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Induction of Ovulation after Artificial Insemination in Rabbits: Intramuscular Injection of Gonadotropin-Releasing Hormone (GnRH) Agonist vs. Intravenous Administration of Mated Doe Serum

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Abstract

BACKGROUND: Rabbits are induced ovulators with the ovulatory process being triggered by neuro-hormonal impulses generated during natural mating. When applying artificial insemination (AI), an array of biostimulation techniques and/or exogenous hormones, such as gonadotropin-releasing hormone (GnRH) or its analogues must be used to induce ovulation. However, the effect of biostimulation techniques and exogenous hormones is not always satisfactory and the use of GnRH analogues is associated with high production costs. Therefore, the development of an alternative inexpensive, efficient, and safe treatment for ovulation induction in artificially inseminated does is urgently needed.

OBJECTIVES: In the present study, we examined and compared the effects of mated doe serum (MDS) and GnRH analogue (Gonadorelin) administered immediately after AI on the circulating concentrations of luteinizing hormone (LH) and fertility in New Zealand does.

METHODS: Forty artificially inseminated does were allocated to four equinumerous groups. The Control G, Treatment G, Control M, and Treatment M groups received 0.2 mL of saline intramuscular (IM), 0.8 µg of Gonadorelin dissolved in 0.2 mL of saline IM, 2.5 mL of mixed-sex normal rabbit serum intravenous (IV),and 2.5 mL of MDS/doe IV, respectively.

RESULTS: A peak in systemic LH concentrations occurred earlier in Treatment M, compared to Treatment G does (71 vs. 107 min post-AI, respectively; $P \leq 0.05$). Mean LH concentrations did not vary ($P \leq 0.05$) from the pre-AI values in neither of the control groups. Serum LH concentrations remained higher ($P \leq 0.05$) in the Treatment M group, in comparison with Treatment G does during30-90 min post-AI. However, LH was higher ($P \leq 0.05$) in the Treatment G group than the Treatment M group 120 and 160 min post-AI. Gonadorelin and MDS injections both resulted in the same kindling rate of 80% at each of the four consecutive AIs (initiated 30 days postpartum) and were significantly greater than that recorded in the control animals (20%).

CONCLUSIONS: It can be concluded that MDS administration is an effective treatment for inducing ovulation in rabbits with repeatability similar to that achieved with a GnRH analogue.

KEYWORDS: GnRH, Induced ovulatory, Mated doe serum, Neuro-hormonal reflex, Rabbit

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Introduction

Artificial insemination (AI) in rabbits has been performed by European farmers since the 1920s mainly to achieve genetic improvement, to establish pregnancies in females that refuse to mate, and/or to avoid the spread of diseases, such as pasteurellosis and Treponema (Morrell, 1995; Theau-Clément, 2010). In addition, the application of AI in rabbits results in a dramatic reduction in the numberof days open and consequently the production of more than 60 weaned offspring (or approximately 120 kg of rabbit meat) per doe annually, which is considered the top performance in the intensive rabbit production systems (Gosling*et al*., 2018). AI is a useful approach to boost production efficiency in rabbit rearing facilities.

Rabbits are induced ovulators in which a coital stimulus is required to trigger the ovulation process (Alekseeva*et al*., 2018; Bakker and Baum, 2000; Castellini, 1996; Castellini *et al*., 2010). When AI is performed in rabbits in the absence of a buck the induction of ovulation is typically achieved by biostimulation (i.e., exposure to male odor, nutritional flushing, alteration of the photoperiod, change of cages, and mother-litter separation prior to AI) or with an injection of exogenous gonadotropin-releasing hormone (GnRH) or GnRH analogues (Castellini *et al*., 2010). Biostimulation techniques have not been fully satisfactory in the reproductive management of rabbits does (Gosling *et al*., 2018). Although several GnRH analogues are currently available on the market, the application of GnRH analogues only resulted in a~30% success rate when used alone in artificially inseminated does (Soliman and El-Sabrout, 2020). Therefore, optimizing ovulation induction in rabbits is pivotal for consistently achieving high fertility rates after AI (Castellini *et al*., 2010; Cervantes*et al*., 2015).

Ovarian function in mammalian species is controlled by the coordinated activity of the hypothalamic-pituitary-ovarian axis. The main signal for ovulation is triggered by anterior pituitary gonadotropins, luteinizing hormone (LH), and follicle-stimulating hormone (FSH). The secretion of gonadotropins is controlled by GnRH released from the hypothalamus. Gonadotropic hormones are released in a pulsatile manner and are transported to the target organs via the bloodstream (Boiti, 2004). In induced ovulating species, both the presence of a male (visual, olfactory, and auditory signals) and mating (physical contact between male and female, intromission, and seminal plasma) initiate the secretion of GnRH by the hypothalamus (Quintela *et al*., 2009). Adams and Ratto (2013) suggested that physical stimulation and seminal plasma content were not the only significant factors contributing to the induction of ovulation in reflex ovulators. They indicated that the interaction of multiple serum constituents, including, but not limited to, lipid depots, non-esterified fatty acids, leptin, gonadotropins, sex hormones (e.g., 17beta-estradiol), and several other unknown factors might also play a role in the control of ovulation and reproductive performance in the females of mammalian species. All these factors could have local and systemic impacts on the induction of ovulation, as well as gamete transport and viability prior to fertilization (Arias-Alvarez *et al*., 2009; Hassanein *et al*., 2021). As a result, it could be proposed that mated doe serum (MDS) contains an array of factors that would facilitate ovulation and fertilization in artificially inseminated rabbits does. Moreover, exogenous LH and, to some extent, GnRH analogues may adversely affect ovarian function and induce immune responses in rabbits. A progressive decline in reproductive efficiency has been reported after applying such hormonal treatments for successive AIs. Following 4-5 AI cycles, some does that received LH or GnRH had to be culled (Castellini *et al*., 2010; Didarkhah*et al*., 2020).

To the best of the authors' knowledge, no study has assessed the possibility of ovulation induction in artificially inseminated does use an injection of MDS or compared it with ovulation induced by a GnRH analogue. Therefore, this experiment was conducted to evaluate and compare the efficacy of MDS and GnRH analogues for repeatedly inducing ovulation in artificially inseminated rabbits.

Materials and Methods

Animals and Experimental Conditions

All chemicals were purchased from Sigma Aldrich unless stated. All instruments were calibrated and maintained according to routine quality control

procedures overseen by the Quality Assurance Department of Razi Vaccine and Serum Research Institute. New Zealand White rabbits (Razi Vaccine and Serum Research Institute, Karaj, Iran) were housed at controlled environmental conditions (20°C±2°C,14:10h light:dark cycle) with *ad libitum* access to food and water. The study design/treatment regimen is summarized in Table 1. Drug dosing and the volumes of sera injected were determined empirically during the preliminary trials based on the general recommendations/guidelines on animal care and welfare.

Table 1. Study design and treatments applied to the rabbit does of the present study.

Group	Treatment	Route of injection	Injection volume/Dose	Time of injection
Control G (n=10)	Saline	Intramuscular	0.2 ml	Immediately after AI
Treatment G (n=10)	Gonadorelin[1]	Intramuscular	0.2 ml/0.8 µg per doe	Immediately after AI
Control M (n=10)	Mixed sex normal rabbit serum	Marginal ear vein	2.5 ml	Immediately after AI
Treatment M (n=10)	Mated doe serum	Marginal ear vein	2.5 ml	Immediately after AI

[1]GnRH agonist

Rabbit Serum Separation

To isolate MDS from blood samples, female rabbits with confirmed fertility (4 groups of 10, 9 months of age, and body weight (b.w.) of ~2 kg) were subjected to protein flushing (24% of crude protein and 2627 kcal of metabolizable energy/kg) 10 days prior to blood collection. The does had not been a part of the regular kindling program for 60 days before this procedure. All does were mated by sexually mature and experienced bucks (9 months age and bodyweight of ~2 kg). Following the successful mating, which is typically associated with the buck's cry and dismounting to one side of the doe, the does were anesthetized by an injection of 0.25 mL of ketamine (15 mg/kg b.w.) and xylazine (10 mg/kg b.w.) mixture into the marginal ear vein(Dadashpour Davachi, 2019). Blood was aspirated from the heart of rabbits with Venoject (BD Life Sciences, Cockeysville, Md, USA) without any anticoagulants. Approximately 100 mL of blood was collected from each doe.

A previously published study reported that the peak of LH surge occurred ~90 min after natural mating in female rabbits (Dufy-Barbe, Franchimont, & Faure, 1973). Therefore, forty mated does used in the present study were sampled 0, 1, 2, and 3h after successful mating. Following the last sampling, all blood samples were pooled and centrifuged at 3000×*g* for 10 min. Next, the supernatant was carefully separated and filtered under sterile conditions and was stored in the refrigerator at 4°C for 24 h before use. Ten male rabbits and ten virgin female rabbits with confirmed fertility (9 months old, mean b.w. of~2 kg) were used to obtain mixed-sex serum.

Semen Collection and Evaluation

Semen samples were collected from 15 clinically healthy and fertile male rabbits (9 months of age and b.w. of ~2 kg) using a homemade artificial vagina pre-warmed to 45°C and a teaser doe (Morrell, 1995). Sperm total and progressive motility were evaluated under a phase-contrast microscope at 37°C and then semen samples were pooled (Didarkhah *et al*., 2020; Smith & Mayer, 1955). Pooled semen samples were diluted with sodium citrate diluents (2.9 g of disodium citrate, 1.25 g of lactose, 0.04g of anhydrouscitric acid, 5 mL of egg yolk, 50000 IU of sodium penicillin, and 50000 µg of streptomycin sulfate/100 m Lof distilled water) at1:10 dilution to a final concentration of 1×10^6 spermatozoa/mL (Morrel, 1995). Sperm concentration in ejaculates ($N\times10^6$/ejaculate) and diluted inseminate doses ($N\times10^6$/mL) was determined using the modified Neubauerhemocytometer slide (Adams*et al*., 2016).

Artificial Insemination

All selected does for AI were housed separately for at least 19 days prior to insemination to avoid the

possibility of pseudopregnancy (Morrell, 1995). Insemination pipettes (~30 cm in length) were prepared by gently bending glass tubes at 45° angle approximately 8 cm away from one end and a 1-mL syringe attached to the opposite end of the pipette to exert proper and controllable pressure during semen deposition. To evaluate doe receptivity in the early morning of the day of AI, the tail was gently lifted and if the animals showed lordosis, they were considered ready for insemination. For semen deposition, all does were restrained in a supine position, and the tip of the pipette was inserted into the vagina at an approximate angle of 45° upward to negotiate the rim of the pelvis. Once the diluted semen was released into the upper vagina and the pipette was removed, the doe was returned to a sitting position (Masoudi and Dadashpour Davachi, 2020; Morrell, 1995).

After AI, defined as gestation day 0, the does were kept individually in standard metal breeding cages at 20°C±2°C and 14:10h light:dark cycle. Food pellets and water were available continuously, and several days before parturition the does were provided with nest boxes and hay. The nest boxes were checked every morning and evening for the presence of the young. The day of birth was defined as post-partum day 0(PP0).At PP30, the does were inseminated again for the next round of the experiment.

Serum Luteinizing Hormone (LH) Measurements

The LH concentrations were first measured in 2-mLsamples of MDS (Treatment M, serum LH level of 1100 ng/mL) and mixed-sex normal rabbit serum (Control M, serum LH level of 19 ng/mL).Blood samples (2 mL) were collected from the ear vein of seven does per group at various times before and after the first AI (–60, 30, 45, 60, 75, 90, 120, and 160 min). A radioimmunoassay technique, as previously described by Dufy-Barbe *et al*. (1973), was employed to measure serum LH concentrations using labeled rat LH (NIAMD-Rat-LH-I), a rabbit anti-ovine LH serum, and a rabbit LH pituitary preparation as the standard (L-LH-B2).The biological activity of the latter, as determined by the ventral prostate test, was 0.208 of the potency of NIH-LH-Si. Antibody was coupled with cyanogen bromide-activated cellulose according to the method described by Dufy-Barbe *et al*. (1973). The antibody-bound hormone was separated from the free hormone by centrifugation at 1000×*g*for 30 min. The assay sensitivity was 0.1 ng/mL with intra-assay coefficients of variation (CVs) of<8% and intra-assay CVs of<12%.

Statistical Analysis

Reproductive performance endpoints recorded in artificially inseminated does were analyzed by the Pearson Chi-square test using the SPSS software version 10 (SPSS Inc., Chicago, Illinois, USA). The mean kindling rate (prolificacy) was calculated using the following formula:

Number of kindling females/Number of artificially inseminated females×100% (Equation 1)

The numbers of live and dead pups were recorded at each birth. A comparison of the remaining single point observations obtained from the treated and control does was conducted using the one-way analysis of variance (ANOVA) at a 95% confidence level (SigmaStat®2.0 for Windows Version 2.03; 1997, SPSS Inc., Chicago, IL, USA). Two-way repeated measures ANOVA (RM-ANOVA) was used to compare the circulating LH over time (–60 to 160 min from AI) among the four groups of studied does. Fisher's protected least significant difference (LSD) was used as a post-ANOVA test to detect differences between individual means. In addition, some characteristics of LH secretion in individual does allocated to Treatment G and Treatment M groups, namely the maximum (peak) concentration, peak amplitude, the time from AI to peak concentration, and the area under curve (AUC) for the serial concentrations of LH were calculated. The AUC was computed using NCSS Statistical Software (Kaysville, UT, USA; version 21.0.2; http://www.ncss.com). The differences in LH secretion characteristics were compared between the two treatment groups by the Student t-test or Mann-Whitney Rank Sum test. The F-test for the equity of variances was used to compare the variability in LH secretory parameters between the Treatment G and Treatment M does. All data are expressed as mean±standard error of the mean (SEM) unless indicated.

Results

Mean serum LH concentrations increased significantly ($P \leq 0.05$) from 60 min before to 30 min after AI/injections in both Treatment G and Treatment M groups. No fluctuations were observed in the circulating LH concentrations of the Control G and Control M groups during the observation period (Figure 1). Treatment M does significantly exceeded ($P \leq 0.05$) the Treatment G animals in terms of mean LH concentrations from 30 to 90 min post-AI, while serum LH concentrations were significantly higher ($P \leq 0.05$) in the Treatment G group, compared to Treatment M from 120 to 160 min post-AI (Figure 1). Although numerically different, the secretory pattern of LH did not vary significantly between the two treatment groups in this study except for the timing of a peak in peripheral LH concentrations that occurred on average 35 min earlier in Treatment G, compared to the Treatment M group ($P \leq 0.05$; Table 2). Variability in mean AUC values for serial LH concentrations was significantly greater in Treatment G than in Treatment M does (Table 2).

Table 2. Changes in the characteristics of LH concentrations in response to the treatments of artificially inseminated does with GnRH analogue (Treatment G) or mated doe serum (Treatment M).

Group	Time from AI to LH peak concentration (min)[1]	Peak LH concentration (ng/ml)[1]	LH peak amplitude (ng/ml)[1]	AUC for serial LH concentrations*,[1]
Treatment G (n=7)	107±6[a]	266±29	247±30	30800±3796[A]
Treatment M (n=7)	71±3[b]	324±9	304±9	36011±475[B]

*Area under the curve;[1]Mann-Whitney Rank Sum test (normality and/or equal variance test failed); [ab]significant difference between the two mean values (Student t-test); [AB]significant difference between the two variances (F-test).

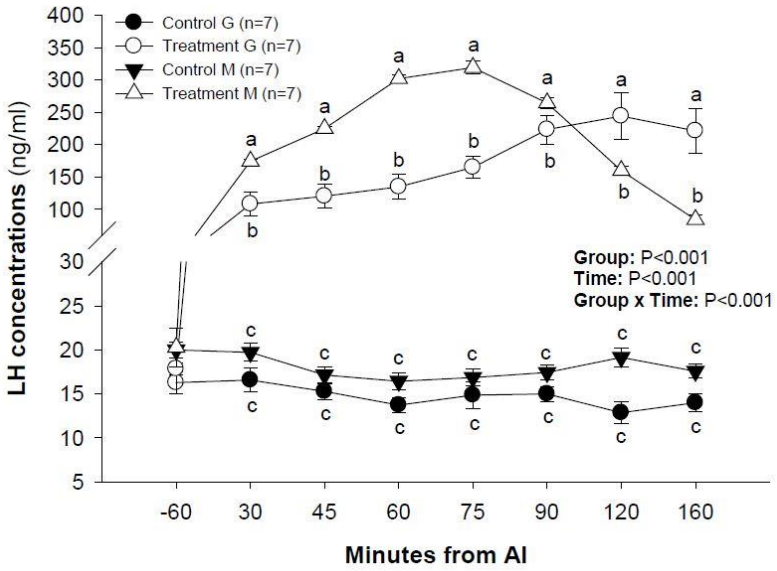

Figure 1. Systemic luteinizing hormone (LH) concentrations from 60 min before to 160 min after treatments/injections of artificially inseminated does (Time 0) that received 0.2 mL of saline i.m. (Control G), 0.8 µg of Gonadorelin in 0.2 mL of saline i.m. (Treatment G), 2.5 mL/doe of mixed sex normal rabbit serum i.v. (Control M) or 2.5 mL/doe of mated does serum i.v. (Treatment M) immediately after AI. Circulating LH concentrations increased significantly from –60 to 30 min in both treatment groups, but no significant fluctuations were observed in control groups. [a-c]denote statistically significant differences among the groups.

Both MDS (Treatment M) and GnRH analogue (Treatment G) significantly augmented kindling rates (4folds) and the prolificacy of rabbits does (Table 3). There were no differences in kindling rate and prolificacy between Treatment G and Treatment M groups nor between Control G (saline) and Control M groups (mixed-sex normal rabbit serum, $P \leq 0.05$; Table 3). Moreover, there were no significant differences between the four groups of female rabbits in terms of the mean mortality of pups at birth (Table 3). Reproductive performance (litter size) in the 4threplicate was significantly lower ($P \leq 0.05$), in comparison with the first three replicates in all groups of animals studied.

Table 3. Reproductive performance (% or mean±SEM) of New Zealand rabbit does after four consecutive ovulation-inducing treatments (study replicates) performed 30 days apart.

Variables/Groups	Replicate	Control G (n=10)	Treatment G (n=10)	Control M (n=10)	Treatment M (n=10)
Kindling rate (%)	1st	20^{a}	80^{b}	20^{a}	80^{b}
	2nd	20^{a}	80^{b}	20^{a}	80^{b}
	3rd	20^{a}	80^{b}	20^{a}	80^{b}
	4th	20^{a}	80^{b}	20^{a}	80^{b}
Prolificacy	1st	$1.2\pm0.8^{a,A}$	$9.6\pm1.6^{b,A}$	$1.3\pm0.9^{a,A}$	$9.8\pm1.6^{b,A}$
	2nd	$1.3\pm0.9^{a,A}$	$9.1\pm1.5^{b,A}$	$1.2\pm0.8^{a,A}$	$9.3\pm1.5^{b,A}$
	3rd	$1.2\pm0.8^{a,A}$	$9.1\pm1.5^{b,A}$	$1.1\pm0.7^{a,A}$	$9.5\pm1.5^{b,A}$
	4th	$0.8\pm0.5^{a,B}$	$7.0\pm1.2^{b,B}$	$0.8\pm0.5^{a,B}$	$7.2\pm1.2^{b,B}$
	Overall	1.1 ± 0.4^{a}	8.7 ± 0.7^{b}	1.1 ± 0.3^{a}	9.0 ± 0.7^{b}
No. of dead pups/doe		0.5±0.2	0.6±0.1	0.6±0.2	0.6±0.1

Different letter superscripts denote significant differences (P<0.05): ^{ab}within rows and ^{AB}within columns.

Discussion

The AI is preferred over natural mating in the intensive rabbit rearing systems and using various ovulation-promoting treatments, such as injecting exogenous GnRH/GnRH analogues or biostimulation are essential in inseminated does. Hormonal treatments have successfully been used in rabbit farms. However, these treatments are expensive and have adverse effects during extended production cycles. Earlier studies on the endocrine changes of the mated doe gave rise to the hypothesis that MDS might induce ovulatory responses in female rabbits undergoing AI. Therefore, the main objective of the present study was to document and compare the efficacy of MDS and GnRH analogue in inducing ovulation in artificially inseminated does. Results of the present study demonstrated that MDS is an effective ovulation-inducing factor in rabbits does post-AI. The fertility traits analyzed in this experiment were positively influenced by MDS treatment and did not vary from those produced by gonadorelin.

The mean LH concentration of the pooled MDS samples collected in the current study was 1100 ng/mL. In a study conducted by Scaramuzzi*et al.* (1972), circulating LH concentrations 1 h after mating in female rabbits had a range of 160-2350 ng/mL. The mean LH level in mixed-sex normal rabbit serum was 19 ng/mL, which is also in agreement with the results obtained by Scaramuzzi *et al.* (1972).Theapplication of mixed-sex normal rabbit serum (Control M) yielded similar results as the saline control (Control G). These observations are supportive of the notion that neurohormonal reflexes associated with mating result in the secretion of an array of biologically active, fertility-enhancing substances into the circulation of does. These hormonal factors, in turn, activate the hypothalamic-pituitary-ovarian axis culminating in multiple ovulations in rabbits.

Comparing the findings of Treatment M and Treatment G does revealed that both treatments were

able to substantially and rapidly elevate serum levels of LH. The peak of LH secretion in the Treatment M group was recorded approximately 35 min earlier than after a GnRH analogue injection (71 vs. 107 min post-AI). Circulating LH concentrations after MDS administration increased at a faster rate, compared to those in Treatment G does. Moreover, total LH (AUC) during the sampling period was more variable in response to gonadorelin injection than after MDS treatment. However, these differences did not impinge on the ovulatory response and post-AI fertility. MDS is a source of readily available LH and its intravascular injection can lead to a dramatic increase in bioavailable lutropin concentrations in the systemic circulation of does.

Mean circulating LH concentrations were significantly higher in Treatment M than in Treatment G does up until 90 min post-AI. Furthermore, the rate of LH decline was greater in the Treatment M group than in the Treatment G group. The average dose of LH injected to MDS-treated rabbits (2.5 mLof MDS or ~2750 ng of LH) does not explain gonadotropin concentrations observed in Treatment M does (rabbits weighing approximately 2 kg hence possessing a total blood volume of 114-130 mL; https://www.nc3rs.org.uk/blood-sample-volumes). It is feasible that MDS injected into Treatment M does and originally obtained from blood samples collected 0-3 h post-mating contained other factors, such as GnRH that stimulate LH secretion. Subsequently, circulating LH in the MDS-injected group exerted negative feedback on GnRH secretion blocking endogenous LH secretion from the pituitary glands of rabbits. This suppression could have occurred more rapidly than in Treatment G does, which is suppressed at higher initial LH levels. Once again, these differences in the pattern of serum LH concentrations between the two subsets of treated does did not affect their reproductive performance.

The body condition score and general metabolic status of female rabbits can also impinge on the GnRH and LH secretion/clearance rate after mating (Gado*et al*., 2015). In the present study, MDS was purified from mated does that were subjected to nutritional flushing 10 days before serum collection. Therefore, their body condition and lipid depot could vary from those in regularly mated does in commercial settings. Castellini (2010) showed that blood leptin concentrations are a key marker of body condition, fat reserve, and energy stores. Circulating leptin concentrations may also influence the reproductive performance of does by directly modulating ovarian activity (Dal Bosco*et al*., 2007). However, the putative role of leptin in the induction of ovulation in rabbits remains to be elucidated.

Based on earlier studies in camelids (Adams and Ratto, 2013; Didarkhah *et al*., 2020),it was proposed that seminal plasma in induced ovulators contained potent ovulation-inducing factor(s). The ovulatory response to seminal plasma in llamas and alpacas is brought about by a surge in the circulating concentrations of LH and this effect of seminal plasma is related to the degree of its absorption from the genital mucosa into the systemic blood circulation (Adams and Ratto, 2013; Adams *et al*., 2016). Moreover, the response to seminal plasma in llamas and alpacas was independent of the degree of physical stimulation of the tubular genitalia. However, the present results indicate that the seminal plasma itself or with the physical stimulation of the vagina during AI only induced ovulation in 20% of does allocated to the control groups. This could, at least in part, be caused by the dilution of inseminating doses used for AI. More confirmative studies are required on the potency of seminal ovulation-inducing factors in rabbit semen.

It is forecasted that the world population will exceed 9 billion in 2050 (Gosling *et al*., 2018). This increment in the global population will necessitate the production of nearly 50% more animal protein (Gosling *et al*., 2018). Rabbits are considered a good potential source of meat and hence a potential solution to the world's rising demand for meat production (Daader*et al*., 2016). The reproductive performance of rabbits is their major advantage, compared toother livestock species as they are highly prolific (30-40 offspring/year), attain sexual maturity relatively early (six to seven months of age), have a short gestational period (30-31 days), and can practically rebreed immediately after kindling (42 days) (Elamin*et al*., 2012).

It can be concluded that the IV administration of MDS is an effective treatment for inducing ovulation in artificially inseminated rabbits making it a suitable replacement for GnRH analogues in rabbit reproductive programs. Therefore, the application of

MDS could be promising for improving the reproductive performance and economic management of rabbits.

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Conflict of Interest

The authors declared no conflict of interest.

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القای تخمک گذاری پس از تلقیح مصنوعی در خرگوش: تزریق عضلانی آگونیست آزاد کننده هورمون گنادوتروپین (GnRH) در مقابل تجویز داخل وریدی سرم خرگوش ماده جفتگیری کرده

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چکیده

زمینه مطالعه: خرگوش‌ها به‌عنوان حیواناتی که روند تخمک‌گذاری القایی دارند شناخته می‌شوند. فرایند تخمک‌گذاری القا شونده به واسطهٔ پیام‌های عصبی-هورمونی که در هنگام جفت‌گیری طبیعی ایجاد می‌شوند، کنترل می‌شود. در زمانی که استفاده از تلقیح مصنوعی برای تکثیر خرگوش‌ها در دستور کار قرار می‌گیرد، به‌منظور القای تخمک‌گذاری در خرگوش‌های ماده باید از مجموعه‌ای از روش‌های تحریک بیولوژیکی و / یا هورمون‌های برونزا (به‌عنوان مثال، هورمون آزادکننده گنادوتروپین (GnRH) یاآنالوگ‌های آن) استفاده شود. با این حال، اثربخشی تکنیک‌های مختلف تحریک زیستی و هورمونی همیشه راضی‌کننده نبوده و استفاده از آنالوگ‌هایGnRH علاوه بر این مشکلات، منجر به تحمیل هزینه‌های بالای تولید و نگهداری خرگوش به‌عنوان یکی از مهمترین حیوانات آزمایشگاهی می‌شود.

هدف: بنابراین، ایجاد یک روش ایمن،کم‌هزینه، وکارآمد به‌عنوان جایگزین درمان‌های هورمونی متداول برای القای تخمک‌گذاری در خرگوش در ادامهٔ انجام تلقیح مصنوعی ضروری است.

روش کار: درمطالعه حاضر، اثرات تزریق سرم خرگوش ماده جفت‌خورده وآنالوگGnRH (گنادورلین) به‌عنوانعوامل احتمالی موثر در القای تخک‌گذاری در خرگوش ماده که مورد فرایند تلقیح مصنوعی قرار گرفته است بررسی می‌شود. خرگوش‌های ماده در چهار گروه ۱۰تایی تقسیم شده و هر گروه به یکی از تیمارهای زیر اختصاص می‌یابند: گروه کنترل ۰/۲ میلی‌لیتر سرم فیزیولوژی (Control G)، ۰/۸ میکروگرم گنادورلین(Treatment G)، ۲/۵ میلی‌لیتر سرم خرگوش نرمال(Control M) i.v. یا ۲/۵ میلی لیتر سرم خرگوش جفت‌خورده (Treatment M) (MDS) i.v.

نتایج: نتایج ثبت‌شده در این مطالعه نشان داد سرژ هورمون LH درزمان‌های آغازین پس از تزریق سرم خرگوش جفت‌خورده در گروه Treatment M درمقایسه با گروه Treatment G به وقوع می‌پیوندد ($P<0/05$) (۷۱ درمقابل ۱۰۷ دقیقه پس از تلقیح مصنوعی). غلظت میانگین LH ($P<0/05$) در مقایسه با غلظت‌های این هورمون قبل از (تلقیح مصنوعی) AI در هر دوگروه کنترل تفاوت معنی‌داری را نشان نداد. همچنین نتایج بررسی سطح سرمی LH نشان داد که غلظتLH سرم در تیمار M در مقایسه با تیمار G از ۳۰ تا ۹۰ دقیقه پس از تلقیح مصنوعی بالاتر بود ($P<0/05$)، درحالی‌که در گروه دریافت‌کنندهٔ تیمار G غلظت LH در مقایسه با گروه دریافت‌کنندهٔ تیمار M در ۱۲۰ و ۱۶۰ دقیقه پس از تلقیح مصنوعی بالاتر بود ($P<0/05$). تزریق گنادورلین و سرم خرگوش مادهٔ جفت‌گیری‌کرده، هر دو منجر به نرخ یکسان تولد زنده (۸۰٪) پس از تلقیح مصنوعی شدند. این نرخ به صورت معنی‌دار از نرخ مشاهده‌شده در گروه‌های کنترل بالاتر بود (۲۰٪).

نتیجه‌گیری نهایی: می‌توان نتیجه گرفت که تجویز سرم خرگوش مادهٔ جفت‌خورده یک درمان موثر برای القای تخمک‌گذاری در خرگوش‌ها است. کیفیت تاثیر این سرم کاملا مشابه با آنالوگGnRH ثبت شد.

واژه‌های کلیدی: خرگوش، رفلکس هورمونی-عصبی، تخمک‌ریزی القایی، هورمون آزادکنندهٔ گونادوتروپین، سرم خرگوش جفت‌گیری‌کرده

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