati G., Federici C., Maranesi M., Gobbetti A., Zerani M., Boiti C. 2006. Hormonal and metabolic adaptation to fasting: effects on the hypothalamic-pituitary-ovarian axis and reproductive performance of rabbit does. *Domest. Anim. Endocrinol., 31: 105-122.* *https://doi.org/10.1016/j.domaniend.2005.09.006*

Brecchia G., Bonanno A., Dall'Aglio C., Mercati F., Zerani M., Di Grigoli A., Boiti C. 2009. Neuroendocrine responses in neonatal mother deprived rabbits. *Brain Res., 1304: 105-112.* *https://doi.org/10.1016/j.brainres.2009.09.057*

Contreras J.L., Beyer C. 1979. A polygraphic analysis of mounting an ejaculation in the New Zealand white rabbit. *Physiol. Behav., 23: 939-943. https://doi.org/10.1016/0031-9384(79)90204-X*

Eiben Cs., Bonanno A., Gódor-Surmann K., Kustos K. 2008. Effect of controlled nursing with one-day fasting on rabbit doe performance. *Livest Sci., 118: 82-91. https://doi.org/10.1016/j.livsci.2008.01.007*

García-Dalmán C., González-Mariscal G. 2012. Major role of suckling stimulation for inhibition of estrous behaviors in lactating rabbits: acute and chronic effects. *Horm. Behav., 61: 108-113. https://doi.org/10.1016/j.yhbeh.2011.11.003*

García Fernández H.L., Chavira-Ramírez D.R., González-Mariscal G. 2019. Long-lasting behavioral and neuroendocrine changes provoked in rabbits by cancelling a single nursing bout in early lactation. *Dev. Psychobiol., 61: 988-998. https://doi.org/10.1002/dev.21882*

González-Mariscal G. 2007. Mother rabbits and their offspring: timing is everything. *Dev. Psychobiol.*, *49: 71-76. https://doi.org/10.1002/dev.20196*

González-Mariscal G., Gallegos J.A. 2007. New Zealand white rabbits show non-selective nursing in various types of nests. *World Rabbit Sci. ,15: 167-172. https://doi.org/10.4995/wrs.2007.592*

González-Mariscal G., Díaz-Sánchez V., Melo A.I., Beyer C., Rosenblatt J.S. 1994. Maternal behavior in New Zealand white rabbits: quantification of somatic events, motor patterns and steroid plasma levels. *Physiol. Behav., 55: 1081-1089. https://doi.org/10.1016/0031-9384(94)90391-3*

González-Mariscal G., Albonetti M.E., Cuamatzi E., Beyer C. 1997. Transitory inhibition of scent-marking by copulation in male and female rabbits. *Anim. Behav., 53: 323-333. https://doi.org/10.1006/anbe.1996.0327*

González-Mariscal G., Cuamatzi E., Rosenblatt J.S. 1998. Hormones and external factors: are they "on/off" signals for maternal nest-building in rabbits? *Horm. Behav., 33: 1-8. https://doi.org/10.1006/hbeh.1997.1425*

González-Mariscal G., Mc Nitt J.I., Lukefahr S.D. 2007. Maternal care of rabbits in the lab and on the farm: endocrine regulation of behavior and productivity. *Horm. Behav., 52: 86-91. https://doi.org/10.1016/j.yhbeh.2007.03.028*

González-Mariscal G., Lemus A.C., Vega-González A., Aguilar-Roblero A. 2013a. Litter size determines circadian periodicity of nursing in rabbits. *Chronobiol. Int., 30: 711-718. https://doi.org/10.3109/07420528.2013.784769*

González-Mariscal G., Toribio A., Gallegos-Huicochea J.A., Serrano-Meneses M.A. 2013b. The characteristics of suckling stimulation determine the daily duration of mother-young contact and milk output in rabbits. *Dev. Psychobiol., 55: 809-817. https://doi.org/10.1002/dev.21071*

González-Mariscal G., García-Dalmán C., Jiménez A. 2015. Biostimulation and nursing modify mating-induced c-FOS immunoreactivity in the female rabbit forebrain. *Brain Res., 1608:66-74.https://doi.org/10.1016/j.brainres.2015.02.033*

González-Mariscal G., Caba M., Martínez-Gómez M., Bautista A., Hudson R. 2016. Mothers and offspring: the rabbit as a model system in the study of mammalian maternal behavior and sibling interactions. *Horm. Behav., 77: 30-41. https://doi.org/10.1016/j.yhbeh.2015.05.011*

Hilliard J., Eaton L.W.M. 1971. Estradiol-17β, progesterone and 20α-hydroxypregn-4-en-3-one in rabbit ovarian venous plasma. II. From mating through implantation. *Endocrinology, 89: 522-527. https://doi.org/10.1210/endo-89-2-522*

Hudson R., Melo A.I., González-Mariscal G. 1994. Effect of photoperiod and exogenous melatonin on correlates of oestrus in the domestic rabbit. *J. Comp. Physiol. A, 175: 573-580. https://doi.org/10.1007/BF00199478*

Maranesi M., Petrucci L., Leonardi L., Piro F., García Rebollar P., Millán P., Cocci P., Vullo C., Parillo F., Moura A., González-Mariscal G., Boiti C., Zerani M. 2018. New insights on a NGF-mediated pathway to induce ovulation in rabbits (*Oryctolagus cuniculus*). *Biol. Reprod. DOI:10.1093./biolre/ioy041 98: 634-643.*

Mc Neilly A.S. 2006. Suckling and the control of gonadotropin secretion. In: Neill J.D. (Ed.), *Knobil and Neill's Physiology of Reproduction, 3d ed. Elsevier, San Diego, 2511-2551. https://doi.org/10.1016/B978-012515400-0/50051-8*

Ramírez V.D., Beyer C., 1988. The ovarian cycle of the rabbit: its neuroendocrine control. In: Knobil E.K., Neill J.D. (Eds.), *The Physiology of Reproduction. Raven Press, New York, 1873-1892.*

Sawyer C.H., Kawakami M. 1959. Characteristics of behavioral and electroencephalographic after-reactions to copulation and vaginal stimulation in the female rabbit. *Endocrinology, 65: 622-630. https://doi.org/10.1210/endo-65-4-622*

Theau-Clément M., Mercier P. 1999. Effect of a doe-litter separation on rabbit doe reproductive performance and growth of the young. *World Rabbit Sci., 7: 177-179. https://doi.org/10.4995/wrs.1999.398*

Ubilla E., Rebollar P.G., Pazo D., Esquifino A., Alvariño J.M.R. 2000. Effects of doe-litter separation on endocrinological and productivity variables in lactating rabbits. *Livest. Prod. Sci., 67: 67-74. https://doi.org/10.1016/S0301-6226(00)00196-2*

Virag, Gy., Kustos K., Szabo L. 1999. Effect of a 48-hour doe-litter separation on rabbit doe's reproductive performance and offspring's growth. *World Rabbit Sci., 7: 155-159. https://doi.org/10.4995/wrs.1999.394*

Yang S.P., Pau K.Y.F., Hess D.L., Spies H.G. 1996. Sexual dimorphism in secretion of hypothalamic gonadotropin- releasing hormone and norepinephrine after coitus in rabbits. *Endocrinology, 137: 2683-2693. https://doi.org/10.1210/endo.137.7.8770887*