International Journal of Veterinary Science and Agriculture Research
Volume 3 Issue 4, November-December 2021
ISSN: 2582-4112, Available at www.ijvsar.com

Mating Behavior of White New Zealand Rabbit Does. Effect of an Opioid Antagonist.

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Abstract: *When does are taken to mate some are ready for mounting, while others show lordosis, cornering and circling. Naloxone treated does were notably calm during handling and mating and behavioral scores were lower compared with saline treated does (P=<0.05It It was concluded that the endogenous opioid system in White New Zealand rabbit does, facilitate sexual behavior and reproduction.*

Key Words: Rabbit Does, mating behavior, naloxone

I. INTRODUCTION

Rabbit does suffer a continuous state of stress during mating, kindling, lactation, pseudo pregnancy and management, factors that affect ovulation rate due to physiological dysfunctions that would affect the secretion of endogenous substances related with reproduction [1] (Castellini et al., 2010).

During mating, rabbit does show variable signs of acceptance and refusal when transferred to the male´s cage [2-3] (Moret 1980; Theau-Clement et a*l*., 2012), if the doe shows immediate acceptance to mate, it means that she is highly receptive and it is rapidly mounted, sign that is of upmost importance for rabbit productivity, correlated with the production of more preovulatory follicles and higher concentrations of estradiol [4-5] (Stoufflet and Caillol, 1988; Szendr**ő,** 2008).

Different methods have been used to improve rabbit doe fertility and prolificacy; including changes in photoperiod and the administration of melatonin [6] (Mousa-Balabel, 2011), eCG[3] (Theau-Clement et al., 2008), combined e CG - hCG treatments [7] (Davoust*et al*., 1994), different lighting programs, controlled lactations and feeding programs [3] (Theau-Clement et a*l*.,1998).

Rabbits are inducedovulatory, when mated (including ejaculation) a rapid LH release is induced due to a postcoital release of GnRH from the Medium Basal Hypothalamus (MBH)

Norepinephrine, neuropeptide Y, and Opioid peptides are some of the neurohormones implicated on the neuronal / humoral feedback regulation that modulates GnRH secretion [8] (Baker and Baum, 2000). GnRH neurons in the preoptic area, control the pulsatile secretion of pituitary LH. On a minute to hourly basis, the GnRH pulse generator is extremely sensitive to stress and other external and internal stimuli[9] (Hill et a*l*., 2008).

There is considerable evidence that endogenous opioid peptides modulate GnRH secretion [10] (Yen et al, 1985). Endogenous opiates (EO) and morphine block GnRH secretion and reduces sexual receptivity, after copulation, endogenous endorphins when released, they attach to μ receptors inhibiting LH surges and increasing prolactin levels;events that might compromise fertility and prolificacy in several species [11](Marongiu and Gulinati, 2008). The infusion of naloxone iv 2 weeks after gonadectomy in rabbits induce a dramatic rise in LH pulse amplitude, together with an increase of POA GnRH mRNA levels [8] (Baker and Baum, 2000). Naloxone was used in doses of 1 mg/kg, and sometimes infused iv continually for several hours, this experiments studied LH secretion after naloxone infusions; changes in sexual behavior was not considered.

The administration of small doses of Naloxone, an opioid antagonist, facilitated the expression of sexual behavior, advanced the preovulatory LH surge and induced a decrease of plasma Prolactin in the ewe [12-13] (Fuentes et al., 2001; Fuentes et al., 2007). In male rabbit's small intermittent doses of naloxone (0.5 mg im) facilitated sexual behavior increased testosterone levels and decreased plasma Prolactin concentration [14-15-16] (Pedron et al.,1996, Pedron et al.,1998, Fuentes et al., 2005). In this study it was considered of interest to study mating behavior of White New Zealand rabbit does treated with small doses of naloxone.

II. Materials and methods

Twenty multiparous receptive New Zealand rabbit does from a local breeder, were chosen at random, and allocated to two groups of ten. Caged individually with natural photoperiod (19º N July August 2013) and free access to a standard diet and water ad lib.

Ethical approval

Welfare of both rabbit groups were handled according to the bioethical animal welfare procedures of the animal research facilities at the Centro Universitario de los Altos, Universidad de Guadalajara, Mexico.

Study design

A technician was appointed to inject, clean and feed both groups of rabbit does to diminish handling stress during the duration of the experiment. While handling and feeding both groups of does, an observer noted all behavioral signs during mating.

Rabbit does (n = 10) were medicated with 0.5 mg naloxone im at 12 hr intervals two days before and two days after mating, the control group was sham treated with saline injections.

Experimental rabbit does were housed at least 100 meters apart from controls to avoid bio stimulation.

A score was designed to evaluate sexual behavior during mating, giving values to different signs during mating. Each behavior had a value of 1, and were listed as follows:

Flat back lordosis, stretched lifting hind Qrts, hunkering down, Cornering, Growling, attacking male, Biting, Loud sniffing, Head shake, Circling, Flat back, no Lordosis.

Statistical analyses

Two sample T test and the Analysis of Variance were used to evaluate the resulting data.

III. Results

During mating some does were restless and intended to avoid handling, when introduced to the male´s cage, some immediately showed lordosis and accepted mounting, while others exhibited different behaviors including

cornering and circling. NX treated rabbit does, scored lower behavioralpoints (P=0.05) and they were calm during handling and mating (Table I).

IV. Discussion

The administration of large doses of naloxone as an opioid antagonist was used to study hypothalamic GnRH release and LH blood levels, large acute doses of naloxone are inadequate because they will couple to a wide variety of endogenous opioid receptors, including those related to LH and GnRH release.

Sometimes, large doses of naloxone causeimmediate distress or/and death in both humans and animals [17-18-19-20-21] (Andre, 1980; Ebling and Lincoln, 1985; Smith and Pinnock, 1985; Yang et al.,1988; Nanda *et al*., 1989;). It is interesting to note that research teams using high doses of naloxone (>1 mg/kg acute or continuously per hour) in experimental animals never report distress after the administration of opioid antagonists and in their objectives they did not considered changes in behavior.

When using small doses of naloxone in male rabbits: libido and mounting was increased, decreased prolactin and increased testosterone levels [14-15-16-22] (Villagran *et al*., 2003, Fuentes et al.,2005; Pedron et al., 1996; 1998). Small doses of naloxone have been also used by other research teams in different farm animals [23-24] (Sciorsci et al., 2001; Audi et a*l.*, 2001) reports that support this study.

Before mating rabbit, some does are reactive to handling and take time to adapt to the hutch and to the male [25] (Maray and Rashwan 2003). The latter behavior causes certain degree of stress, releasingendorphins that produce a certain degree of inhibition of LH release, factor that would reduce ovulation rate [26] (Byrmes et al 2000) effect that can account for the lower kints born in control does as compared with naloxone treated does.

Does treated with Naloxone were extremely calm when transferred to the male´s hutch and immediately receptive, they showed and immediate acceptance of the male and mating. In low doses naloxone inhibits the action of endorphins at the μ receptor level [27] (Sobor et al., 2011) making them oblivious to handling and accepting the male approach and mating.

Opioid receptors exist in two forms, one without the cations Ca^{2+} and Na^{+} with high affinity for agonists and the other form, which is more rich in Ca^{2+} and Na^{++} with a high affinity for antagonists [22-28] (Sciorci et al 2000, 2001). β endorphin inhibits calcium passage by influencing cytosolic Ca^{2+} in critical waves, where the frequency of Ca^{2+} oscillations rises with the degree of stimulations [29] (Goldbeter 2002). Naloxone when present in low doses, controls calcium turnover via down-regulation/desensitization of opioid receptors [30-31] (Minoia and Sciorsci 2001, Wang and Burns, 2009). The latter leads to postulate that small doses of naloxone displace β endorphins from their receptor site for a long period of time, and that Ca^{2+} oscillations at the μ receptor take time to return to normal levels, facilitating changes in sexual behavior.

In this work we submit a method for scoring mating behavior in rabbit does, and the use of an opioid antagonist, such as naloxone, facilitatingmating. Findings that would be useful to improve reproductive performance of rabbit does when mated or using artificial insemination [5] (Szendro 2008).

V. Conclusion

It was concluded that the endogenous opioid system in rabbits plays an important role in sexual behavior and reproduction in rabbit does.

Conflict of interest

None of the authors has any financial or personal relationships that could inappropriately influence or bias the content of the paper.

VI. Acknowledgements

Thanks are given to the staff of the Centro Universitario de los Altos for all the support given

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Table I

The effect of small doses of naloxone (0.5 mg/12hrs 2 days before and 2 days after mating) on receptivity of rabbit does during mating

Doe #	1	2	3	4	5	6	7	8	9	10
Lordosis &mating	√	√	√	√	√	√	√	√	√	√
Stretching&mating	√			√				√		
Hunkering										
Cornering					√				√	
Growlig										
Attaking back										
Biting										
Sniffing										
Head Shake										
Circling			√					√		
Flat										
Total score	2	1	3	2	2	1	1	3	2	1
Conception	+	+	+	+	+	+	+	+	+	+

Table II The effect of saline solution injections on receptive rabbit does during mating

Doe #	1	2	3	4	5	6	7	8	9	10
Lordosis &mating	√	√	√	√	√	√	√	√	√	√
Stretching&mating	√	√	√	√			√		√	
Hunkering	√				√					
Cornering	√	√		√		√		√	√	
Growlig										√
Attaking back							√			
Biting										
Sniffing			√							
Head Shake										√
Circling		√		√		√			√	
Flat										√
Total score	4	4	3	4	2	3	3	2	4	4
Conception	+	+	+	+	+	+	+	+	+	+

Table III. Acummulated results of mating Behavior

	Groups	MEAN	VARIANCE	SD	n	t	Critical value	P
Behavior at mating	NX Treated control	1.8 3.3	0,6222 0.6778	0.7888 0.8233	10 10	-4.60	>2.01	0.05