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Secretaría de Desarrollo Agropecuario del Gobierno del Estado de México, Secretaría de Agricultura, Ganadería, Desarrollo Rural,
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**EFFECT OF DIFFERENT GnRH ANALOGUE TREATMENTS ON THE
PERFORMANCE OF LACTATING RABBITS**

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ABSTRACT

Reproductive traits of 210 rabbits inseminated on postpartum day 11 and induced to ovulate by i.m. 0.84 µg or 1.26 µg buserelin acetate (Receptal[®] or Suprefact[®]) or 20 µg gonadorelin (Fertagyl[®]) or by i.vag. 25 µg GnRH analogue [des-Gly10, D-Ala6]-LHRH ethylamide in 0.5 mL semen extender (MRAbit[®]) were studied according to reproductive status under farm practice (only light stimulation). The data were evaluated by the chi-squared test or by ANOVA using the Statgraphics 6.0 (1992) statistical software. Pregnancy and kindling rates and the number of live born kits per litter were not affected by the GnRH treatments but differed ($P < 0.05$) with parity and receptivity (94%, 89%, 11.7 in multiparous receptive vs. 77%, 69%, 9.42 in primiparous non-receptive, or 10.2 kits in multiparous non-receptive does, respectively). Global productivity (number of live born kits per 100 AI) with Receptal[®] in primiparous receptive or non-receptive or in multiparous receptive or non-receptive does were 930, 450, 1020, 787, with Suprefact[®] 1064, 670, 1209, 895, with Fertagyl[®] 489, 763, 1003, 832 and with MRAbit[®] 715, 600, 1010, 850, respectively. With the studied i.m. and i.vag. GnRH analogue treatments, the lactating does had good and similar performance under the farm practice of light stimulation with no eCG use before insemination. Reproduction was influenced by doe physiological status. Multiparous receptive does had superior productivity.

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Key words: intravaginal ovulation induction, buserelin, gonadorelin, LHRH ethylamide, prolificacy



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Introduction

With appearance of GnRH synthetic analogue products of various agents and efficacies a novel way of ovulation induction in rabbits is possible (Dal Bosco et al., 2011). Intramuscular (i.m.) or subcutaneous (s.c.) injection of GnRH analogue at insemination (AI) can be substituted by intravaginal (i.vag.) absorption by supplementing the semen extender with GnRH analogue. The method improves the welfare at AI (no injection) and, faster or more rabbits can be inseminated (Viudes-de-Castro et al., 2007). The disadvantage is that a higher i.vag. dose is needed to get efficacy similar to that of the i.m. usage (Viudes-de-Castro et al., 2014).

The success of i.vag. GnRH analogue treatment for ovulation induction can be affected by several factors. Besides agent and its concentration, the composition of semen extender and doe physiological status are important. The i.vag. application was studied only with receptive does (Viudes-de-Castro et al., 2007; Vicente et al., 2008, 2011) or rabbits pre-injected with eCG hormone (Quintela et al., 2008, 2012; Zhang and Qin, 2012). To our knowledge, in Hungary the i.vag. ovulation induction by GnRH analogue of rabbits has not yet been tried and its reproductive results reported.

Our aim was to compare different, intramuscular or intravaginal GnRH analogue treatments and to investigate their effects on reproduction under the nursing and lighting programs of farm practice.

Materials and Methods

The experiment was conducted at the rabbit farm of S&K-Lap Ltd. in Galgamácsa between 21 February and 24 March, 2014. In the heated (18-20°C) building with windows, the rabbits were housed in wire-net breeding cages (80 x 53 cm with 90 cm height) equipped with a plastic mat, an elevated platform (40 x 53 cm) at 25 cm height, a gnawing stick and an outer nest (23 x 53 cm) with metal sheet walls.

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Lactating Hycole PS rabbit does (n=210) were distributed into four groups with respect to parity and number of reared kits (primiparous (n=91): 8.02±0.08 kits per litter or two- to three times kindled (n=119): 9.77±0.07 kits per litter). Heterospermic pooled semen from Hycole bucks and four different GnRH analogue treatments were used (Table 1). The control (i.m. 0.84 µg buserelin acetate) corresponded with today's routine practice (Zapletal et al., 2008).

Group:	1. (Control)	2.	3.	4.
Treatment*:	i.m.	i.m.	i.m.	i.vag.
Dose:	0.2 mL=0.84 µg	1.0 mL=1.26 µg	0.2 mL=20 µg	0.5 mL=25 µg
GnRH analogue:	buserelin acetate Receptal A.U.V. [®]	buserelin acetate Suprefact [®]	gonadorelin Fertagyl A.U.V. [®]	LHRH ethylamide in semen extender
Semen extender:	Merck III [®]	Merck III [®]	Merck III [®]	MRabit [®]
Semen dose:	0.5 mL/doe	0.5 mL/doe	0.5 mL/doe	0.5 mL/doe
Sperm per doe	30 million	30 million	30 million	30 million

* Injected into muscle (i.m.: *intramuscular*) or with semen extender in the vagina (i.vag.: *intravaginal*)

Table 1: Different GnRH analogue treatments in lactating rabbits at AI on postpartum day 11

We also planned an i.vag. treatment with Suprefact[®] but when adding it to Merck III semen extender we observed the destruction of sperm, so this intravaginal GnRH treatment failed. Quintela et al. (2004) reported a successful i.vag. use of Suprefact but they used MA 24 semen extender.

Controlled nursing was applied by opening the metal-sheet nest door between 9:00 to 10:00 h from postpartum day 1 to 14 and free nursing thereafter. AI was done on postpartum day 11 three hours after nursing. At AI does with red / violet and turgid vulvas were judged to be receptive.

There was no hormonal pre-injection to synchronize estrus but on day 8 before AI, the daily lighting of 9 h (light) L/15 h (dark) D (8 a.m. to 5 p.m.) was abruptly increased by 7 hours, to 16L/8D (6 a.m. to 10 p.m.). The lighting was reduced by 2 hours on days 3 and 4 after AI



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(14L/10D, 6 a.m. to 8 p.m. and 12L/12D, 8 a.m. to 8 p.m.) and by 3 hours on day 5 after AI (9L/15D, 8 a.m. to 5 p.m.) returning to the 9 h daily lighting.

Rabbit does were fed *ad libitum*, with a single diet (10.0 MJ/kg DE, 17.5% CP, 3.80% EE, 14.9% CF, 7.70% ash).

The effects of different GnRH analogue treatments and reproductive status on pregnancy and kindling rates were evaluated by the chi-squared test and on number of kits born by ANOVA using the Statgraphics 6.0 (1992) statistical software.

Results and Discussion

At AI, 61% of the lactating does had red or violet, and 73% turgid vulva. The receptivity rate was 57%. However, the pregnancy rate was very good, 89% and, 81% of the rabbits kindled (Table 2). The check of receptivity is more precise with the male (lordosis position of the doe) but this cannot be used in big farms. In ovulation the neural and genital-somato-sensory stimuli associated with AI (catching, catheter intromission, etc.) can also be important (Rebollar, 2011; Rebollar et al., 2012), explaining the contradiction between the lower receptivity and good pregnancy.

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The ovulation induction method did not significantly affect the pregnancy (84-93%) and kindling rates (76-87%) or the number of live and total born kits (10.1-11.0 and 10.5-11.1; Table 2).



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Group	No. of does	Pregnancy rate, % (no.)	Kindling rate, % (no.)	Litter size	
				Live born	Total born
Ovulation induction					
i.m. 0.84 µg buserelin acetate (Receptal A.U.V. [®])	50	88 (44/50)	82 (41/50)	10.8±0.5	11.0±0.5
i.m. 1.26 µg buserelin acetate (Suprefact [®])	55	93 (51/55)	87 (48/55)	11.0±0.4	11.1±0.4
i.m. 20 µg gonadorelin (Fertagyl A.U.V. [®])	50	92 (46/50)	76 (38/50)	10.8±0.5	11.0±0.5
i.vag. 25 µg LHRH ethylamide in semen extender (MRabit [®])	55	84 (46/55)	80 (44/55)	10.1±0.4	10.5±0.4
Reproductive status					
Primiparous lactating receptive	56	91 ^{ab} (51/56)	79 ^{ab} (44/56)	10.6±0.4 ^{ab}	10.7±0.4 ^a
Primiparous lactating non-receptive	35	77 ^a (27/35)	69 ^a (24/35)	9.42±0.6 ^a	9.62±0.6 ^a
Multiparous lactating receptive	64	94 ^b (60/64)	89 ^b (57/64)	11.7±0.4 ^b	12.0±0.4 ^b
Multiparous lactating non-receptive	55	89 ^{ab} (49/55)	84 ^{ab} (46/55)	10.2±0.4 ^a	10.5±0.4 ^a
Total	210	89 (187/210)	81 (171/210)	10.5±0.2	10.7±0.2

Values in the same column with unlike superscripts (a, b) differ (P<0.05)

Table 2: Reproductive traits of rabbits inseminated and induced to ovulate by i.m. buserelin acetate or i.m. gonadorelin or i.vag. GnRH analogue [des-Gly10, D-Ala6]-LHRH ethylamide semen extender

The performance of lactating does was influenced by reproductive status (Table 2, Figure 1)

The multiparous receptive rabbits had 17% better pregnancy rate (94 vs 77%; P<0.05) and among them 20% more kindled (89 vs 69%; P<0.05) than from the primiparous non-receptive does. They also spawned 2.3 or 1.5-kit larger litters than the primiparous non-receptive or the multiparous non-receptive rabbits (11.7 vs 9.42 or 10.2; P<0.05). For the farmers global productivity (number of live born kits per 100 AI) is a very important trait that is determined by kindling rate and born alive.

The productivity of multiparous receptive rabbits was by 64% better than the primiparous non-receptive does' (1051 vs 643 rabbits; Figure 1).

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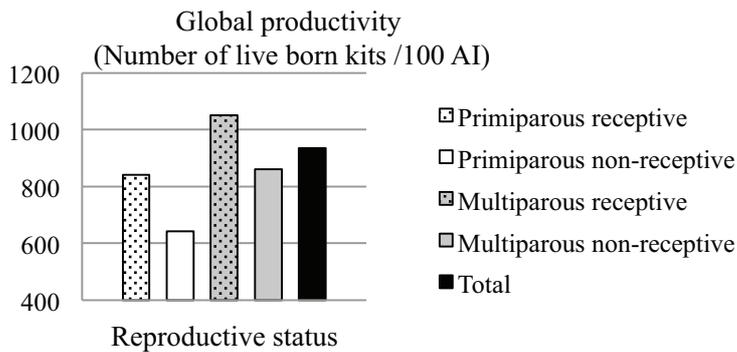
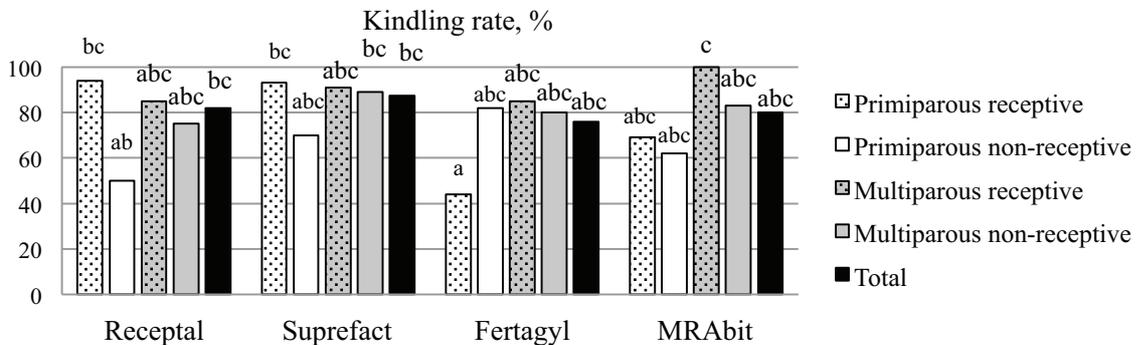


Figure 1: Global productivity according to the reproductive status of lactating does

At AI, there are does in different physiological status. It raises the question whether there are differences in the effects of various GnRH analogue treatments if the above-mentioned influence of physiological status is considered (Figures 2-4).



Values within treatments with unlike superscripts (a, b, c) differ ($P < 0.05$)

Figure 2: Kindling rate (%) depending on GnRH analogue used and lactating does' reproductive status

The kindling rate of multiparous receptive i.vag. LHRH ethylamide (MRabit) treated does was higher than the primiparous non-receptive i.m. 0.84 μ g buserelin acetate (Receptal) treated or primiparous receptive i.m. 20 μ g gonadorelin (Fertagyl) treated rabbits (100 vs 50 or 44%; $P < 0.05$; Figure 2). Of note, the latter had a good, 89% pregnancy rate based on abdominal

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palpation but only half of them delivered. Because of the small number of does these findings should be taken with caution. However, our results verify the producer’s recommendation that with the GnRH analogue containing MRAbit semen extender, only receptive rabbits should be inseminated.

The number of live born kits within the groups was not significantly affected by the physiological status. That is explained by the different result found with the i.vag. (MRAbit) compared with the i.m. treatments (Figure 3).

In the i.m. GnRH analogue treatments (Receptal, Suprefact, Fertagyl) the number of live born kits per litter of primiparous rabbits was lower than that of the multiparous does and, the receptive does produced more live kits. Contrary to this, with the i.vag. treatment (MRAbit) good litter size was found independent of doe physiological status (Figure 3). The advantage of this can be that fewer rabbits need to be fostered to alien does.

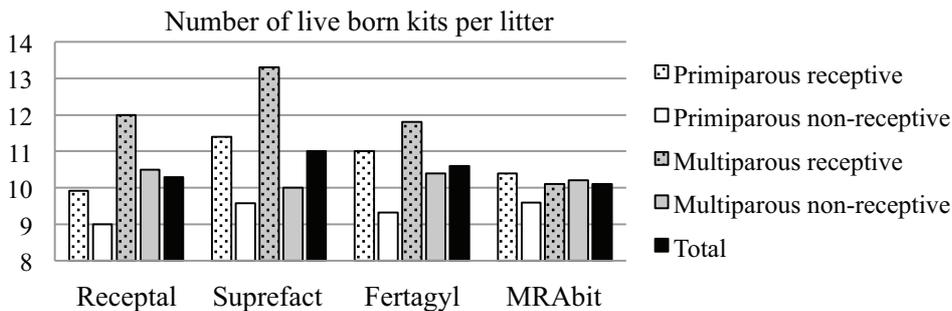


Figure 3: No. of live born kits per litter depending on GnRH analogue used and doe reproductive status

Within the groups we calculated the global productivity according to the physiological status (Figure 4). Based on this the i.m. 1.26 µg buserelin acetate (Suprefact) treatment was the most

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effective, mainly in the receptive does (primiparous: 1064 rabbits, multiparous: 1209 rabbits). Global productivity was similar by lower dose of i.m. 0.84 µg buserelin acetate (Receptal) or by i.m. 20 µg gonadorelin (Fertagyl) or by i.vag. 25 µg LHRH ethylamide (MRAbit). In productivity the superiority of receptive rabbits versus non-receptive does is evident.

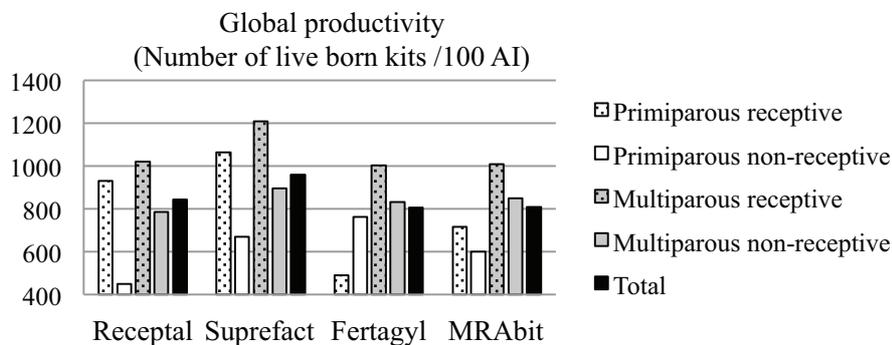


Figure 4: Global productivity according to GnRH analogue used and reproductive status

Perrier et al. (2000) applied i.m. 0.4 or 0.8 µg buserelin (Receptal). Reproduction did not differ with the GnRH concentration but varied significantly with the physiological status. Theau-Clément et al. (1990) used i.m. 0.8 µg buserelin (Receptal) or 20 µg gonadorelin (Fertagyl) and reported similar numbers of live born kits per litter but the litter size was higher in receptive than in non-receptive does. Quintela et al. (2001) treated multiparous lactating does with i.m. 20 µg gonadorelin (Fertagyl) and noted 78-80% kindling rate and 10.6-10.9 kits per litter depending on the lighting regime. In their other studies (Quintela et al., 2008, 2009) the i.m. 20 µg gonadorelin (Inducel) treatment or the i.vag. 25 µg LHRH ethylamide used via semen extender resulted in similar 86 or 91% kindling rates, 10.3 or 10.8 live born kits per litter and productivity (949 or 1029 rabbits) in pre-injected rabbits. Quintela et al. (2012) using 0.5 mL/doe MRAbit semen extender or i.m. lecirelin (0.2 mL Dalmarelin) obtained identical productivity. Our results are in agreement with those reports.

Conclusions

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Under the farm practice with the studied i.m. and i.vag. GnRH analogue treatments and without pre-injection the reproductive performance of lactating does was similar and good. The reproductive traits were affected by doe physiological status. The productivity of multiparous receptive rabbits is over average. For the producers, the use of the i.vag. GnRH analogue treatment can be recommended in receptive rabbits.

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References

- Dal Bosco A., Rebollar P.G., Boiti C., Zerani M., Castellini C., 2011. Ovulation induction in rabbit does: Current knowledge and perspectives. *Anim. Reprod. Sci.*, 129, 106-117.
- Perrier G., Theau-Clément M., Jouanno M., Drouet J.P., 2000. Reduction of the GnRH dose and inseminated rabbit doe reproductive performance. *In: Proc. 7th World Rabbit Congr, Valencia, Spain, pp. 225-230.*
- Rebollar P.G., 2011. Factors affecting efficacy of intravaginal administration of GnRH analogues for ovulation induction in rabbit does. *In: Proc. 4th Italian Rabbit Days, Forli, Italy, pp. 35-45.*
- Rebollar P.G., Dal Bosco A., Millán P., Cardinali R., Brecchia G., Sylla L., Lorenzo P.L., Castellini C., 2012. Ovulating induction methods in rabbit does: The pituitary and ovarian responses. *Theriogenology*, 77, 292-298.
- Quintela L.A., Barrio M., Prieto C., Gullón J., Vega M.D., Sánchez M., García N., Becerra J.J., Peña A.I., Herradón P.G., Hernandez-Gil. R., 2012. Field test of the effectiveness of rabbit semen extender MRA-bit® incorporating GnRH analogue. *In: Proc. 10th World Rabbit Congr, Sharm El-Sheikh, Egypt, pp. 431-433.*
- Quintela L., Peña A., Barrio M., Vega M.D., Díaz R., Maseda F., Garcia P., 2001. Reproductive performance of multiparous rabbit lactating does: effect of lighting programs and PMSG use. *Reprod. Nutr. Dev.*, 41, 247-257.
- Quintela L.A., Peña A.I., Vega M.D., Gullón J., Prieto M.C., Barrio M., Becerra J.J., Maseda F., Herradón P.G., 2004. Ovulation induction in rabbit does submitted to artificial insemination by adding buserelin to the seminal dose. *Reprod. Nutr. Dev.*, 44, 79-88.
- Quintela L.A., Peña A.I., Vega M.D., Gullón J., Prieto C., Barrio M., Becerra J.J., Herradón P.G., 2008. Ovulation induction in rabbit does by intravaginal administration of the GnRH analogue [des-Gly10, D-Ala6]-LHRH ethylamide: field trial. *In: Proc. 9th World Rabbit Congr, Verona, Italy, pp. 427-430.*
- Quintela L.A., Peña A.I., Vega M.D., Gullón J., Prieto C., Barrio M., Becerra J.J., Herradón P.G., 2009. Reproductive performance of rabbit does artificially inseminated via intravaginal administration of [des-Gly10, D-Ala6]-LHRH ethylamide as ovulation inductor. *Reprod. Dom. Anim.*, 44, 829-833.
- Statgraphics ® 1992. Reference Manual, Version 6.0, Manugistics Inc., Rockville, MD, USA
- Theau-Clément M., Bolet G., Roustan A., Mercier P., 1990. Comparaison de differents modes d'induction de l'ovulation chez les lapines multipares en relation avec leur stade physiologique et la receptivite au moment de la mise a la reproduction. Mem. *In: Proc. 5emes J. Rech. Cunic Paris, France 1 (Communication 6).*



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Vicente J.S., Lavara R., Lavara F., Marco-Jiménez F., Viudes-de-Castro M.P., 2008. Rabbit reproductive performance after insemination with buserelin acetate extender. *Livest. Sci.*, 115, 153-157.

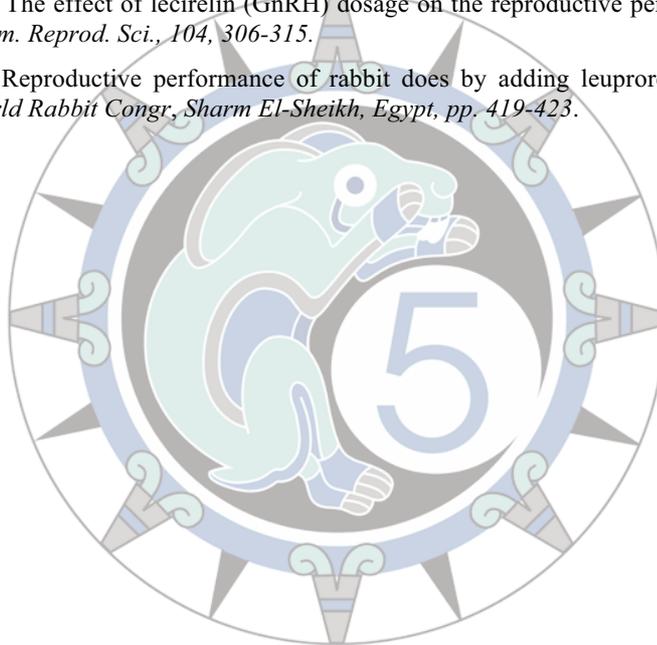
Vicente J.S., Lavara R., Marco-Jiménez F., Viudes-de-Castro M.P., 2011. Detrimental effect on availability of buserelin acetate administered in seminal doses in rabbits. *Theriogenology*, 76, 1120-1125.

Viudes-de-Castro M.P., Lavara R., Marco-Jiménez F., Cortell C., Vicente J.S., 2007. Ovulation induced by mucosa vaginal absorption of buserelin and triptorelin in rabbit. *Theriogenology*, 68, 1031-1036.

Viudes-de-Castro M.P., Mocé E., Lavara R., Marco-Jiménez F., Vicente J.S., 2014. Aminopeptidase activity in seminal plasma and effect of dilution rate on rabbit reproductive performance after insemination with an extender supplemented with buserelin acetate. *Theriogenology*, 81, 1223-1228.

Zapletal D., Pavlik A., 2008. The effect of leirelin (GnRH) dosage on the reproductive performance of nulliparous and lactating rabbit does. *Anim. Reprod. Sci.*, 104, 306-315.

Zhang Y.Q., Qin Y., 2012. Reproductive performance of rabbit does by adding leuprorelin in semen to induce ovulation. In: *Proc. 10th World Rabbit Congr, Sharm El-Sheikh, Egypt*, pp. 419-423.



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