MANUAL INDUCTION OF LORDOSIS AND DETECTION OF OESTRUS IN THE DOMESTIC RABBIT (*Oryctolagus cuniculus*)

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ABSTRACT

Receptive female mammals adopt the lordosis posture to facilitate fertilization during copulatory interaction. The present paper shows the results of experiments carried out to find mounting-associated stimuli that actually activate the lordosis in female domestic rabbits. The involvement of estrogens in such activating stimuli was tested. Eight females were ovarioectomized (experimental condition 1) and subsequently estrogenized with estradiol benzoate (experimental condition 2). In each condition, females were subjected to two kinds of stimulus: a) pressure upon the rump, sacs filled with sand (weighting either 0.5, 1.0, 2.0, or 3.0 kg) simulated the bodyweight of the male during mounting; b) perineal beatings, subtle finger-beatings (3 per second) simulated the penile exploration of a male attempting penetration. Pressure exerted upon the rump is capable of activating the lordosis response when it is ≥1.0 kg. The lordosis response to the pressure stimulus is entirely dependent on body levels of estrogens. Seemingly, the effectiveness of this stimulus is strongest when the pressure approximates the weight of a young semental male: the difference between responses to 1.0 kg and 2.0 kg was significant (P=0.003). On the other hand, the lordosis response is induced by the beating stimulus in a particularly accentuated manner. Interestingly, the effectiveness of the beating stimulus is estrogen-independent. Similar responses were obtained with ovarioectomized and estrogenized females. Our findings suggest that different nervous mechanisms induce the lordosis reponse. We propose the induction of the lordosis response as an alternative to current indicators of sexual receptiveness, which are either ambiguous or relatively difficult to notice. Induction of lordosis can be achieved by subtly pushing the rump of females with an open hand.

Key words: Rabbit, Lordosis, Oestrus, Reproduction, Sexual behaviour.

INTRODUCTION

Sexual receptivity is the behaviour displayed by female mammals to allow copulatory interaction and fertilization. One action carried out by receptive females is adopting the posture of lordosis as a response to the male mounting: the male approaches round behind, stands on the hindlegs and rests his body on the rump of the female. While holding her flanks with the forelegs, the male makes pelvic thrusts to achieve penetration. In response, the female raises the hindlegs to expose the perineal region, thus facilitating the coincidence of the vulva and penis (Dewsbury, 1979; Sachs and Meisel, 1988). The lordosis has been observed in many species and is considered to be representative of the estrous cycle (Worden, 1962; Hafez, 1970; Dewsbury, 1979; Sachs and Meisel, 1988). It is also known that its manifestation depends on body levels of estrogens (Pfaff and Schwartz, 1988; Kow and Pfaff, 2004; Pfaff, 2005). However, the studies of lordosis in rats are by far the most detailed (Pfaff and Schwartz, 1988; Sachs and Meisel, 1988). In rats, the male's embrace has been observed to activate the lordosis response. Such activating stimulus can be simulated manually with a proper fingering technique (Dewsbury, 1979). It is believed that female rabbits respond to the male's embrace and pelvic thrusts on the rump in the same way as rats do (Dewsbury, 1979; Ramírez and Beyer, 1988). The estrous cycle is not clearly defined in domestic rabbits. Some authors consider that signs of nervousness and

restlessness are indicators of oestrus. These conductual indicators are vague and subjective. Sexual receptivity is clearly indicated by a purplish and moist vulva (Dewsbury, 1970; Hafez, 1970; Ambarino, 1993). However, performing the inspection of the vulva requires some effort.

We are interested in the biological processes associated with the regulation of the copulatory activity in domestic rabbits. Accordingly, we have characterized aspects of copulation in males, including the duration and frequency of pelvic thrust trains, the activity of the seminal vesicle at ejaculation, and the involvement of androgens (Contreras and Beyer, 1979; Beyer *et al*., 1982). We have attempted to induce lordosis in females by simulating the male's embrace in a way similar to that of the fingering technique. However, the lordosis response is not activated by this stimulus. Negative results were also obtained after simulating pelvic thrusts upon the rump. Such observations prompted us to find mounting-associated stimuli that actually induce lordosis in female rabbits. To this aim, we made experiments simulating the pressure exerted by males on the rump of females during mounting. Similarly, we explored the involvement of penile stimulation associated with the male's pelvic thrusts; early observations indicated us to stimulate the perineal region of females.

MATERIALS AND METHODS

Eight female domestic rabbits (*Oryctolagus cuniculus*) weighting 3 to 4 kg were used throughout this study. The females were ovarioectomized, thus providing a first experimental condition. For a second condition, the ovarioectomized females were estrogenized artificially with daily injections of 20 μg of estradiol benzoate. Receptivity was verified by allowing trained males to interact with females in both ovarioectomized and estrogenized conditions; the receptivity coefficient for each condition was 0.0 and 0.8-1.0, respectively. In each condition, females were subjected to two kinds of stimulus:

1.- Pressure stimulus. Cloth sacs (20 x 30 cm) filled with sand (weighting either 0.5, 1.0, 2.0, or 3.0 kg) were used to simulate the varying levels of pressure that the bodyweight of a male can exert on a female's rump during copulatory interaction. The females received each sac one time.

2.- Beating stimulus. Subtle finger-beatings were exerted upon the perineal region of females to simulate the penile stimulation. In a previous work, we have reported that the frequency of the male's pelvic thrusts averages about 13 movements per second (Contreras and Beyer, 1979). Notwithstanding this observation, we have found that subtle finger-beatings (about 3 per second) in the perineal region can be enough to induce lordosis in a receptive female. Each female received this stimulus three times.

The complete experimental session was conducted twice. The corporal responses to the pressure and beating stimuli were recorded. Absence of lordosis was considered as a negative response (–). Varying manifestations of the lordosis response were categorized as follows: +, if body weight rested on the metatarses; ++, if hindlegs were partially extended; +++, if hindlegs were completely extended. The statistical significance of the results was evaluated with the Sign Test (Siegel, 1972; Conover, 1980), a non-parametric test suitable for the comparison of observations that can be paired under a natural basis (in the case of this study, each female can be considered to be its own control).

RESULTS AND DISCUSSION

Table 1 shows the responses to pressure stimulation. Clearly, pressure upon the rump did not induce lordosis in females deprived of estrogens. In two sessions, ovarioectomized females exhibited negative responses to varying levels of pressure. If all recorded responses are distributed among 4 pressure levels, then the 25% of responses corresponding to each level were negative. In contrast, pressure was positively correlated with lordosis in estrogenized females. The only exception was in the range of 0.5 kg, which accounted for 25% of all negative responses in this condition. In the range of 1.0 kg, the percentage of negative responses (12.5%) equaled the sum of positive ones (+, 7.8%; ++, 3.1%; +++, 1.5%). Nevertheless, when pressure was further increased, negative responses became insignificant

(1.5% for 2.0 and 3.0 kg). We tested whether responses tended to be stronger as pressure increased. Responses to 1 and 2 kg were significantly different (P=0.003, α=0.05), whereas those to 2.0 and 3.0 kg were similar (P=0.5). We suggest that the effect of the pressure stimulus is strongest when it approximates the weight of a young semental male.

Table 1: Responses of females subjected to pressure stimulus

Session	Female	Condition							
		Ovarioectomized				Estrogenized			
		Pressure (kg)				Pressure (kg)			
		0.5	1.0	2.0	3.0	0.5	1.0	2.0	3.0
I	1	–	–	–	–	–	+	+++	++
	2	–	–	–	–	–	–	++	++
	3	–	–	–	–	–	++	++	++
	4	–	–	–	–	–	–	–	+
	5	–	–	–	–	–	++	++	++
	6	–	–	–	–	–	–	++	+
	7	–	–	–	–	–	+	+++	++
	8	–	–	–	–	–	+	+	++
II	1	–	–	–	–	–	+	++	+++
	2	–	–	–	–	–	–	+	++
	3	–	–	–	–	–	+	+++	+++
	4	–	–	–	–	–	+++	++	+++
	5	–	–	–	–	–	–	+	–
	6	–	–	–	–	–	–	+	+++
	7	–	–	–	–	–	–	++	+
	8	–	–	–	–	–	–	++	+

Table 2 shows results obtained when females were subjected to beating stimulation upon the perineal region. Interestingly, ovarioectomized females did show lordosis. Only 2% of the recorded responses were negative. Positive responses showed level variations (+, 27%; ++, 27%; +++, 44%). A significant number of positive responses were very strong. Perineal stimulation was associated with the activation of a strong lordosis response when females were estrogenized (+, 2%; ++, 37.5%; +++, 60.5%). Seemingly, the effectiveness of the beating stimulus is estrogen-independent. An enhancing effect of estrogens on the lordosis response cannot be ruled out, as indicated by the comparison of the responses in the ovarioectomized and estrogenized conditions (P=0.004, α=0.05).

The lordosis responses were further described as incomplete (+), complete (++), or pronounced (+++) on the basis of relevant posture indicators. Incomplete lordosis was showed by receptive females subjected to pressure stimulation. In this scenario, the female is relaxed and lies flat on the ground. Weak attempts to extend her hindlimbs can be noticed, but the bodyweight rests entirely on the metatarses. The tail is elevated, although the vulva stays oriented downwards and inaccesible. The incomplete posture is considered as lordosis because non-receptive females arch the body upwards and show signs of muscular tension when subjected to the pressure stimulus. The incomplete lordosis is noticeable by subtly pushing the rump of a receptive female with an open hand. This response was also presented by ovarioectomized females exposed to the beating stimulus. Complete lordosis is characterized by the elevation of the rump due to a partial extension of the hindlegs. The female lifts the tail and exposes the perineal region.

This posture was achieved by receptive females with both stimuli. The most effective level of pressure was 2.0 and 3.0 kg. This effectiveness can be correlated with the average bodyweight of a young semental male. Also, we consider that complete lordosis bears resemblance to the posture that female rats adopt in response to either the male's embrace or the fingering technique (Dewsbury, 1979). Pronounced lordosis was strongly induced by simulating the exploratory beatings of the penis over the perineal region in both conditions. This posture included complete extension of hindlegs with withdrawal of the tail. Moreover, if the beating stimulus is moved around the perineal region, the female rotates the rump accordingly. The female follows the stimulus with wide movements. This lordosis response is maintained as long as the beating continues.

Table 2: Responses of females subjected to beating stimulus

Session	Female	Condition					
		Ovarioectomized			Estrogenized		
		Beatings (3 per second)			Beatings (3 per second)		
		I	II	III	I	II	III
I	1	+	+	+	+++	++	++
	2	++	+	+++	++	++	++
	3	+++	++	+	+++	+++	+++
	4	+++	+++	+++	+++	+++	+++
	5	+++	+++	+++	+++	+++	+++
	6	++	+++	+++	+++	+++	+++
	7	++	++	+	++	++	++
	8	+++	+++	+++	++	++	++
II	1	++	++	++	+++	+++	++
	2	++	+	+	+++	+++	+++
	3	++	++	+++	+++	+++	+++
	4	+++	+++	+++	+++	+++	+++
	5	–	+	+++	+++	+++	+++
	6	+	+++	++	+++	+++	++
	7	+	+	+	++	++	++
	8	+++	++	+++	++	+	++

As determined by the interaction with males, ovarioectomized females do not show a significant receptive behaviour. This is consequence of the depletion of autogenous estrogens. Accordingly, lordosis is not observed in ovarioectomized females exposed to a pressure stimulus, unless estrogens are exogenously suministered. Clearly, a conspicuous aspect of mounting, i.e., pressure upon the rump of females, induces lordosis in estrogen-dependent manner. Nevertheless, the ovarioectomized females adopt the posture of lordosis if a beating stimulus is exerted upon the perineal region. This response is observed independently of the presence of estrogens. These findings indicate that the lordosis response induced by penile exploration does not depend on estrogens.

CONCLUSIONS

Our findings suggest that lordosis is a composite response that depends on more than one nervous mechanism. Stimuli known to induce lordosis in rats are extrapolated to rabbits because the copulatory behaviour of both species resemble each other in essential aspects. As a consequence of this generalization, behavioural traits unique to female rabbits have been ignored. These traits must have biological significance. Succesful copulatory interactions are responsible of ovulation in rabbits. Probably, activating mechanisms of lordosis are superimposed to enhance reproduction.

In rabbit farms, the recognition of receptive females relies largely on behavioural indicators which are subjective, e.g., nervousness and restlessness. A direct way to determine oestrus in females is checking the coloration and moisture of the vulva. However, the female has to be lifted from the ground to perform this inspection. This effort is time-consuming and not suitable for inspecting large numbers of individuals. Alternatively, the lordosis can be detected easily by subtly pushing the rump with an open hand.

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