INFLUENCE OF TYPE OF STIMULATION ON THE SUCCESS OF ARTIFICIAL INSEMINATION IN RABBITS DOES OF THE SYNTHETIC STRAIN

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Abstract

The objective of the study is to determine the impact of applying different stimulation modes in rabbits to achieve fertile insemination (positive palpation). A total of 30 rabbits of the synthetic strain aged 6.5±1.4 months, with an average weight of 3607.2 ±241.6 g, were divided into three experimental batches (10 rabbits/batch), according to the stimulation method used: hormonal (either GnRH or PMSG) and mechanical stimulation by the use of sterile males. The effect of the determined stimulation mode on the reproductive parameters of the rabbit conducted in AI was analyzed during the three consecutive reproduction cycles, in order to determine the most reliable and cost-effective method to recommend to improve the fertility and productivity of the rabbits... The rabbits were monitored and their reproductive parameters recorded. The receptivity of rabbits before insemination is recorded. All data obtained from three reproductive cycles revealed that, apart from stillbirth, other reproductive parameters were not affected by the mode of ovulation induction.

Keywords: *Hormonal stimulation, infertile male, ovulation, fertility, prolificacy, mortality*

INTRODUCTION

The rabbit's particular situation in animal production confers him a special status in the area of reproductive biotechnology, in particular artificial insemination, a biotechnology that has proved its worth over the past 15 years in rabbits. Today, 86% of farmers worldwide use it with an average fertility of 77% (Theau-Clement, 2005). According to Theau-clément and *al* (2011), ovulation induction, necessary in the case of artificial insemination, by GnRH analogues is the more efficient method in the short and long term. However, their protein and exogenous nature associated with their high molecular weight has led to fears of significant immunogenicity, thus reducing their efficacy in prolonged use in this species. Thus, Adams (1972) showed that 87% of rabbits no longer ovulate after the fifth injection.

Indeed, the predictable evolution of regulations on the use of exogenous hormones requires the search for alternative methods to improve the sexual receptivity of rabbits and consequently their productivity. For these reasons, methods called "Biostimulations" have been proposed. These methods, applied immediately prior to insemination, must be easy to apply, inexpensive, compatible with animal welfare and well adapted to band driving.

In this context, the present study aims to stimulate ovulation of rabbits, conducted in artificial insemination, by hormonal methods (GnRH and PMSG) compared to a method of ovulation induction by the use of a sterile male. It also aims to study the effect of the mode of ovulation induction on certain reproductive parameters in rabbits undergoing artificial insemination.

1. MATERIALS AND METHODS

1.1 Animal material and experimental conditions

The experiment took place in the animal house hutch of the Department of Agricultural Sciences and Biotechnology of Hassiba Ben Bouali University (Chlef), during a period from May 2 to September 21, 2017. The breeding building consists of a breeding room containing the maternity cages, a room for the cages of the breeding males and an analysis laboratory where the various analyses carried out

during the experiment took place. Ventilation is provided by two exhaust fans, which are switched on every morning to remove harmful gases. Lighting is provided by artificial light, the duration of illumination of the maternity cell is 16 hours/day.

Thirty rabbits of the Algerian synthetic strain "ITELV 2006", aged 6.5 ±1.39 months, were inseminated with the seed of a male belonging to the same genetic type, aged 08.5 months and a half and weighing 2975 g. Characteristics of this strain were also described by Lebas and *al* (2010) and Zerrouki and *al* (2014). Females are supplied with a commercial pelleted feed, composed of alfalfa, from flour mills, maize, sunflower meal, molasses, poly-vitamins, trace elements, calcium carbonate, phosphates, amino acids, vitamin E, D3, A. The chemical analysis of the feed yielded 14.5% raw protein and 9.5% crude fiber. Water was always available from automatic nipple drinkers. Lighting was natural.

1.2 Methods

1.2.1 Evaluation of male sperm parameters

An analysis of the macro and microscopic characteristics of ejaculate collected from the male used was performed by visual observation, volume, pH and color measurement on the graduated tube weaned at collection. Sperm concentration, mass and single motility, viability and rate of sperm abnormalities were also assessed. After the semen has been analysed, the semen was diluted 10 x with a commercial "Galape" diluent.

1.2.3 Receptivity control

It is carried out by color observation and vulva turgidity before insemination. Females with turgid red or purple vulva were reported as receptive, while females with pink or white vulva were not receptive.

1.2.4 Constitution of lots

The 30 rabbits were divided into three experimental groups of 10 rabbits, depending on how ovulation was induced.

First group (1): Ovulation was induced by the hormonal method by administering 0.3ml of GnRH, injected intramuscularly, 1 hour before the insemination .- Second group (): Ovulation was induced by the hormonal method by the administration of 0.3ml of GnRH, injected intramuscularly, 1 hour before the insemination.

Second group (2): Ovulation was hormonally induced by administering 40 IU of PMSG, by intramuscular injection, 2 days before insemination.

Third group (3): Ovulation induction in rabbits was performed using a sterile male with the usual sexual characteristics (overlap). The infertile male was left with a female for 15 minutes, which was deemed sufficient to induce overlapping of the female, and therefore ovulation.

Females from the three lots were inseminated by a dose of 0.3ml of semen that was collected and analysed. 12 days following the AI, abdominal palpation was performed on inseminated rabbits to diagnose pregnancy. At birth, the females are weighed, the litters are counted, weighed and the dead born are withdrawn. Weaning is carried out 30 days after birth. The weaned young rabbits are weighed.

1.2.5 Zootechnical parameters used

Reproductive parameters studied are: receptivity, fertility, litter size at birth and at weaning (total born, alive born and number of weanlings), mortality of kits at birth and during the birth-weaning phase, litter and kits weights at birth and at weaning are recorded.

1.2.6 Statistics analysis

Variables that were measured and calculated were statistically processed by XL stat plus version 5 software, with an Analysis of Variance Model (ANOVA), taking the fixed effect of ovulation induction mode in the female (3 levels). A descriptive statistical study was made with the same

software. The means were compared 2 to 2 by the Fisher test. The presentation of all results is made in tables and graphs, using Excel software version 2007.

2. RESULTS AND DISCUSSION

2.1 Characteristics of semen

In the experimental study period, 20 collections of sperm were carried out. Mean values of macro and microscopic analyses of semen used to inseminate females are presented in Table 1. The semen volume determined is 0.52ml, higher than the sperm volume values reported by Theau Clément (2007) when estimating the fertility of rabbit semen from INRA strains (0.4 ml), and by on New Zealand white rabbit (0.49ml). On the other hand, Boulbina (2011) notes that rabbits of local Algerian population have a higher sperm volume (0.86 ml).

The color of samples is pearly white (BN). According to the Roca et al. table, 1993, this semen color was given a score of 3. The pH of the sperm used in the experiment is 6.76, which is slightly lower than that found by Brun and *al* (2006) who reported an average pH of 7.3. A large variability in pH of sperm from males of different strains and breeds has been reported by many authors. Indeed, Brun and *al*, (2006) recorded an average pH of 7.13 on a seed collected from males of the local population, aged between 19 and 33 weeks. Similarly, Lavara and *al.(*2008) published pH values ranging from 5.6 to 7.8, similar to those recorded by Garcia- Tomas and *al.* (2006); Brun and *al.* (2009), from 6.94 to 7.63. In addition, these authors reported the effect of the environment in pH variation. The overall movement of a sample taken from the collected ejaculate was estimated at 5.25 according to Petitjean's (1965) grid (Boussit 1989) ranging from 0 to 9.

Seed analysis parameters	**Evaluation**
Volume	0.52 ml
Color	3 (BN)
pH	6.76
Massale motility	5.25
Individual motility	3.5
Concentration	513.12x 106 spz/ml
Anomalies	9.8

Table 1. Semen characteristics of males rabbits of synthetic strain

Indeed, during the investigation of mass mobility by microscopy, we noted a movement of most spermatozoa, which allowed us to determine a good motility of the used semen.

The spermatozoa observed under a microscope (x40) are moving quickly through a small diameter helix. We attributed this individual mobility a score of 3.5 on the Andrieu scale (1974) (Boussit 1989).

The mean concentration of sperm in collected ejaculates is evaluated at 513.12x 106 spz/ml, which is higher than the value recorded by Nabi (2013) on the semen of white buck rabbits (428.94x 106 spz/ml) and lower than on the semen recorded by Boulbina (2011) (734.9×106 spz/ml). The rate of abnormal sperm observed is 9.8%.

Comparison of the semen characteristics used in this study with the bibliographic data revealed that our females were inseminated with good quality semen.

Lankri and *al* (2019), on the same strain of males, reported comparable characteristics to our results (502±29.106 spz/ml, 0.48 ±0.11 ml, 5.21±0.77, 3.36±0.56, 6.86±0.12 respectively for concentration,

volume, mass motility note, individual motility note and pH) has a better quality for artificial insemination.

2.2 Reproductive parameters of rabbits according to the rabbit's mode of stimulation

Table 2 summarizes the reproductive performance of rabbits according to the mode of ovulation induction in rabbits.

Table 2. Reproductive performance according to the mode of ovulation induction

	stimulation mode			
Variables	GnRH	Infertile male sterile	PMSG	P
Receptivity(%)	73,33	63,33	70	0.1
Pregnancy rate (%)	86,66	73,33	80	0.1
Kindling rate(%)	68,33	61,66	65	0.1
Total born	9,63	10,23	10,2	0.4
	n=289	n=307	n=306	
Alive born	8,06	7,63	7,2	0.37
	n=242	n=229	n=216	
Weaned	7,33	6,76	6,5	0.32
	n=220	n=203	n=195	
litter birth weights (g)	486,18	433,48	407,25	0.1
Kitsbirth weight	60,785	56,946	58,03	0.13
Mortinatality (%)	$16,53^{a}$	$26,26^{b}$	$29,4^{b}$	<0.01
Mortality birth- weaning (%)	7,93	10,33	9,73	0.7

2.2.1. Stimulation mode and receptivity effect

An average receptivity rate of 68.9% was recorded during the experiment, lower than the rates obtained by Moulla and Yakhlef, 2007 and by Zerrouki et al, (2001) on local population rabbits that were naturally mated. They recorded an average rate of 89.87% and 80% respectively. These lower receptivity rates are attributed to the lactation state of females. This observation was confirmed by Theau-Clement and Roustan (1980) who recorded 73% in lactating females and 84% in non-lactating females. Similarly, Roustan and Maillot (1990) reported that nulliparous females are more receptive, accounting for our rabbits' relatively low rates, the most of them in the 2nd or 3rd cycle (secondiparous and multiparous).

Females in which ovulation is induced by GnRH have a higher receptivity rate (73.33%) than those in which we used the PMSG (70%), but the differences are not statistically significant. Many authors have showed that the receptivity is greater following the use of GnRH analogues than the PMSG, which appears to be reducing it. However, Theau-Clément (2005) showed that the administration of 20 IU of PMSG per rabbit, 48 hours before artificial insemination increases receptivity.

Salvetti (2008) shows that an intramuscular injection of a dose of GnRH allows a higher rate of receptivity compared to a mixture of the same hormone with the semen, i.e. a rate of 90% versus 60% for both pathways, respectively. Other researchers have indicated that the use of equine chorionic gonadotropic hormone (ECG) would have a positive effect on rabbit susceptibility (Alabiso and *al.*, 1994; Mirabito and *al.*, 1994; Theau-Clement and Lebas, 1996; Theau-Clement and *al.*, 1998).

The attempt to trigger ovulation directly by unfertilizing mating with the use to a sterile male resulted in a lower receptivity rate (63.33%) than the other two batches. This was confirmed by Kustos and *al* (2000), who reported that the overlap by a sterile male has no effect on improving rabbit receptivity.

2.2.2 Fertility effects

For several years now, the impact of hormonal methods on female rabbits has been the object of several studies. Indeed, Hulot and Poujardieu (1976) reported that injections of GnRH, hCG or LH have been shown to induce ovulation well; however, repeated injections with these hormones lead induce immunization and decreased efficiency after the 4th or 5th injection.

In our study, the hormonal effect on fertility is clearly observed, despite an absence of significativity ($P<0.1$). Indeed, we recorded higher palpation and parturition rates (86.66 and 68.33%) with GnRH injections than with PMSG (73.33 and 61.66%). GnRH appears to be increasing fertility rates.

This result is in agreement with the one reported by Theau-Clément (2008) who indicates that repeating injections of PMSG are generally followed by a reduced fertility rate in female rabbits. On the other hand, Quintela and al. (2004) show that adding 25g of GnRH analog in the semen gives a fertility higher than that of the rabbits obtained following an intramuscular injection of 20g Gonadorelin (GnRH, 270 IA) or 91.1% and 85.6%, respectively.

Fertility rates recorded in rabbits following the use of a sterile male to stimulate ovulation are similar to those recorded with the other two methods (80 and 65% respectively for pregnancy and calving rates).

However, Kustos and *al* (2000), show that the introduction of a male among females 4 days before artificial insemination was not modify fertility. In addition, Hulot and Poujardieu (1976) noted that mechanical stimulation of the vagina by action on the brain can caused ovulation, but the results were very random (14.7%).

Theau-Clément and Roustan (1980) point out that in artificial insemination, the fertility rate is influenced by the receptivity rate of rabbits. In contrast, Theau-Clément and Poujardieu (1994); Theau-Clément and Lebas (1996); Rodriguez de Lara and Fallas (1999), Theau-Clément (2001), report that the fertility of receptive rabbits is significantly higher (>75%) than that of non-receptive ones. Our results insofar as rabbits stimulated by the administration of GnRH have receptive levels and consequently comparatively high fertility.

In general, we recorded average values of 80% and 65% respectively for gestation and farrowing rates without considering the effect of the stimulation mode. These results are higher than those recorded by Hounton (2008), who reported a fertility rate of 52.11%. Tanimomo, (2007) recorded a rate of 69.50%.

The fertility of rabbits can be influenced by many factors, highlighting the parity of females (the multiparous majority in our study), indeed, Perrier et al. 2000; Theau-Clément (2005) show that the fertility rate of primiparous women is generally $\leq$ 70% lower than that of nulliparous women. This was confirmed by Questel (1984) who recorded a fertility rate of 81% for nulliparous females and 57% for multiparous females.

It should be noted, however, that no statistically significant difference ($p<0.1$) was found between palpation or calving rates depending on the method of ovulation induction.

2.2.3 Effect on prolificity

The number of kits In fact, the hormonal method using GnRh seems to give the lower total number (9.63). The other two groups have comparable values (10.23 and 10.2 for sterile male and PMSG groups). In terms of alive births and number of weaned, the situation is reversed. The rabbits in the GnRH group have the greatest numbers of kits However, the statistical analysis did not show any statistically significant variation ($P<0.3$-0.4) between the three modes of ovulation induction. Hormonal induction mode (GnRh and PMSG) does not increase prolificity in females as compared to induction by the sterile male.

A similar observation was made for the average litter weight and mean birth weight of kits. Indeed, the females of the three groups produce litters with an average weight of 486.2g, 433.5g and 407.2g with an average birth weight of approximately 60.8g, 57.0 and 58.0 g respectively for the females in the GnRH, sterile male and PMSG groups.

This result is confirmed by Hulot and Poujardieu (1976), Theau-Clément and Lebas (1996), Vicente et al. (2009), demonstrated that the improvement in the prolificity of hormone-treated rabbits is in fact only associated with an increase in the percentage of susceptible rabbits.

Also, Quitela and al (2004) and (2008) reported that using GnRH directly in the semen dose for insemination is more effective compared to intramuscular injection.

Theau-Clément (2005),Mehaisen and al (2005) noted that using eCG tends to increase the ovulation rate. Several studies, examining the relationship between the different treatments used to induce ovulation, have found a variation in their effect on prolificacy. Indeed, according to Bechstedt and Hattenhaur (1984), GnRh is more effective than treatment with HCG. In addition, Kennlly and Foote (1965); Maurer and *al.* (1968); Besenfelder and *al.* (2002); Mehaisen and *al.* (2005) demonstrate a higher response to super ovulation treatment with FSH use compared to eCG use.

2.2.4 Effect on mortality

Many authors have noted a negative influence of hormone use on embryo quality (kanffiman and al., 1998; Mehaisen and al., 2006). Indeed, in the present study, this negative effect is observed on the stillbirth rate, which is the only parameter affected by method of inducing ovulation (P=0.01). Rabbits in GnRH lot have a lower rate (16.53%), compared to rabbits in PMSG lot (29.4%) and sterile male lot (26.26%). Our results are consistent with those reported in the bibliography. Indeed, Canali and al., 1991; Alabiso and al., 1994; Maertens and Luzi,(1995), noted that the use of hCG can have a negative effect on fertilisation rates and birth viability rates.

Moreover, this justifies the higher stillbirth rate from rabbits for which ovulation was induced by PMSG. Contrary to stillbirth, mortality recorded between birth and weaning was not affected by the method of ovulation induction (P=0.7). Although the GnRH lot registered the lowest rate (7.93%), compared to the two other lots (10.33 and 9.73 respectively for the sterile male lot and PMSG).

Many studies report that induction mode only has an effect on increases in the number of receptive females at the time of insemination (Theau-Clément and Lebas, 1996; Salvetti, 2008).

CONCLUSION

From these results, it appears that:

Rabbits' receptivity is influenced by the mode of ovulation induction. Indeed, the rabbits in which ovulation is induced by GnRH have a significantly greater receptivity and fertility rates and prolificacy compared to females for which PMSG and the infertile male have been used.

Except for the stillbirth rate, no reproductive variables were significantly affected by the ovulation induction mode. This is an interesting result as an alternative method which is easily applied, cheap, compatible of animal welfare and well-adapted to driving in a band, such as that using a sterile male, can substitute hormonal methods which show a high immunogenic capacity in time and which can reduce their effectiveness if used for a long period of time in this species.

Females for which ovulation is induced by the sterile male have a good fertility rate and prolificity, in accordance with bibliographic data. This allows us to say that this method will be able to substitute these hormonal methods, thus allowing more safety, while taking into account the regulations aimed at reducing the use of exogenous hormones in all animal species.

Finally, it would be very interesting to continue this work on a larger number of females in order to confirm our results and the robustness of the methods used as an alternative to hormone use.

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