

## BUCK EFFECT ON RABBIT OESTROUS: VULVA COLOUR, VAGINAL LUMEN CELLS AND OVARIAN FOLLICLE POPULATIONS

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**ABSTRACT:** The aim of this study was to determine the response to combinations of male stimuli in the oestrous doe in terms of changes in vulva colour, exfoliated cells in the vaginal lumen and the ovarian histology. Twelve oestrous heterogeneous rabbits does (6 multiparous and 6 nulliparous) were allotted equally to 3 experimental treatments: VIC-Male exposure with visual contact; NVC-Male exposure without visual contact and; NOC-Females completely isolated and not allowed any contact with the male. The does were placed under these treatments for 30 consecutive days during which vulvar colour was checked and vaginal smears were taken daily for each animal. All animals were sacrificed on the 30<sup>th</sup> d and their ovaries processed for histological examination. Does with NVC and VIC exposure displayed either red or purple vulva in 95 and 90% of cases, respectively, the remaining cases being pink coloured but never a whitish vulva ( $P<0.05$ ), whereas does with NOC exposure displayed pink vulva on 80% of the days checked and another 11.7% cases with white vulvae but never a reddish vulva ( $P<0.05$ ). The proportions of superficial, intermediate and parabasal epithelial cells in the vagina smears showed that superficial cells usually contribute less than 16% of the total and are also less abundant ( $P<0.05$ ) irrespective of the doe's exposure type or parity status. All categories of follicles were seen on ovarian sections from NVC and VIC does, but corpora lutea were also present on the sections from VIC does, whereas only follicles below 0.5 mm diameter were seen on the sections from NOC does. In conclusion, these results suggest that the rabbit does require some form of stimulus from the male to remain sexually attractive. Moreover, ovulation appeared to have been induced in the does with visual contact with the male, probably in conjunction with auditory and olfactory, but without tactile or mating contacts.

**Key Words:** rabbit, oestrous, ovulation, male effect.

## INTRODUCTION

Oestrous cycle pattern varies widely among both wild and domesticated animal species. For instance, in 2 wild species of guinea pigs (*Cavia aperea* and *Galea musteloides*), while oestrus, ovulation, and corpus luteum activity occur spontaneously in the former, the latter exclusively requires visual, acoustic and olfactory contacts (but not necessarily tactile or mating contact) with the male to induce oestrus, after which ovulation and corpus luteum activity occur spontaneously (Weir, 1973; Touma *et al.*, 2001). In the prairie vole (*Microtus ochrogaster*), exposure to male or male urine is sufficient to induce oestrus in the female, while actual copulation is further required to induce ovulation (Carter *et al.*, 1980, 1987; Cushing and Hite, 1996). Oestrus in goats and ewes is also affected by stimuli from the male or 'male effect' (Chemineau, 1987).

The sexual responses of the female rabbit (*Oryctolagus cuniculus*) to visual, acoustic/auditory and olfactory stimuli from the male are not properly understood, but ovulation is believed to be induced by mating through a neuro-endocrinological reflex which provokes an LH surge (Voloschin and Gallardo, 1976; Bakker and Baum, 2000). Frank (1966) reported the possibility of certain pheromones secreted by the sebaceous glands of rabbit bucks which could induce sexual receptivity in the does. Much later, some authors (Vodermayer, 1989; Hudson and Distel, 1990; McNitt, 1992) suggested that the rabbit doe emits specific signals containing information on her sexual state that attract males. To date, the precise nature of the pheromones or signals supposedly exchanged between the male and female rabbits is unknown. However, it appears that male presence is beneficial to receptivity and fertility in nulliparous does (Lefèvre *et al.*, 1976; Berepudo *et al.*, 1993) but not in lactating does (Kustos *et al.*, 2000; Eiben *et al.*, 2001; Bonanno *et al.*, 2003).

In the absence of mating or hormonal stimulation, successive preantral follicles would become atretic and be reabsorbed into the ovarian cortex (Kranzfelder *et al.*, 1984; Boiti, 2004; Arias-Alvarez *et al.*, 2010). Interestingly, spontaneous ovulation and pseudo-pregnancy in rabbits not mated or hormonally induced have been a puzzle for many years (Walton and Hammond, 1928; Templeton, 1940; Sawyer, 1959; Staples, 1967; Theau-Clément *et al.*, 2000; Rebollar *et al.*, 2008). So far, there has been no clear explanation for these observations. It thus becomes imperative to investigate the cause of spontaneous ovulation in rabbit does not mated or hormonally induced to ovulate.

We hypothesise that the precise effect of the male stimuli on sexual signals, oestrus, ovulation and corpus luteum function in the female rabbit has not been elucidated, probably because the modern system of commercial rabbit production employs artificial insemination (AI) methods. Thus, we set out to determine the effects of different male stimuli on oestrous in does. In this pilot study we monitored changes in vulva colour, vaginal lumen cells and structure of ovarian sections in twelve does under 3 different types of exposure to the male.

## MATERIAL AND METHODS

### *Experimental animals and management.*

Twelve heterogeneous (extensive cross breeding has occurred over the years and there are no longer pure breed rabbits available in Nigeria) rabbit does comprising 6 multiparous and 6 nulliparous together with 2 intact adult bucks were used for the study. The study was conducted in the rabbitry section of the University's Teaching and Research farm which is located 7° 28'N and 4° 33'E at an altitude of 240 m above sea level. Animals were housed in buildings that exposed them to the prevailing daylight cycle of around 12:12 light:dark hours and were fed on a compounded diet that supplied 2400 kcal digestible energy/kg DM and 18% of crude protein as well as mixture of *Panicum maximum*, *Gliricidia sepium* and *Tridax procumbens* foliages. Water was also given *ad libitum*. Prior to randomisation into treatments, all animals were housed individually in cage compartments measuring 30×24×20 cm. At the time of study, the multiparous and nulliparous does averaged 1.92±0.29 kg and 24.6±4.5 mo and 1.29±0.13 kg and 9.8±3.2 mo in body weight and age, respectively.

### *Experimental design and data collection*

The does were randomised into 3 treatments, each containing 2 multiparous and 2 nulliparous does, namely: VIC-Male exposure with visual contact; NVC-Male exposure without visual

contact and NOC-Complete isolation of does from bucks (control group). VIC and NVC does were housed in pairs with a male between both cages in a separate central compartment. In the NVC group, visual contact between the male and female pairs was blocked with a solid dark partition. In the NOC group, does were housed in pairs without male, in another building more than 200 m away. Animals were maintained in this position for 14 d before data collection commenced.

Vulvar colour was checked in all does for 30 consecutive days between 08:00 and 10:00 a.m. with a fabricated colour chart containing 4 shades (white, pink, purple and red).

Immediately after the vulvar colour check, a smear of vaginal lumen was taken with a sterile swab stick and rolled on a clean glass slide in 2 parallel tracks. The smear was immediately fixed in absolute methanol, allowed to dry for about 5 min and then dipped 2-3 times in staining jar containing Leishman dye. This method has been successfully used to stain vaginal lumen cells in goats (Ola *et al.*, 2006). The stained smears were later viewed at 100× and the epithelial cells classified into superficial, intermediate, and parabasal cell types as described by Tsiligianni *et al.* (2004). From 10 different fields, each cell type was counted and then expressed as percentage of the total as previously described (Ola and Oyegbade, 2008). A field at 100× magnification contains 10-30 epithelial cells.

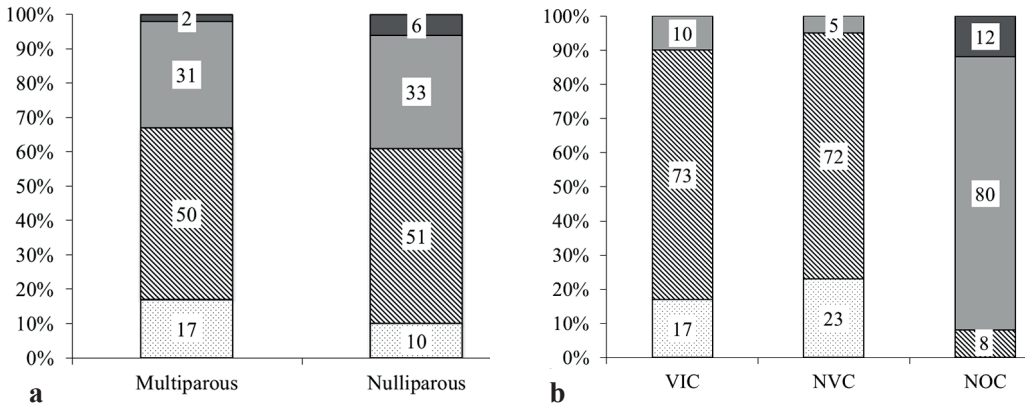
On the 30<sup>th</sup> d of experiment, all 12 does were slaughtered. Ovaries were collected, weighed and placed into Bouin's fluid. The fixed ovaries were later dehydrated in ethyl alcohol, embedded in paraffin wax, sectioned longitudinally in 5 µm slices and then stained with haematoxylin and eosin. Three sections (one each from the middle and the edges of the ovary) from each ovary were selected for follicle counting. Sections were viewed at 100× and follicle diameter was measured with a micrometer. Follicles were then categorised into 5 different sizes i.e. primordial/primary (<0.1 mm), early secondary (0.1-0.24 mm), late secondary (0.25-0.49 mm), early tertiary/antral (0.5-0.9 mm) and late tertiary or Graafian (>1 mm). All follicles measuring 0.1 mm and above were counted.

Data from the vulva colour measurement were expressed as percentages of occurrence and statistically compared by chi-square procedure, while the quantified vaginal epithelial cells and the follicle population were statistically compared between the treatments by Analysis of Variance and Duncan test using SPSS 13.0 package.

## RESULTS AND DISCUSSION

### *Vulva colour appearance*

The frequencies of occurrence of the 4 different vulva colours are shown in Figure 1. Overall, purple was the most observed colour accounting for 51% of all observations, followed by pink, red and white with 32, 13 and 4%, respectively. Across the different exposure type, both multiparous and nulliparous does showed similar pattern of vulva colour appearance (Figure 1a). Purple colour was more frequently exhibited (about 50% in both,  $P<0.05$ ) than pink colour (31.1 and 33%) in multiparous and nulliparous does, respectively. Display of reddish vulva in multiparous does almost doubled that of the nulliparous does (17 and 10%, respectively) while the reverse was the case with whitish vulva appearance (2 and 6%, respectively). However there were significant disparities in the observations between the exposure types (Figure 1b). Purple was the most frequent vulva colour in VIC (72.4%) and NVC does (71.7%), as against 8% in the



**Figure 1:** Frequency of occurrence of vulva colour types in rabbit does in function of (a) parity order and (b) different stimuli from male (VIC, visual contact; NVC, no visual contact; NOC, no contact). ■ White, ■ Pink, ▨ Purple, ▩ Red.

NOC does ( $P < 0.05$ ). VIC and NVC does did not display whitish vulva throughout the study, and NOC did not display red vulva.

The differences observed in the vulva colour of these does suggested that cues from the male may have affected sexual receptivity in the female rabbits. Red or purple and turgid vulva are generally used as indicators of sexual receptivity or oestrus in rabbit does especially under AI programme (Caillol *et al.*, 1983; Rodriguez *et al.*, 1989). Although rabbit does may accept mating from the buck at any period, including during pregnancy and immediately post partum (Diaz *et al.*, 1988; Stoufflet and Caillol, 1988), sexual receptivity and fertility are higher when the vulva appears purplish-reddish and turgid (Theau-Clément and Roustan, 1992, Ubilla and Rebollar, 1995). As in most other mammals, sexual receptivity in the rabbit is associated with higher oestrogen levels (Caillol *et al.*, 1983; Ubilla and Rebollar, 1995; Ubilla *et al.*, 2000; Rebollar *et al.*, 2006). However, receptivity was found to be maintained for a prolonged period in many ovariectomised-adrenalectomised rabbits, indicating that sex steroids may not be the sole activator of sexual behaviour (Beyer *et al.*, 2007). When there is a contact with the male (at least olfactory or auditory) rabbit does exhibited a high level of coloured vulva (oestrus indicator). In contrast, does remaining without any contact with bucks displayed pale vulva, which is an indicator of dioestrus (Rodriguez *et al.*, 1989). However, the precise mechanism by which these contacts influence receptivity in the does needs to be established.

#### *Vaginal smearing for exfoliated epithelial cells.*

The different exfoliated epithelial cell types (superficial, intermediate and parabasal) in the smears were statistically analysed with respect to the doe's parity status and the type of exposure to the male, (Table 1).

The occurrence of the different epithelial cells was similar for both multiparous and nulliparous does, which followed the pattern observed for VIC does. In the smears from NVC does, the dominating epithelial cells were of intermediate types whereas the parabasal cells were the most abundant in the smears from NOC does. On the other hand, both intermediate and parabasal cells were quantified similarly in the VIC does. In all the exposures types, superficial cells were the least observed cells ( $P < 0.05$ ). Vaginal smearing is an unreliable procedure to determine

**Table 1:** Epithelial cell types in the vaginal lumen of rabbit does under varying conditions (%).

Treatment Group	No.	Superficial cells	Intermediate cells	Parabasal cells
<b>Parity</b>				
Multiparous	180	10.24±0.83 <sup>B</sup>	44.86±1.93 <sup>A</sup>	44.90±1.92 <sup>A</sup>
Nulliparous	176	12.30±0.83 <sup>B</sup>	40.71±2.13 <sup>A</sup>	46.99±2.01 <sup>A</sup>
<b>Male exposure<sup>1</sup></b>				
NVC	120	9.20±0.63 <sup>Ca</sup>	59.44±1.71 <sup>Aa</sup>	31.37±1.59 <sup>Ba</sup>
VIC	116	11.06±1.02 <sup>Bab</sup>	40.07±2.63 <sup>Ab</sup>	48.87±2.54 <sup>Ab</sup>
NOC	120	13.52±1.25 <sup>Cb</sup>	28.82±1.21 <sup>Bc</sup>	57.66±1.59 <sup>Ac</sup>

<sup>ABC</sup> In each treatment grouping, means within the same row with different superscript are significantly different ( $P<0.05$ ).

<sup>abc</sup> In each treatment grouping, means within the same column with different superscript are significantly different ( $P<0.05$ ).<sup>1</sup> VIC, visual contact; NVC, no visual contact; NOC, no contact.

oestrus period in the rabbits (Kunde and Proud, 1929; Ypsilantis *et al.*, 1996; Ola and Oyegbade, 2008) because the exfoliated cells do not appear in a cyclic manner as seen with guinea pigs, rats and other spontaneous ovulators. Although the prairie vole, like rabbit, also shows induced oestrus and ovulation had predictable vaginal oestrus (Cushing and Hite, 1996), our results again confirmed the unreliability of vaginal smears for predicting sexual receptivity in rabbit, since the pattern of cells occurrence varied little between does under different conditions.

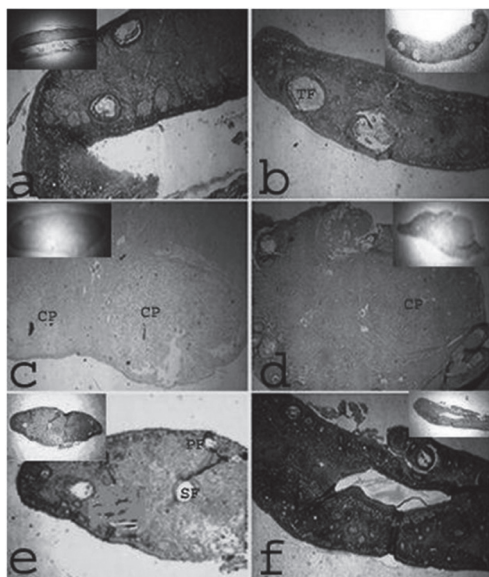
*Ovarian histology and follicle populations.*

The mean population of the different categories of follicle in the 3 groups of rabbit is presented in Table 2. Micrographs of the representative samples of the ovarian sections are also shown in Figure 2. Only follicles with diameter greater than 0.1 mm (i.e. growing primary follicles and later stages, Fortun-Lamothe *et al.*, 2000) were counted while the much smaller ones (i.e. primordial and early primary follicles) were subjectively estimated and quantitatively represented with + sign. Even though the ovaries of multiparous does were larger than those of nulliparous does, there were no differences in the population of the different categories of the follicles on the ovaries. On the contrary, the ovarian weight as well as the follicle population were greatly

**Table 2:** Mean follicle populations (per ovary) of rabbit does under different stimuli from the male or parity.

Group	No. of ovary	Mean Ovarian wt (g)	Corpus luteum	Follicle size categories (mm)				
				>1	0.5-0.9	0.25-0.49	0.1-0.24	<0.1 <sup>1</sup>
<b>Parity:</b>								
Multiparous	12	0.265 <sup>a</sup>	0.58	0.67	0.67	5.58	10.67	++
Nulliparous	12	0.075 <sup>b</sup>	0.42	0.33	0.75	7.25	11.58	+++
<b>Male exposure<sup>2</sup></b>								
NVC	8	0.180 <sup>a</sup>	0.00 <sup>b</sup>	0.00 <sup>b</sup>	0.63 <sup>b</sup>	2.88 <sup>b</sup>	8.62 <sup>b</sup>	++
VIC	8	0.218 <sup>a</sup>	1.50 <sup>a</sup>	1.50 <sup>a</sup>	1.50 <sup>a</sup>	4.75 <sup>b</sup>	9.62 <sup>b</sup>	++
NOC	8	0.112 <sup>b</sup>	0.00 <sup>b</sup>	0.00 <sup>b</sup>	0.00 <sup>c</sup>	11.63 <sup>a</sup>	15.12 <sup>a</sup>	++
	SEM	0.03	0.16	0.17	0.17	0.94	1.04	

<sup>abc</sup> In each treatment grouping, means within the same column with different superscript are significantly different ( $P<0.05$ ). <sup>1</sup> Subjective estimation and quantification. <sup>2</sup> VIC, visual contact; NVC, no visual contact; NOC, no contact.



**Figure 2:** Ovarian histology of rabbit does exposed to different male stimuli. Follicle stages up to tertiary antral follicles (TF) were seen in sections from both multiparous (a) and nulliparous (b) does with no visual contact with male, NVC. In addition to all the follicle stages, corpus luteum (CP) was also seen on the ovaries from multiparous (c) and nulliparous (d) does with visual contact with the male, VIC. In the non exposed does, NOC, only follicles up to secondary size (SF) were seen (e and f) with a large pool of primary (PF) and primordial follicles. All micrographs were taken at 30× while the insets were the whole ovarian section taken at 10×.

Dutch Belted rabbit does. Interestingly, ovulation did however occur among submissive oestrous does if they were repeatedly mounted by oestrous or anoestrous does while caged in pairs for periods ranging from 1 to 17 d.

In our experiment, the does were housed in pairs and were also vaginally stimulated daily during smear collection. We could thus hypothesise that since all the does in our experiment were similarly manipulated, the presence of ovulation sites on the ovaries of the VIC does could suggest that ovulation may have been induced in the rabbit does through an interplay of visual contact with the male, vaginal stimulation and interaction with the pen mate. Both Kustos *et al.* (2000) and Rodriguez De Lara *et al.* (2003) reported that short time exposure (8-10 h) to male just prior to AI had no effect on the sexual receptivity (as judged by lordosis posture) in the female. However, the latter authors reported larger litter size in the male-exposed does and were of the opinion that visual cues around insemination might have induced a greater sensitivity of

influenced by the type of contact/exposure the female had with the male. Ovaries were significantly smaller in does not exposed to male. All follicle categories, including corpora lutea, were seen in all the ovaries from VIC does. On the contrary, the ovaries of the NVC and NOC does were mainly populated with follicles below 0.5 mm diameter. While some antral follicles (0.5-0.9 mm) were seen on some of the sections from NVC, ovaries from NOC does had significantly larger number of follicles below 0.5 mm size (late primary and early secondary follicle)

The structure of the ovaries of the rabbit does with (VIC) or without (NVC) visual contact (but assumingly with auditory and olfactory contacts) with the male were typical of the oestrous doe (Garcia-Garcia *et al.*, 2009; Arias-Alvarez *et al.*, 2010) except for the presence of the corpus luteum in the VIC, which indicated a pseudo-pregnant status. Theau-Clément *et al.* (2000) found 46% of lactating primiparous does to be pseudo-pregnant at the point of insemination, with no clue to the causes. Mechanical stimulation of the vagina and/or the cervix (Yamane and Egashira, 1925; Shibata, 1931; Carlyle and Williams, 1961) or mere rubbing of the vulva (Hammond, 1925) was reported to cause ovulation in a few oestrus rabbits. Staples (1967) showed that neither the act of mounting and ejaculation without intromission by buck nor the close proximity to and mounting by male was sufficient to cause ovulation in

the pituitary gland to exogenous GnRH, thus enhancing ovarian follicular development and the ovulation process. The absence of large antral follicles (>0.9 mm) in the ovaries of the NVC does further reinforces our suspicion that a visual cue from the male may be an activator for follicle selection and final ovulation in the female rabbits. The rabbit does completely isolated from the male (NOC) presented ovaries significantly reduced in size and lacked antral follicles, displaying a pale vulva (Figure 1). Taken together, these findings suggest that the does were in a state of reproductive quiescence.

Ample reports have shown that all the characteristics of the oestrous cycle i.e. behavioural receptivity, ovulation and corpus luteum activity, can occur spontaneously or be induced by external stimuli. Among the rodent family for instance, *Microtus ochrogaster* require copulation to induce ovulation (Carter *et al.*, 1980, 1987; Cushing and Hite, 1996) whereas in *Galea musteloides*, after oestrus was induced by visual, acoustic and olfactory contacts with the male, ovulation and corpus luteum activity occurred spontaneously (Touma *et al.*, 2001). Moreover, in mice, rat and hamster, even though oestrus and ovulation occur spontaneously, development of a functional corpus luteum depends on stimulus from copulation (Conaway, 1971; Nelson, 2000).

Our proposition from this pilot study is that visual in conjunction with acoustic and olfactory contacts with an intact male and/or vaginal stimulation of rabbit does could play a role in oestrus and ovulation. However, the precise mechanism for non coitus induced ovulation in rabbit remains unknown. Even though our findings require further testing on a larger scale, the information is of practical importance in intensive operations that employ either AI or natural service to guard against incidence of pseudo-pregnancy and the consequent reduced fertility.

## CONCLUSION

Our results will likely reinvigorate studies on the causes that can induce ovulation in the rabbit without coital stimulation. The so called “male effect” on oestrous and ovulation in rabbits may also need to be revisited. In other words, coitus may not necessarily be the only means of natural ovulation in the rabbit as generally accepted and if behavioural (not hormonal) ways of ovulation induction could be found, they may bring to an end the use of hormones for this purpose in AI.

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## REFERENCES

- Arias-Álvarez M., García-García R.M., Torres-Rovira L., Gonzalez-Bulnes A., Rebollar P.G., Lorenzo P.L. 2010. Influence of hormonal and non hormonal estrus synchronisation methods on follicular and oocyte quality in primiparous lactating does at early post partum period. *Theriogenology*, 73: 26-35. doi: 10.1016/j.theriogenology.2009.07.017
- Bakker J., Baum M.J. 2000. Neuroendocrine regulation of GnRH release in induced ovulators. *Front. Neuroendocrin.*, 21: 220-262. doi: 10.1006/frne.2000.0198
- Berepudo N.A., Nodu M.B., Monsi A., Amadi E.N. 1993. Reproductive response of prepubertal female rabbit to photoperiod and/or male presence. *World Rabbit Sci.*, 1: 83-87. doi: 10.4995/wrs.1993.199
- Beyer C., Hoffman K.L., González-Flores O. 2007. Neuroendocrine regulation of estrous behavior in the rabbit: Similarities and differences with the rat. *Horm. Behav.*, 52: 2-11. doi: 10.1016/j.yhbeh.2007.03.027
- Boiti C. 2004. Underlying physiological mechanism controlling the reproductive axis of rabbit does. In *Proc.: 8<sup>th</sup> World Rabbit Congress, 7-10 September, 2004. Puebla, Mexico.* 186-206.
- Bonanno A., Mazza F., Alabiso M., Di Grigoli A., Alicata M.L. 2003. Effects of bio-stimulation induced by contact with buck on reproductive performance of rabbit does. In *Proc.: A.S.P.A. 15th Congress, Parma, June 18-20, 2003. Ital. J. Anim. Sci.* 2: 133-135.

- Caillon M., Dauphin-Villemant C., Martinet L. 1983. Oestrous behaviour and circulating progesterone and oestrogen levels during pseudopregnancy in the domestic rabbit. *J. Reprod. Fert.*, 69: 179-186. doi: 10.1530/jrf.0.0690179
- Carlyle A., Williams T. D. 1961. Artificially induced ovulation in the rabbit. In *Proc. of the Physiological Society: Bristol Meeting, 14-15 April 1961*. *J. Physiol-London*, 157: 42p.
- Carter C.S., Getz L.L., Gavish L., McDermott J.L., Arnold P. 1980. Male-related pheromones and the activation of female reproduction in the prairie vole (*Microtus ochrogaster*). *Biol. Reprod.*, 23: 1038-1045. doi: 10.1095/biolreprod23.5.1038
- Carter C.S., Witt D.M., Schneider J., Harris Z.L., Volkening D., McEwen B.S. 1987. Male stimuli are necessary for female sexual behavior and uterine growth in prairie voles (*Microtus ochrogaster*). *Horm. Behav.*, 21: 74-83. doi: 10.1016/0018-506X(87)90032-8
- Chemineau P. 1987. Possibilities of using bucks to stimulate ovarian and oestrus cycles in anovulatory goats: A review. *Livest. Prod. Sci.*, 17: 135-147. doi: 10.1016/0301-6226(87)90059-5
- Conaway C.H. 1971. Ecological adaptation and mammalian reproduction. *Biol. Reprod.*, 4: 239-247.
- Cushing B.S., Hite R. 1996. Effects of estradiol on sexual receptivity, wheel-running behavior, and vaginal estrus in virgin prairie voles. *Physiol. Behav.*, 60: 829-832. doi: 10.1016/0031-9384(96)00084-4
- Diaz P., Gosalvez L.F., Rodriguez J.M. 1988. Sexual behaviour in the postpartum period of domestic rabbits. *Anim. Reprod. Sci.* 17: 251-257. doi: 10.1016/0378-4320(88)90062-0
- Eiben Cs., Kustos K., Szendrő Zs., Theau-Cément M., Gódor-Surmann K. 2001. Effect of male presence before artificial insemination on the receptivity and prolificacy in lactating rabbit does. In *Proc.: 12<sup>th</sup> Symposium on Housing and Diseases of Rabbits, Furbearing Animals and Pet Animals, Celle, 1-6*.
- Fortun-Lamothe L., Powers S., Coulet A., Read K., Mariana J.C. 2000. Effects of concurrent pregnancy and lactation in rabbit does on the growth of follicles in daughters' ovaries. *World Rabbit Sci.* 8, 1: 33-40. doi: 10.4995/wrs.2000.415
- Frank H. 1966. Ablation des bulbes olfactifs chez la lapine impubère. Répercussions sur le tractus génital et le comportement sexuel. *Soc. Biol.* 160: 389-390.
- García-García R.M., Arias-Alvarez M., Rebollar P.G., Revuelta L., Lorenzo P.L. 2009. Influence of different reproductive rhythms on serum estradiol, testosterone levels, features of follicular population and atresia rate, and oocyte maturation in controlled suckling rabbits. *Anim. Reprod. Sci.*, 114: 423-433. doi: 10.1016/j.anireprosci.2008.10.007
- Hammond J. 1925. *Reproduction in the rabbit*, pp. 44 and 70. *Oliver & Boyd, Edinburgh*.
- Hudson R., Distel H. 1990. Sensitivity of female rabbits to changes in photoperiod as measured by pheromone emission. *J. Comp. Physiol. A*, 167: 225-230. doi: 10.1007/BF00188115
- Kranzfelder D., Korr H., Mestwerdt W., Maurer-Schulze B. 1984. Follicle growth in the ovary of the rabbit after ovulation-inducing application of human chorionic gonadotropin. *Cell Tissue Res.*, 238: 611-620. doi: 10.1007/BF00219879
- Kunde M.M., Proud T. 1929. The ineffectiveness of vaginal smears in predicting the oestrous cycle in the rabbit. *Am. J. Physiol.*, 88: 446-452.
- Kustos K., Eiben Cs., Szendrő Zs., Theau-Cément M., Gódor S-Né., Jováncai Zs. 2000. Effect on reproductive traits of male presence among rabbit does before artificial insemination. In *Proc.: 7<sup>th</sup> World Rabbit Congress, 4-7 July, 2000, Valencia, Spain*. Vol. A: 161-166.
- Lefèvre B., Martinet L., Moret B. 1976. Environnement et comportement d'oestrus. In *Proc.: 1<sup>er</sup> Congrès International Cunicole, Dijon (France), Communication n°61*.
- McNitt J.I. 1992. Endocrinological approaches for commercial rabbit production. *J. Appl. Rabbit Res.*, 15: 364-397.
- Nelson R.J. 2000. *An Introduction to Behavioral Endocrinology*, 2<sup>nd</sup> ed, Sinauer Associates, Inc., Sunderland, Massachusetts.
- Ola S.I., Sanni W.A., Egbunike G.N. 2006. Exfoliative vagina cytology during the oestrous cycle of West African Dwarf goats. *Reprod. Nutr. Dev.*, 46: 87-95. doi: 10.1051/rnd:2005067
- Ola S.I., Oyegbade M.O. 2008. The influence of the different contact levels with male on the vaginal cytology in rabbits under the tropical humid condition. In *Proc.: 9<sup>th</sup> World Rabbit Congress, 10-13 June, 2008, Verona, Italy*: 417-421.
- Rebollar, P.G., Milanés, A., Pereda, N., Millán, P., Cano, P., Esquino, A.I., Villarroel, M., Silván, G., Lorenzo, P.L. 2006. Oestrus Synchronization of rabbit does at early post-partum by doe-litter separation or eCG injection: reproductive parameters and endocrine profiles. *Anim. Reprod. Sci.*, 93: 218-230. doi: 10.1016/j.anireprosci.2005.06.032
- Rebollar P.G., Bonanno A., DiGrigoli A., Tornambe G., Lorenzo P.L. 2008. Endocrine and ovarian response after a 2-day controlled suckling and eCG treatment in lactating rabbit does. *Anim. Reprod. Sci.*, 104: 316-328. doi: 10.1016/j.anireprosci.2007.02.018
- Rodriguez J.M., Agrasal C., Esquifino A. 1989. Influence of sexual receptivity on LH, FSH and prolactin release after GnRH administration in female rabbits. *Anim. Reprod. Sci.*, 20: 57-65. doi: 10.1016/0378-4320(89)90113-9
- Rodriguez-De Lara R., López-Fallas M., Rangel-Santos R., Mariscal-Aguayo V. 2003. Influence of short-term relocation and male exposure on sexual receptivity and reproduction in artificially inseminated lactating doe rabbits. *Anim. Reprod. Sci.*, 78: 111-121. doi: 10.1016/S0378-4320(03)00064-2
- Sawyer C. H. 1959. Seasonal variation in the incidence of spontaneous ovulation in rabbits following estrogen treatment. *Endocrinology*, 65: 523-525. doi: 10.1210/endo-65-3-523
- Shibata S. 1931. [The oestrous cycle and ovulation in the rabbit.] *Nihon Chikusan Gakkaiho*, 11: 309. doi: 10.2508/chikusan.5.69
- Staples R.E. 1967. Behavioural induction of ovulation in the oestrus rabbit. *J. Reprod. Fert.*, 13: 429-435. doi: 10.1530/jrf.0.0130429
- Stoufflet I., Caillon M. 1988. Relation between circulating sex steroid concentrations and sexual behaviour during pregnancy and post partum in the domestic rabbit. *J. Reprod. Fert.*, 82: 209-218. doi: 10.1530/jrf.0.0820209
- Templeton G. S. 1940. Pseudopregnancy in domestic rabbits. *Wildlife, Circ. U.S. Dep. Int.* 1940 No. 4: 13 pp.
- Theau-Clément M., Roustan A. 1992. A study on relationship between receptivity and lactation in the doe, and their influence on reproductive performance. *J. Appl. Rabbit Res.*, 15: 412-421.
- Theau-Clément M., Boiti C., Mercier P., Falières J. 2000. Description of the ovarian status and fertilizing ability of primiparous rabbit does at different lactation stages. In *Proc.: 7<sup>th</sup> World Rabbit Congress, 4-7 July, 2000, Valencia, Spain*. Vol. A: 259-272.
- Tsiligianni Th., Sratsi A., Besenfelder U., Anastasiadis A., Vainas E., Saratsi Ph., Brem G. 2004. The use of cytological examination of vaginal smears in the selection of rabbits for superovulation. *Theriogenology*, 61: 989-995. doi: 10.1016/S0093-691X(02)01293-1
- Touma C., Palme R., Sachser N. 2001. Different types of oestrous cycle in two closely related South American rodents (*Cavia aperea* and *Galea musteloides*) with different social and mating systems. *Reproduction*, 121: 791-801. doi: 10.1530/rep.0.1210791
- Ubilla E., Rebollar P.G., Pazo D., Esquino A.I., Alvarino J.M.R. 2000. Pituitary and ovarian response to transient doe-litter separation in nursing rabbits. *J. Reprod. Fert.*, 118: 361-366. doi: 10.1530/jrf.0.1180361



- Ubilla E., Rebollar P.G. 1995. Influence of the postpartum day on plasma estradiol-17 $\beta$  levels, sexual behaviour, and conception rate, in artificially inseminated lactating rabbits. *Anim. Reprod. Sci.*, 38: 337-344. doi: 10.1016/0378-4320(94)01366-T
- Vodermayer T. 1989. Wechselwirkungen von Photoperiode, Pheromonen und oestrichen Korrelaten bei weiblichen Kaninchen. *Thesis, Ludwig - Maximilians - Universität, München.*
- Voloschin L.M., Gallardo E.A. 1976. Effect of surgical disconnection of the medial basal hypothalamus on post coital reflex ovulation in the rabbit. *Endocrinology*, 99: 959-962. doi: 10.1210/endo-99-4-959
- Walton A., Hammond J. 1928. Observations on ovulation in the rabbit. *J. Exp. Biol.* 6: 190-204.
- Weir B.J. 1973. The role of the male in the evocation of oestrus in the cuis, *Galea musteloides* (Rodentia: Hystricomorpha). *J. Reprod. Fertil.*, 19: 421-432.
- Yamane J., Egashira T. 1925. [On the relation of copulation to ovulation in the rabbit as shown by means of artificial insemination.] *J. Jpn. Soc. Vet. Sci.* 4, 110. doi: 10.1292%2Fjvms1922.4.101
- Ypsilantis P., Tsiligianni Th., Karagiannidis A. 1996. The use of cytological examination of vaginal smears for the determination of domestic rabbit's oestrus cycle. *J. Hellenic Vet. Med. Soc.*, 47: 186-190.
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