Effect of buserelin acetate and gonadorelin treatments on reproductive performance in Croatian Big Silver Marten rabbits after natural mating

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Abstract

The goal of this study was to determine the effects of GnRH analogue treatments on reproductive performance of naturally mated Croatian Big Silver Marten (CBSM), an indigenous rabbit breed. A total of six fertile bucks and 36 sexually mature CBSM rabbit does (8-10 months of age) were divided into three groups for this study. Two groups were treated after natural mating: the first group (*n*=12) received 0.25 mL Receptal® (RPL) intramuscularly, containing 0.84 µg buserelin acetate, while the second group (*n*=12) received 0.25 mL Fertagyl® (FGL) containing 20 µg gonadorelin. The third group (*n*=12) was comprised of untreated does as the control (CON) group. Each group of does mated with a male from another group after the next lactation. After 35 days of lactation, kits were weaned. All females (*n*=36) had three litters with an average litter size of 8.01 newborns (dead or alive), or 865 in total following 108 successful matings. Total neonatal losses until weaning at 35 days of age were 30.52% (*n*=263): stillbirth 9.71% (*n*=84), 8.79% died within first 7 days of life (*n*=76), 6.24% died from 8 to 21 days (*n*=54), and 5.78% died from 22 days until weaning at 35 days (*n*=50). Nulliparous does had a lower litter size than primiparous and secundiparous does (7.78 *vs*. 8.06 and 8.19, respectively). Reproductive performance, including kindling rate and litter size at birth and weaning (at 35 days of age), was significantly better in the RPL and FGL groups than in the CON group.

***Key words**: Buserelin; Gonadorelin; Preweaning mortality; Rabbit; Reproduction*

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Introduction

The reproduction of rabbit does is regulated by a neurohormonal system, in which the hypothalamus and pituitary gland play a leading role (Heba-T-Allah et al., 2016). Gonadotrophin releasing hormone (GnRH), produced by the hypothalamus, stimulates the anterior part of the pituitary gland to synthesise and release follicle stimulating hormone (FSH) and luteinizing hormone (LH) that target the ovaries (Wei et al., 2012). The hormone responsible for follicular growth is FSH, while LH controls the final follicular maturation and induction of ovulation (Heba-T-Allah et al., 2016). In the rabbit doe, ovulation does not occur spontaneously, but is induced by a neurohormonal reflex initiated during mating (Mobarak et al., 2015). When using artificial insemination (AI) in the absence of a male, ovulation has to be induced by artificial hormonal stimulation (Kalaba and Abdel-Khalek, 2011). The appearance of GnRH synthetic analogue products of various agents and efficiencies has given a novel means of inducing ovulation in rabbits (Dal Bosco et al., 2011). The ovulation inducing method most frequently used is intramuscular application of GnRH (Quintela et al., 2004; Rebollar et al., 2012; Eiben et al., 2014). The success of the AI technique in rabbits depends mainly on parity number, physiological status and sexual receptivity at the moment of insemination (Castellini, 1996). Intramuscular or subcutaneous injections of a GnRH analogue at insemination could be substituted by intravaginal absorption by supplementing the semen extender with a GnRH analogue, though at higher doses (Quintella et al., 2004; Viudes-de-Castro et al., 2007).

The Croatian Big Silver Marten (BSM) is relatively rare and is the only Croatian indigenous rabbit breed (Posavi et al., 2004). In 1982, rabbit breeders in the town of Bjelovar, Croatia obtained certain combinations by mating Great Chinchilla rabbits and received an extremely beautiful mutation (Kapitan, 2006). Under professional supervision and standardization, this rabbit breed has become very popular and attractive to many breeders in Croatia and abroad. Twenty-five years ago, it was a giant breed, with an average weight over 6 kg (Đuričić et al., 2011). Today BSM belongs, by European standards, to the medium breeds (body weight 3.6-5.5 kg). The Croatian BSM rabbit has a large, muscular and harmonious body of cylindrical shape, meaning that it is approximately equal in width and height. It has strong, solid and straight legs, a very developed head with a broad forehead and full cheeks. The ears are fleshy and hairy, and 13-14.5 cm in length. The Big Silver Marten rabbit breed has three colour variations: Black, Blue and Chocolate (or Havana) with white and/or tan markings, plus white ticking on the lower body. The Black Big Silver Marten rabbit is the most popular variety of this breed. They have white tags around the nares, eyes, cheeks and edges of the ears. The forelegs, inner side of the hindquarters, lower part of the tail and belly are also white. Eye colour is dark brown (Black and Havana) or dark blue (Blue). The lower coat colour is dark blue (Kapitan, 2006).

The goal of this study was to determine the effects of two GnRH analogues (buserelin acetate and gonadorelin) treatments on reproductive performance of the indigenous rabbit breed, Croatian Big Silver Marten, under natural mating conditions.

Materials and methods

All animals were handled in strict accordance with good animal practices as defined by the relevant national and/or

Table 1. Mating schedule of 36 female Croatian Big Silver Marten (CBSM) rabbit does arranged in 3 groups: Receptal group (RPL) (1-12), Fertagyl group (FGL) (13-24) and control group (CON) (25-36) with 6 CBSM males (A-F)

females		nulliparous		primiparous		secundiparous	
group	doe	male	*repeated	male	*repeated	male	*repeated
RPL	1	A	2	C		E	
	2	B		D	1	F	
	3	A		C		E	1
	4	B	1	D		F	
	5	A		C	2	E	
	6	B		D		F	
	7	A		C		E	
	8	B	3	D		F	
	9	A		C	1	E	
	10	B		D		F	
	11	A		C		E	1
	12	B		D		F	
FGL	13	C		E		A	1
	14	D	2	F		B	
	15	C		E	1	A	
	16	D	1	F		B	
	17	C		E		A	
	18	D		F		B	2
	19	C		E	3	A	
	20	D		F		B	
	21	C		E		A	1
	22	D		F		B	
	23	C	1	E		A	
	24	D		F		B	
CON	25	E		A	1	C	
	26	F	1	B		D	1
	27	E		A		C	
	28	F	2	B		D	
	29	E		A	2	C	1
	30	F		B		D	
	31	E	2	A		C	
	32	F		B	1	D	
	33	E		A		C	1
	34	F	3	B		D	
	35	E		A	1	C	
	36	F	1	B		D	2

*number of repeated matings every next day until copulation (with the same male)

local animal welfare bodies. The research protocol and animal management were in accordance with Directive 2010/63/EU (European Union 2010) on the protection of animals used for scientific purposes. A total of 36 sexually mature Croatian Big Silver Marten rabbit does (8–10 months of age, 3.6–5.1 kg at the beginning of the study) and 6 fertile rabbit bucks (10–12 months of age, 4–5.5 kg) were used in this study. Each breeding animal was identified by its ear-tattoo. All does were kept under similar management and husbandry systems, separated in boxes with combined floors (fully and partially wired), feeders and nipples for automatic drinking water and equipped with an outer nest (53 x 30 x 30 cm) with metal sheet walls. Controlled light-dark cycles (16 h light: 8 h dark) were applied during the study. Rabbits were fed a commercial balanced diet (14% crude protein and 16% crude fibre) with 350 g meadow hay per rabbit per day. Does were divided into three groups. Two groups were treated after natural mating: the first group (n=12) received 0.25 mL Receptal® (RPL) (MSD, Animal Health, New Zealand) intramuscularly, containing 0.84 μg buserelin acetate, while the second group (n=12) received 0.25 mL Fertagyl® (FGL) (Merck, Animal Health, USA) containing 20 μg gonadorelin. The third group (n=12) was comprised of untreated does as the control (CON) group. Each doe was mated with another male (A-B, C-D or E-F) after the next lactation when estimated to be receptive (vulva was turgid and red or violet with positive copulatory reflex) (Table 1). Artificial insemination (AI) is commonly used in rabbit husbandry to improve breeding management and avoid contact between animals. For AI, mixed ejaculates from several bucks are typically used to create a heterospermic pool, then routinely diluted in a commercial insemination diluent (Vasicek et al., 2014). Due to the relatively low number of CBSM rabbits and to avoid genetic mixture by heterospermic pools, this study used natural breeding.

Pregnancy was controlled 12–14 days by palpation after mating. Non-pregnant does were mated again. After 35 days of lactation, kits were weaned and the procedure was repeated. When does were estimated to be receptive, they mated with another male, and again after the third lactation. After parturition, kindling rate and litter size were recorded at birth (live and dead) and weaning at 35 days of age.

All data were statistically analysed using StatSoft, Statistica 9 and Tukey test of post-hoc analysis. Statistically significant results were considered at $P < 0.05$.

Results

All females (n=36) from each group had three litters (nulliparous, primiparous and secundiparous) with an average litter size of 8.01 newborns (dead or alive), for a total of 865 offspring in 108 successful matings. Total neonatal losses until weaning at 35 days (Table 2) were 30.40% (n=263): stillbirth 9.71% (n=84), 8.79% died within first 7 days of life (n=76), 6.24% died from day 8 to 21 days (n=54), and 5.78% died from 22 days until weaning at 35 day of life (n=50). Nulliparous rabbit does had a lower litter size in comparison to primiparous and secundiparous (7.78 *vs.* 8.06 and 8.19, respectively). A total of 22.22% non-receptive does from the RPL and the FGL groups and 36.01% from the CON group repeatedly mated with same buck every day until copulated (Table 3).

Discussion

In accordance with the literature (Singh et al., 2004; Xiccato et al., 2004; Rebollar et al., 2009; Tuma et al., 2010), the reproductive performance of does

Table 2. Number of newborns (live or dead), stillbirth, neonatal losses (until 7 days, 8-21 and 22-35 days of life) in three groups of Croatian Big Silver Marten rabbit does treated with Receptal (RPL), treated with Fertagyl (FGL) and non-treated or control group (CON).

		RPL		FGL		CON	
		n	%	*n*	%	*n*	%
	nulliparous:						
	live newborns	64	66.67	61	64.21	59	66.29
	stillbirth	11	11.46	8	8.42	8	8.99
	died in first 7 days	11	11.46	9	9.47	12	13.48
	died from 8 to 21 days	6	6.25	10	10.53	7	7.87
	died from 22 to 35 days	4	4.16	7	7.37	3	3.37
	neonatal losses	32	33.33	34	35.79	30	33.71
	primiparous:						
	live newborns	70	71.43	72	72.73	65	69.89
	stillbirth	11	11.22	9	9.09	11	11.83
	died in first 7 days	6	6.12	6	6.06	8	8.60
	died from 8 to 21 days	5	5.10	5	5.05	4	4.30
	died from 22 to 35 days	6	6.12	7	7.07	5	5.38
	neonatal losses	28	28.57	27	27.27	28	30.11
	secundiparous:						
	live newborns	73	73.00	70	70.71	67	69.79
	stillbirth	8	8.00	10	10.10	8	8.33
	died in first 7 days	8	8.00	8	8.08	8	8.33
	died from 8 to 21 days	6	6.00	5	5.05	6	6.25
	died from 22 to 35 days	5	5.00	6	6.06	7	7.29
	neonatal losses	27	27.00	29	29.29	29	30.21
*total live newborns		207[a]	70.41	203[a]	69.28	191[b]	68.71
total neonatal losses until 35 days		87	29.59	90	30.72	87	31.29
*total newborns (dead or alive)		294[a]		293[a]		278[b]	

*Values with different superscripts in the same column differ significantly ($P < 0.05$)

Table 3. Number and percentage of non-receptive Croatian Big Silver Marten does and repeated natural matings according groups treated with Receptal (RPL), treated with Fertagyl (FGL) and nontreated control group (CON) and according parity

parity		RPL	FGL	CON
1. nulliparous	does	3	3	5
	%	25.00	25.00	41.67
2. primiparous	does	3	2	4
	%	25.00	16.67	33.33
3. secundiparous	does	2	3	4
	%	16.67	25.00	33.33
total (non-receptive)	does	8	8	13
*repeated matings	%	22.22[a]	22.22[a]	36.11[b]

*Values with different superscripts in the same column differ significantly ($P < 0.05$)

is affected by parity order (Zapletal and Pavlik, 2008). In the current study, there were fewer live newborns in nulliparous than in primiparous (-23) or secundiparous does (-26). Contrary to our study, a lower kindling rate was reported in primiparous does than in nulliparous and multiparous does, which could be explained by the negative energy balance of simultaneously lactating and pregnant primiparous does (Xiccato et al., 2004). Đuričić et al. (2011) concluded that parity does not significantly influence litter size in Croatian Big Silver Marten rabbits.

Preweaning mortality of rabbits depends on the health status of breeding, the breed, litter size, body condition of the doe, duration of lactation, age of kits at weaning, ruling of females, hygienic conditions and microclimate factors in rabittaries (Feki et al., 1996; Depres et al., 1997; Castellini et al., 2003; Chineke, 2006).

According to Koehl (1995), the average litter size in the temperate climate zone for medium-large rabbit breeds is 9.5 live or dead kits per female (per litter), which is 1.31 more newborns than in our study. Although CBSM is a rabbit breed that originated from a gene mutation of the Great Chinchilla breed, their reproductive data were different. Namely, Bolet et al. (2004) recorded 74.6% weaned kits of Great Chinchillas (n=97) or 5% more than in our study, while Đuričić et al. (2011), recorded 64.75% weaned kits in CBSM (n=52), which is less than in the current study. The average litter size (live newborns) was 5.73 in Great Chinchilla (Bolet et al., 2004), which is similar to our result of 5.56 live newborns in CBSM. It is possible that the preweaning mortality of CBSM rabbits in this study was higher than in other commercial breeds of rabbits because as this is a new rabbit breed without established properties, in addition to the fact that each breed was initially created by inbreeding. The fertility rate in rabbits is usually high, exceeding 90 to 95% (Peiró et al., 2014), while in CBSM it was 91.23% (Đuričić et al., 2011) under natural mating conditions. Recently, the induction of ovulation in rabbit does induced by GnRH or buserelin showed similar results to those obtained by natural mating (Rebollar et al., 2012; Gogol, 2016). The effect of GnRH in rabbit does injected with an GnRH analogue (RPL) at a level of 0.25 mL/doe at mating on day 10 post-mating resulted in improvement of reproductive performance, in terms of a higher kindling rate, litter size and kit viability rate at birth and weaning (El-Ratel et al., 2017). In the present study, it can be concluded that reproductive performance including kindling rate and litter size at birth and weaning (at 35 days of age) were significantly better in the RPL and the FGL groups than in the CON group.

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Učinak aplikacije buserelin acetata i gonadorelina na reprodukcijske pokazatelje u hrvatskog bijelog bijeloopaljenog kunića nakon prirodnog parenja

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Cilj ovog istraživanja bio je ustvrditi učinke davanja GnRH analoga na reprodukcijske pokazatelje u prirodno parenog hrvatskog velikog bijelopaljenog (HVBO) kunića, izvorne pasmine. Za ovo istraživanje ukupno je šest plodnih mužjaka i 36 spolno zrelih HVBO ženki kunića (u dobi od 8-10 mjeseci) bilo podijeljeno u tri skupine. Dvije su skupine bile tretirane nakon prirodnog parenja pa je prva skupina (*n*=12) primila intramuskularno 0,25 mL Receptala® (RPL) koji je sadržavao 0,84 µg buserelin acetata, dok je druga skupina (*n*=12) primila 0,25 mL Fertagyla® (FGL) koji je sadržavao 20 µg gonadorelina. Treća se skupina (*n*=12) sastojala od netretiranih ženki, i poslužila je kao kontrolna (KON) skupina. Svaka je skupina ženki kunića bila parena s mužjakom iz različite skupine nakon slijedeće laktacije. Nakon 35 dana laktacije, kunići su bili odbijeni. Sve su ženke (*n*=36) imale po tri legla, s prosječnom veličinom legla od 8,01 novorođenčadi (mrtvo ili živo rođenih), ili ukupno 865 nakon 108 uspješnih parenja. Ukupni gubitci novoređenčadi do odbića u dobi od 35 dana bili su 30,52 % (*n*=263), a sastojali su se od: mrtvorođenih 9,71% (*n*=84), 8,79 % je uginulo u prvih 7 dana života (*n*=76), 6,24 % je uginulo od 8. do 21. dana (*n*=54), a 5,78 % od 22. dana do odbića 35. dana (*n*=50). Nuliparne ženke imale su manja legla od primiparnih i sekundiparnih ženki kunića, odnosno 7,78 prema 8,06 i 8,19 mladunčadi. Reprodukcijski pokazatelji, uključujući stopu poroda i veličinu legla po rođenju i odbiću (u dobi od 35 dana), bili su značajno bolji u RPL i FGL skupinama u odnosu na KON skupinu.

Ključne riječi: *buserelin, gonadorelin, smrtnost prije odbića, kunić, reprodukcija*