

# Artificial insemination in rabbits: factors that interfere in assessing its results



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Received: January 24, 2020 • Accepted: February 23, 2020 • Published Online: February 28, 2020

**Abstract** Understanding the rabbit's sexual behavior and reproduction process is very important when using artificial insemination (AI) instead of natural mating in intensive rearing systems. Ovulation in rabbit females not occur spontaneously/ induced ovulatory animals, but it has to be induced through a neuro-hormonal reflex, which is stimulated during mating. The mating effect of satiety on specific measures of rabbit female sexual behavior has not been investigated. When applying AI, in the absence of a male, ovulation has to be induced by exogenous hormonal analogs. Bio-stimulation techniques are suggested as a substitute for hormones in that respect. One of the most effective approaches to distribute ejaculates from bucks of superior quality or genetic value is the systematic use of AI in intensive rabbit production. Rabbit's sexual receptivity has a significant influence on fertility at insemination. In addition, a limitation factor for rabbit AI spread is related to their semen preservation. Fresh diluted semen has been used but its quality can be maintained only for a short period. However, there are few articles available have indicated the negativity of AI usage on rabbit, and the factors that can influence the AI process including the sexual desire behavior. Therefore, the objective of this review is to identify the important factors that directly or indirectly affect AI process success, meanwhile interfering with and/or relying on AI assessment.

**Keywords:** bio-stimulators, natural mating, semen collection, sexual behavior, welfare

## Introduction

The world population has constantly grown, as expected will reach more than eight billion in 2030, and maybe more than 9 billion in 2050. This will require an increase in food production of nearly 50 percent. Therefore, the world will be required more than 50% increase in global meat production (da Costa et al 2011). The rabbit is a good potential meat source as a solution to fulfill demand for animal proteins (Mailafia et al 2010; Daader et al 2016). With an

intensive breeding program, a rabbit doe (female) can be expected to produce 60 weaned young per year, representing a total of 120 kg rabbit meat at market age (Lukefahr and Cheeke 1990). Moreover, rabbits have a high reproductive rate compared to other livestock. They become sexually mature earlier, relatively short gestation period, produce higher litter size at birth and can be rebred immediately after kindling and short generation interval (Hassan et al 2012).

The artificial insemination (AI) of rabbit females appear on European farms in the late 1980's (Theau-Clement 2007). It used previously to obtain the genetic improvement of the animals and to solve sanitary problems. However, significant improvements in both productive and economical aspects were later achieved, such as the acceleration of the desirable traits of high genetic value, which supported by the use of biotechnologies for semen and embryos preservation (da Costa et al 2011).

Maintaining animals by improper management includes some certain negative effects may cause abnormal behavior (Lawrence and Rushen 1993). Understanding rabbit behavior is necessary to determine their needs and the consequent adaptation of commercial rearing systems include AI. Normal behavior is among animal welfare regulations identified by the Farm Animal Welfare Council (FAWC 2001). For appropriate evaluation of rabbit welfare, physiology parameters, disease, behavior and performance indicators should be considered (Trocino and Xiccato 2006). Additionally, understanding factors affect rabbit sexual behavior is very important because they influence its sexual efficiency directly (El-Sabroust 2017). In general, there are few articles available have indicated the negativity of AI usage on rabbit, and the factors that can influence the AI process including the sexual desire behavior. Therefore, the objective of this review is to identify the important factors that directly or indirectly affect AI process success, meanwhile interfering with and/or relying on AI assessment.

## Natural Mating

Rabbit's sexual behavior and breeding potential are influenced by a wide board of external and internal stimuli factors (Ajuogu and Ajayi 2010). Sexual behavior of rabbit buck consists of performing a single mount, followed by a series of pelvic thrusts during which intromission occurs, and ultimately resulting in semen ejaculation inside the doe vagina (Coy et al 2012). Rabbit bucks have a remarkable capacity for sexual activity (Jiménez et al 2012) and their average frequency of mating 2.43 in 15 minutes (Gado et al 2015). Rabbit bucks with sexually used perfectly and high-quality semen are required throughout the year to achieve maximum productivity and libido, either through AI (Rodríguez-de Lara et al 2008) or natural mating (Saleh et al 2010).

Rabbit doe is considered poly-estrus or having no cycle or regular estrus (Aduku and Olukosi 1990) and ovulation occurs after mating (Vella and Donnelly 2012). Rabbit uterus morphology represents a duplex of the uterus consisting of two separate, fully functional uterine horns and cervixes opening into a single vagina (Fischer et al 2012). Periods of doe receptivity last anywhere from 5 to 14 days and are followed by one to two days in which the doe will refuse mating with buck (Vella and Donnelly 2012). Rabbit does are in heat (estrus) when their vulva is wet, red or slightly violet color and swollen (Maertens and Luzi 1995; Szendrő et al 2006), this is followed by the peak luteinizing hormone (LH) in their blood (Maertens et al 1983).

In natural mating, the breeder enters the female into the male cage, since they live commercially in separately. Natural mating is common and suitable on small and medium farms with less than 100 does (Szendrő et al 2012). Ovulation occurs in the ovaries between 10 and 13h after mating (Vella and Donnelly 2012). Within the same time, the sperm move inside the doe reproductive system to the fertilization area (ampule) to meet oocyte and fertilization takes place (Coy et al 2012). The fertilization rate is generally high, about 95% (Peiró et al 2007).

## Artificial Insemination

Artificial insemination technique, in general, showed to be most suitable for both small and large commercial rabbit farms than natural mating (Shuji 2009). In the last two decades, the productivity of rabbit farms has increased by the implementation of AI due to intensive cycled production and genetic selection for increased prolificacy (Castellini et al 2010). However, AI is commonly used at large rabbit farms in Europe and include five steps: collecting semen from the bucks, examining the semen visually and microscopically, dilution of semen 10 to 20 times, inseminating does by pipette, and injecting Gonadotropin-Releasing Hormone (GnRH) analog into the hind leg muscle at the moment of

insemination (Szendrő et al 2012). Rabbit reproduction in most European farms carried out by AI, known as "single batch". In this system, all rabbit does subjected to a specific intervention on the same day (e.g. all does are inseminated on one day), and the next will takes place 6 wks later. Here the economic value of fertility is increased since the doe that was not fertilized will remains not pregnant for 6 wks (Theau-Clément et al 2015). Consequently, the palpation, parturition and kits weaning make on the same day.

In France, the rabbit does in over 90% of the farms are inseminated 11 days after parturition (Azard 2006). This is known as the 42-d reproduction cycle, which comprises the gestation period (31 days) and time for rebred (thereafter 11 days). In this case, kits are weaned at 28 to 32 days of age and the growing rabbits are sold at the age of 70 to 74 days, with average weight from 2.4 to 2.5 kg (Jentzer 2008). From the animal welfare viewpoint, the 56-d cycle was more favorable than 42-d cycle, for doe body condition and survival of does, but number of kits/doe/year of the 42-d exceeded that of the 56-d by 19 to 23% which has a high economic impact the rabbitry benefits (Szendrő et al 2012).

When AI applies the does are in the lactating period, the conception rate depends on their receptivity. Theau-Clément et al (2006) observed a strong inverse relationship between lactation and reproductive functions in non-receptive does. However, hormonal treatment [pregnant mare serum gonadotrophin (PMSG)] is sometimes recommended for increasing lactating doe receptivity.

## Factors interfered with the evaluation of AI results

AI technology allows breeders to keep a greater number of breeding does whereas reducing the number of bucks required to be housed, thereby minimizing the spread of disease from mating and improving production rates (Morrell 1995). In addition, the AI technique includes prolonging fertility during hot times of the year, better breeding programs, favorable gene selection, health monitoring improvements and finally cycle-based production (Carluccio et al 2004), and rabbit embryo developmental (Obasi et al 2016).

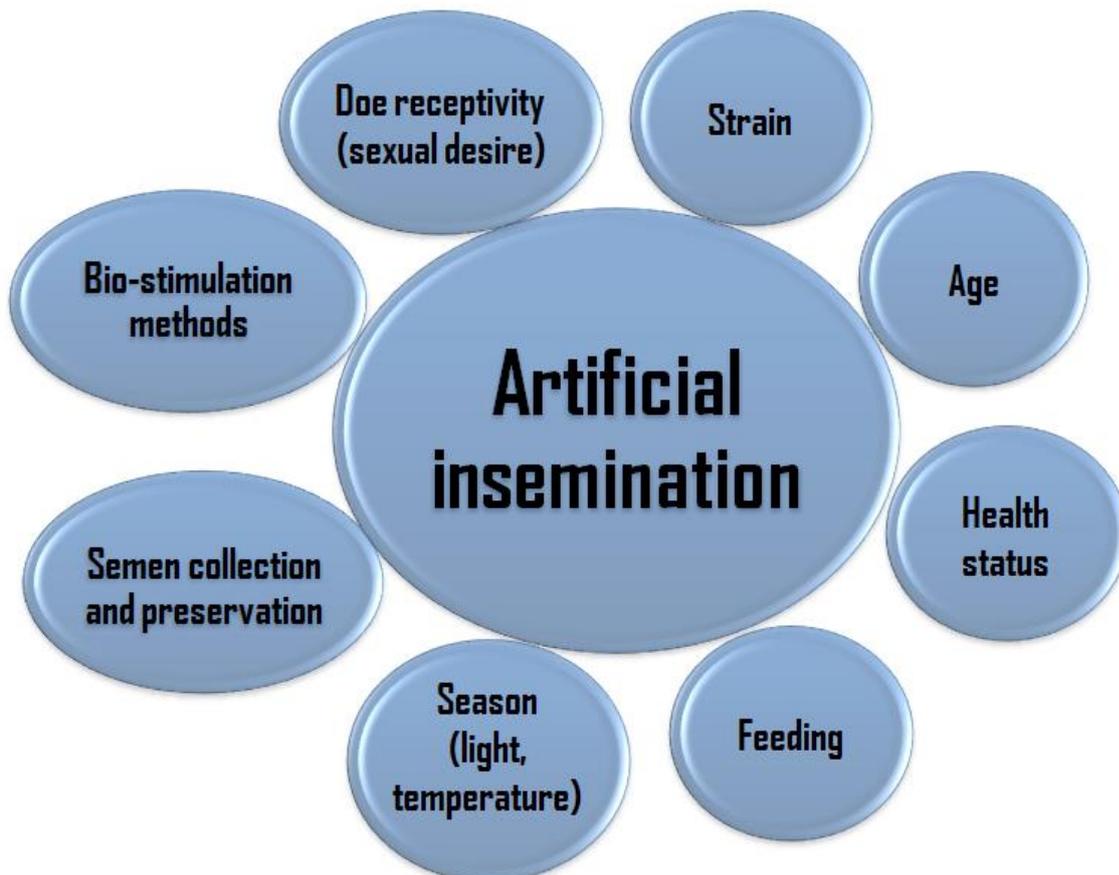
Several factors are affecting the AI success and reproductive performance of inseminated does (Figure 1). However, it is important to highlight that most of these factors also have an impact on the reproductive efficiency of rabbits submitted to natural mating.

### *Doe receptivity (libido) and Buck effect*

Fertility affects greatly by sexual receptivity of rabbit does at AI, which could be induced by hormones or "bio-stimulation" techniques. There is high individual variability of sexual receptivity of non-lactating rabbit does maintained without any bio-stimulation or hormonal treatment (Theau-Clément et al 2011; 2015). The receptivity and fertility in the

rabbit have a high phenotypic relationship (Theau-Clément 2008) whereas fertility has low heritability value (Piles et al 2004). Theau-Clément et al (2015) found that the heritability of receptivity was 0.04, which indicated to be not heritable, which lead to a lack of selection response. Moreover, they reported that the pseudo-pregnancies due to uncontrolled ovulations could have interfered with receptivity.

Receptive does at insemination produce three to four times more rabbits at weaning than those non-receptive ones, particularly when they are lactating (Theau-Clément 2008). Therefore, receptivity is often induced by injection of PMSG and/or bio-stimulation method (Renouf and Klein 2008). However, Theau-Clément et al (2008) showed that lower fertility would not have been compensated by PMSG due to its inefficiency in pseudo-pregnant does.



**Figure 1** Factors that could affect the artificial insemination (AI) success.

Sexual receptivity, parity, lactation status, pseudo pregnancy (Theau-Clément et al 2012) and physiological status (Castellini 1996) affected the reproductive performance of rabbit doe at the time of insemination. Sexual receptivity is determined at the day of AI using three methods: color of vulva (red or pink represent receptive rabbit doe, while purple or white represent non-receptive rabbit doe); rectal temperature, and vagina cytology (the percentage of keratinized cells), according to Ola and Oyegbade (2012).

On the other hand, buck existence could influence the AI success. Hammond and Asdell (1926) reported early that AI followed by sterile mating resulted in a higher pregnancy rate than AI alone (91% versus 4%). The presence of rabbit

buck near the doe affect the fertility of the doe was reported by Berepudo et al (1993) and Bonanno et al (2003).

Cervantes et al (2015) documented that the effect of physical/social interaction on ovulation in New Zealand White (NZW) rabbits, and did not support the hypothesis that seminal plasma stimulates ovulation in rabbits. Short-term (2 h) bio-stimulation of NZW rabbit does (exposed to male odor or an adult male) resulted in the appearance of various behavioral responses followed by differences in conception rates after routine AI (EL-Azzazi et al 2017). In addition, high reproductive performance in the inseminated rabbit does is depends on the physiological and behavioral experience of does at the time of insemination (Theau-Clément 2007). The

lower rates of receptive does with AI result in a less successful insemination process (Theau-Clément et al 2015).

In a recent study, Ola and Oyegbade (2012) reported that does exposed to visual contact with the buck (in conjunction with olfactory and auditory contacts) showed reddish or pinkish vulva most of the time, an indication of high state of sexual receptivity. It has been concluded that certain male signs other than mating can also stimulate ovulation in the doe rabbit. Ola and Olatunbosun (2013) indicated the buck's presence improved the doe's sexual receptivity. However prolonged visual and/or tactile contacts between the two opposite sex appeared to be detrimental to the breeding efficiency and overall productivity of the doe.

In respect of bucks, authors suggest that rabbits exchange sex pheromones of either male or female origin to influence reproductive behavior in the opposite sex (Hudson and Distel 1990; McNitt 1992). Introductions of female pheromones cause significant elevation in male sex hormones that regulate spermatogenesis in male Syrian hamsters (Richardson et al 2004). Pheromones are chemical elements emitted by one individual and received by another from the same species, raises different behavioral and endocrine activities (Hegab et al 2015). Rabbit doe exposure of bucks could be a low-cost bio-stimulation option that benefits AI in commercial rabbit farms by improving buck rabbit's reproductive performance (improves sexual drive, sperm production and quality).

#### *Light effect, bio-stimulation methods, and PMSG*

To increase receptivity and conception rates at 11 days after kindling, rabbit does are generally treated 2-3d prior to AI by PMSG. The PMSG treatment, depending on the dose and frequency, may induce antibody production which can negatively influence the kindling rate, and in consequence, culling rate will increase (Castellini 1996; Theau-Clément 2007). For these reasons, researchers and breeders would like to replace the PMSG with alternative bio-stimulation methods.

The optimal interval between PMSG injection and AI was studied by Alvarino (2005) that concluded that fertility decreases when the interval reaches 96 h, without any significant differences of fertility from 24 to 72 hours. Moreover, Rebollar et al (2006) found a positive effect of PMSG only during the first four injections. It is commonly accepted that a dose of 20-25 IU of PMSG at 48 h before insemination of lactating does at 11 days post-partum increases the percent of receptive does at insemination. In addition to their high costs, the use of hormones has become unfavorable from the welfare point. The PMSG has been used for about 15 years to synchronize rabbit doe estrus (Daader et al 2016). For the previous conditions, researchers and breeders would like to replace the PMSG treatments with alternative

bio-stimulation methods (Theau-Clément 2007), some of these methods already used in practice, such as dam-litter separations prior to insemination, changing the nursing method or lighting schedule.

Sexual bio-stimulation methods (act through sensory signals) have their effect on both receptivity and fertility of rabbit does. The hormonal regulation of receptivity in rabbit does showed to be strongly related to estrogen levels, as it is generally used to assess follicular growth and ovarian functions (Marongiu and Dimauro 2013). Bio-stimulated rabbit does yielded higher pregnancy rates and litter size at birth and at weaning compared to untreated does. Different bio-stimulation techniques have been used effectively in initiating and enhancing the sexual behavior and reproductive cycles in rabbits (Rodríguez-de Lara et al 2010; González-Mariscal et al 2015). Furthermore, the number of offspring produced in a litter is the result of the interaction of several variables including both maternal and male effects (Schmidova et al 2016).

It was proven that increasing the daily lighting schedule from 8 to 16 h 7-8 d prior to AI leads to higher receptivity and kindling rates (Gerencsér et al 2008; Matics et al 2012) and kindling rate (Szendrő et al 2016). However, Maertens and Luzi (1995) increased the lighting period from 10 to 16 h/day five days before doe AI but there is no significant change observed in the conception rate. Normal program of light (16 Light: 8 Dark) is optimal and has no significant effect on any reproductive traits of inseminated rabbit does compared with different examined light programs (Gerencsér et al 2012) and also semen reproductive traits of bucks (Marai et al 2002). Gerencsér et al (2008) concluded that light stimulation could be an alternative to PMSG treatments. Daily lighting period, in general, differs according to year-seasons. Rodríguez-de Lara et al (2010) showed that semen characteristics of rabbit bucks differed by season and reproductive potential in spring was significantly greater than those in winter.

In addition, Szendrő et al (2016) reviewed that rabbits are able to see well during the night; however, their color vision is restricted. Rabbits which exposed to a periodic light and dark environment generate a 24 h rhythm of their body functions. Moreover, Gerencsér et al (2011) suggested that light color's effect on the rabbit production can be perspective. According to the literature the rabbits perceive the red light (its wavelength) less compared to other light colors. In general, bio-stimulation methods are very natural and less expensive compared to the hormonal treatments (Lorenzo et al 2014).

#### *Vaginal barrier and seminal plasma*

Rabbit vaginal fluids have some amount of water since any substance intended for vaginal delivery requires a certain

degree of solubility (Hussain and Ahsan 2005). Recently, the GnRH recommended adding directly to the seminal dose of AI. This method provides new perspectives on the absorption capacity of different GnRH analogs from the doe reproductive system, meanwhile reducing the time of hormone injections and improving the welfare of rabbit does (Quintela et al 2009). Factors represent a vaginal barrier such as the dissolution degree in vaginal lumen, the membrane thickness, the degradation or blockage due immunoglobulins, cytokines, proteases, the leucocytosis, the fast or slow transport to the uterus by means muscle contractions, the hormone profile, the conductivity, etc. (Vicente et al 2008; Rebollar 2011). In addition, factors influencing the absorption of GnRH by the vaginal mucosa include the state of the mucosa (affected by sexual receptivity), extender composition and the capability of sperm to incorporate foreign molecules (Vicente et al 2008).

GnRH is a hypothalamic multi-peptide that plays a necessary role in the regulation of reproductive processes (Conn and Crowley 1994). GnRH is a member of a larger family of peptides that is present in every vertebrate class examined thus far (Tsai and Zhang 2008). Several GnRH analogs with different pharmacological properties and potencies are commercially available, such as triptorelin, leuprolide, buserelin, gonadorelin, dalmarelin, etc. are used to induce ovulation in rabbit does. The PMSG and GnRH application studied by Dimitrova et al (2009) showed that fertility was 28.57% when applied only GnRH, while it was 62.50% when both applied. Furthermore, NZW does showed higher level of fertility than Californian does.

Seminal plasma also contains other particles of different size which affect the spermatozoa behavior during the transit along the female reproductive tract. Strain, feeding, health status, rearing condition, season, age and collection frequency are most factors of affected seminal characteristics, thus contributing to the large variability in semen traits (Alvariño 2000). Paolicchi et al (1999) suggested that some factor(s) in the seminal plasma could contribute to the secretion of LH and, consequently to the induction of ovulation in receptive rabbit doe. Ratto et al (2010) have concluded that ovulation-inducing factor in seminal plasma is a protein molecule that is resistant to heat and enzymatic digestion with proteinase K, and has a molecular mass approximately 30 kDa. The stimulatory chemical compound in seminal plasma which ovulation-inducing effect is a protein called beta nerve growth factor ( $\beta$ -NGF). It is suggested that  $\beta$ -NGF (ovulation-inducing factor, OIF) induces its effects on the hypothalamo-pituitary axis (Adams et al 2016). This protein was found in abundant amount in rabbit seminal plasma and induced ovulation in 80% of female alpacas following administration of 1 mg. (Kershaw-Young et al 2012). It was concluded that  $\beta$ -NGF may provide an alternative mechanism for the induction of ovulation in alpacas and; reduces the need for synthetic hormones and

partially improves fertility in combination with AI. In addition, Silva et al (2015) mentioned that  $\beta$ -NGF from llama seminal plasma origin stimulate level followed by ovulation and the development of functional corpora lutea, regardless of the route of administration. Adams et al (2016) reported that the ovulatory response to  $\beta$ -NGF seminal plasma in llamas and alpacas is brought an increase of LH level in the bloodstream and their function depend on the degree of absorption of this factor from the genital mucosa into circulation and not a response to physical stimulation of the tubular genitalia itself.

#### *Semen quality and site of insemination*

Generally, the success in AI depends on many factors as semen quality, insemination dose, live and normal spermatozoa percentages, duration between semen collection and AI and depth of semen deposition in the female reproductive tract (Boiti et al 2005) and also receptive females and perfect inseminator (Rowida et al 2016). The rabbit vagina is a favorable environment for prolonged sperm survival, after mating or AI, sperm capable of fertilization are transported beyond the cervixes leaving the seminal plasma behind (Drobniz and Overstreet 1992). Only 5% of semen deposited transported beyond the uro-vaginal valve (Overstreet and Cooper 1978). The vagina, cervix, and uterus each maintain a population of progressively motile spermatozoa for at least 16 h after mating, by which time ovulation occur and 98% of oocytes are fertilized (Cooper et al 1979). Therefore, one of the most important factors associated with high fertility with AI is the site of semen deposition inside the doe vagina. There is a difference in the length of the rabbit vagina which was showed to be 10.0 cm (Frandsen 1981), 20.8 cm (Morrell 1995), 14 to 19 cm long (Rebollar 2011), and 14.5 to 19.5 cm (Rowida et al 2016). These differences may be due to strain and age of rabbit does. Louis (2009) found that the pipette should be inserted 10-15 cm into rabbit vagina to ensure good fertility. The optimal number of spermatozoa per insemination will vary upon the procedures to which the spermatozoa are subjected prior to storage (Farrell et al 1993) and their motility (Lavara et al 2005). The good required insemination dose depends on semen handling as diluters and preservation storage time as well as the genetic strain and physiologic state of the female (Brun et al 2002). It showed to be 0.5 ml of diluted fresh semen containing about  $1.6 \times 10^6$  motile sperm (Chen et al 1989), more than  $50 \times 10^6$  motile sperm (Seleem 2003), diluted 1:3 with  $80-100 \times 10^6$  spermatozoa/ml (López-Gatius et al 2005), diluted 1:5 with  $6 \times 10^6$  spermatozoa/ml (Lavara et al 2005), diluted 1:6 with  $60 \times 10^6$  spermatozoa/ml (Zeidan et al 2008). Rowida et al (2016) found that increasing the number of spermatozoa ( $45.04 \times 10^6/0.5$  ml) in dilution rate 1:5 and deposit inter vagina at 12 cm improved conception and kindling rates while litter size was not affected.

Sperm numbers required for optimal fertility can be reduced in case of deep vaginal-cervical insemination as an important factor in commercial AI of rabbits (Tantasuparuk et al 2011). Moreover, Anderson et al (2004) and Kurykin et al (2006) reported that a low number of spermatozoa significantly increased the pregnancy rate, when the semen deposited in the uterus. On the other hand, Chelmońska et al (2007) showed that deep insertion of the posterior region of the uterus creates unfavorable conditions for spermatozoa and consequently, fertility efficacy. According to Araujo et al (2013), using suitable insemination dose and dilution rate are very important because very small volumes may result in less effective mechanical drainage, while highly concentrate semen may be more irritating because of more contacts between spermatozoa and endometrium, resulting in an intense inflammatory response. Theau-Clément et al (2016) showed that the presence or absence of gel, volume, percentage of progressive sperms, curvilinear velocity and beat frequency of the flagellum, did not affect fertility or productivity of inseminated INRA1001 rabbit doe. However, mass motility and the percentage of motile cells (>84%) significantly influenced rabbit doe productivity, but there was a non-linear relationship. On the other hand, Ndors et al (2015) showed that three/day AI frequency with NZW does has significantly better conception and litter size compared to 1/day and 2/day frequencies. However, gestation length and litter weight traits were not significantly affected.

#### *Preservation of rabbit semen*

Commercial rabbit meat production is usually used AI with fresh diluted semen, yielding pregnancy rates similar to natural mating (Morrell 1995) and performed within 6-12 h of collection (Daniel and Renard 2010). Rabbit semen preservation is one of the main problems for a wide use of AI in rabbitries (Alvariño 2000). However, the demand for stored semen to be used in AI programs of livestock animals (Zhao et al 2009) and rabbit (Di Iorio et al 2014) is increasing. Storage of rabbit semen for longer than 24 to 48 h causes damage of semen quality with a decrease in fertility (Rosato and Iaffaldano 2011; Di Iorio et al 2014). Rabbit spermatozoa are more sensible to hypertonic solutions, causing a reduction in storage ability and consequently a decrease in kindling rates (Seleem and Rowida 2005). Moreover, the freezing process of rabbit semen is associated with a reduction in motility, viability, and fertility or prolificacy after AI (Castellini et al 2006). This is partially due to the membrane lipid peroxidation caused to increase the level of reactive oxygen species, which in turn would affect lipids, proteins, nucleic acids and sugars within the sperm (Bansal and Bilaspuri 2011; Kim et al 2011). Moreover, rabbit semen does not respond to dilution as in other species mainly because of its sensibility to hypertonic

solution, and to cry-protective agents containing hydroxyl groups such as glycerol (Ndors et al 2015).

The attempts to improve rabbit semen extenders and storage conditions to prolong the storage duration of semen still going on and the negative and positive results are obtained. Unfortunately, rabbit spermatozoa had a minimum capacity to chilling (Rosato and Iaffaldano 2011) or frozen (Mocé and Vicente 2009) storage. Echegaray-Torres et al (2004) found that the maximum period of NZW rabbit semen preservation at 15 °C in gelatin supplemented extender was 72 h, without affecting seminal characteristics. Adding 2% gelatin to tris-buffer extender of APRI rabbit semen enhanced freezing ability, efficiency, and fertility of spermatozoa in thawed semen (El-Sherbieny et al 2012). Similarly, López-Gatius et al (2005) and Raga-Ayat et al (2012) showed that motility and viability of spermatozoa are maintained for a longer time when gelatin is added to fresh rabbit semen extenders. Only a few reports have shown satisfactory results with frozen semen (Iaffaldano et al 2012; Rosato and Iaffaldano 2013). However, the freezing technique is more expensive and need more equipment than with chilling technique.

Of recent attempts, the results of Hong et al (2012) indicated that sperm preparation from cauda epididymis direct collection of sperm for AI was faster and more efficient for inseminated rabbits does than the conventional method. Chilled semen storage studied by Johnke et al (2015), and found that chilled with  $30 \times 10^6$  spermatozoa/ml may provide an appropriate balance degree between motility and  $H_2O_2$  production to best maintain overall sperm function. Additionally, Rowida et al (2015) indicated that sodium chloride is effective as a diluents to obtain good results at 37 °C. The Tris extender pH 7.5 is of great value for the preservation of NZW rabbit semen at 5 °C. Moreover, the enrichment of pooled rabbit semen tris-basic extender with 1.2-1.6 mg propolis ethanolic extract/5 ml maintain the sperm characteristics in good condition all over 72 h of chilling (El-Seadawy et al 2017).

In addition to the genetic makeup of the rabbit (strain effect) (Heba Allah et al 2016), there are many environmental/managements factors reported in the literature to influence the reproductive characteristics of doe and/or buck and maybe interfere with the evaluation of AI success, e.g. the differences in mating frequency (Ajuogu and Ajayi 2010); age of doe (Rodel et al 2004), dietary supplementations (Alsenosy and Abd El-Aziz 2019), light (Mousa-Balabel and Mohamed 2011; Szendrő et al 2016), season (Szendrő et al 2016), climates/heat stress (Kumar et al 2013; Marco-Jimenez et al 2013), housing (Rommers et al 2006), and suckling mortality (Hamilton et al 1997).

Finally, the overall view of the comparison between nature mating and AI in rabbits should include: (1) the

prospective of damage caused by the use of exogenous hormones before and after AI on the hormonal status and/or reproductive functions of does. (2) Doe behavioral changes resulting from the non-practice of natural mating (buck effect/ doe libido/ doe sexual practice), which deprived them of natural sexual desire (welfare aspect matter). We may suggest further researches in the future on these two important points.

### Final Considerations

Artificial insemination in rabbits (with/without hormones) has been reported in several previous studies with different rabbit's strains, applications, and environments that have been partially reviewed in this review. The AI process could be difficult for the smallholder of rabbit farms to be carried out accurately/ no potential because of the abundance of factors affecting the success of this process, in term of semen collection, dilution degree, protocol of hormone injection date, equipment, duration of semen storage, technical of AI procedure, as well as the costs of these steps. This may suggest the need for private companies for rabbit semen collection, dilution, conservation and AI application in small farms, especially in developing countries that depend on small rabbit flocks. In addition, the review confirms that AI is useful in large rabbit farms taking into account the factors mentioned to achieve the highest fertility results. However, further researches are needed in order to not/minimize future use of hormones (PMSG & GnRH) for AI in rabbits, either for the availability of bio-stimulation methods to synchronize rabbit doe estrus or natural substances in seminal plasma for rabbit ovulation.

### Acknowledgments

Authors of this review are grateful to their respective university for its technical assistance and valuable support in the completion of this review.

### Conflict of Interest

The authors declare no conflict of interest.

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